University of Agronomic Sciences
and Veterinary Medicine of Bucharest
Faculty of Horticulture

SCIENTIFIC PAPERS
SERIES B. HORTICULTURE

Volume LXIX, No. 1



2025
Bucharest

SCIENTIFIC PAPERS
SERIES B. HORTICULTURE
VOLUME LXIX, NO. 1, 2025


EX·TERRA·AVRVM

University of Agronomic Sciences
and Veterinary Medicine of Bucharest
Faculty of Horticulture

SCIENTIFIC PAPERS
SERIES B. HORTICULTURE

Volume LXIX, No. 1

2025
Bucharest

SCIENTIFIC COMMITTEE

- Bekir Erol AK - University of Harran, Sanliurfa, Turkey
- Arina Oana ANTOCE - University of Agronomic Sciences and Veterinary Medicine of Bucharest, Romania
- Adrian ASĂNICĂ - University of Agronomic Sciences and Veterinary Medicine of Bucharest, Romania
- Liliana BĂDULESCU - University of Agronomic Sciences and Veterinary Medicine of Bucharest, Romania
- Valentina BOZHKOVA - Fruit Growing Institute, Plovdiv, Bulgaria
- Daniela CICHI - University of Craiova, Romania
- Mirela CORDEA - University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, Romania
- Sina COSMULESCU - University of Craiova, Romania
- Elena DELIAN - University of Agronomic Sciences and Veterinary Medicine of Bucharest, Romania
- Alin DOBREI - University of Life Sciences "King Mihai I" from Timişoara, Romania
- Elisabeta DOBRESCU - University of Agronomic Sciences and Veterinary Medicine of Bucharest, Romania
- Lucia DRAGHIA - "Ion Ionescu de la Brad" University of Life Sciences of Iași, Romania
- Franco FAMIANI - Universita degli Studi di Perugia, Italy
- Claudia FABIAN - University of Agronomic Sciences and Veterinary Medicine of Bucharest, Romania
- Mihaela Ioana GEORGESCU - University of Agronomic Sciences and Veterinary Medicine of Bucharest, Romania
- Gheorghe GLĂMAN - President of the Romanian Horticultural Society, Romania
- Luca Corelli GRAPPADELLI - University of Bologna, Italy
- Dorel HOZA - University of Agronomic Sciences and Veterinary Medicine of Bucharest, Romania
- Károly HROTKÓ - Corvinus University of Budapest, Hungary
- Olimpia IORDĂNESCU - University of Life Sciences "King Mihai I" from Timişoara, Romania
- Karsten KLOPP - Obstbauversuchsanstalt Jork, Germany
- Mekjell MELAND - Norwegian Institute of Agricultural and Environmental Research - Bioforsk, Norway
- Carmen MANOLE - University of Agronomic Sciences and Veterinary Medicine of Bucharest, Romania
- Orsolya BORSAI- University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, Romania
- Adrian PETICILĂ - University of Agronomic Sciences and Veterinary Medicine of Bucharest, Romania
- Silviero SANSAVINI - University of Bologna, Italy
- Radu SESTRAŞ - University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, Romania
- Alvaro STANDARDI - University of Perugia, Italy
- Florin STĂNICĂ - University of Agronomic Sciences and Veterinary Medicine of Bucharest, Romania
- Dorin SUMEDREA - National Research-Development Institute for Biotechnology in Horticulture Stefanesti-Arges, Romania
- Florin TOMA - University of Agronomic Sciences and Veterinary Medicine of Bucharest, Romania
- Valerica TUDOR - University of Agronomic Sciences and Veterinary Medicine of Bucharest, Romania
- Cristos XILOYANNIS - University of Pisa, Italy

EDITORIAL BOARD

General Editor: Adrian ASĂNICĂ
Executive Editor: Adrian PETICILĂ
Members: Bekir Erol AK, Valentina BOZHKOVA, Luca CORELLI GRAPPADELLI, Mihaela Ioana GEORGESCU, Elisabeta DOBRESCU, Károly HROTKÓ, Karsten KLOP

PUBLISHERS:

University of Agronomic Sciences and Veterinary Medicine of Bucharest - Faculty of Horticulture
Address: 59 Mărăşti Blvd, District 1, 011464, Bucharest, Romania
E-mail: journal@horticultura-bucuresti.ro, Webpage: www.horticultura-bucuresti.ro

CERES Publishing House
Address: 106 Izbiceni Street, District l, Bucharest, Romania
E-mail: edituraceres@yahoo.com, Webpage: www.editura-ceres.ro

Copyright 2025

To be cited: Scientific Papers. Series B. Horticulture, Vol. LXIX, No. 1, 2025

The publishers are not responsible for the opinions published in the Volume. They represent the authors' point of view.

Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

International Database Indexing: Web of Science Core Collection (Emerging Sources Citation Index), Index Copernicus, CABI, Ulrich's Periodicals Directory (ProQuest), PBN, Scientific Indexing Service, Cite Factor (Academic Scientific Journals), Scipio, OCLC (WorldCat), Research Bible, Google Scholar.

SUMMARY

FRUIT GROWING

1. BIOCOMPATIBLE MANAGEMENT ON POSTHARVEST DISEASES - **Diyana ALEKSANDROVA, Gabor ZSIVANOVITS**..... 17
2. EFFECTS OF BAGGING ON FRUIT DEVELOPMENT AND QUALITY IN 'KANZI' APPLES - **Orsolya BORSAI, Andrei CRISTEA, Sándor RÓZSA, Tincuța-Marta GOCAN, Cosmin Alexandru MIHAI, Viorel MITRE, Lehel LUKÁCS** 23
3. DIGITAL MANAGEMENT OF ARONIA CROP: THE BENEFITS OF HUMIDITY AND TEMPERATURE MONITORING THROUGH SPECIFIC SENSORS - **Anca Maria CHIOREAN, Zsolt JAKAB- ILYEFALVI, Ioan ZAGRAI, Smaranda ROȘU- MAREȘ, Georgeta Maria GUZU, Claudiu MOLDOVAN, Adrian ASĂNICĂ** 33
4. FRUIT CHARACTERISTICS OF DIFFERENT AUTOCHTHONOUS CHERRY GENOTYPES SELECTED FOR THE INTERACTION WITH THE PLUM POX VIRUS - **Florentina DOBRINESCU, Viorel Cătălin OLTENACU, Ligia ION** 39
5. STUDY ON THE IMPACT OF SEMI-TRANSPARENT PHOTOVOLTAIC PANELS IN HORTIVOLTAIC SYSTEMS ON MICROCLIMATE AND STRAWBERRY PRODUCTION IN ROMANIA - **Damian DRAGOMIR, Cătălin-Viorel OLTENACU, Dragos Vasile OFRIM, Cristian CĂLINIȚĂ, Bogdan Alexandru OFRIM, Dragoș Mihai OFRIM** 44
6. PLANT MATERIAL IN SOME STONE FRUIT SPECIES ROOTSTOCKS - **Elena Andreea VOCHIN (DUMITRESCU), Valentina ISAC, Florin STĂNICĂ** 54
7. EFFECTS OF DWARFING ROOTSTOCKS ON THE VEGETATIVE AND GENERATIVE DEVELOPMENT OF SEVERAL CHERRY CULTIVARS IN PEDOCLIMATIC CONDITIONS OF BISTRITA FRUIT REGION - **Zsolt JAKAB-ILYEFALVI, Daniela-Larisa VLASIN, Anca Maria CHIOREAN, Georgeta-Maria GUZU, Claudiu MOLDOVAN, Smaranda ROȘU-MAREȘ** 60
8. POMOLOGICAL AND CHEMICAL CHARACTERIZATION OF SOME SWEET CHERRY VARIETIES GROWN IN URBAN ORCHARD - **Lehel LUKACS, Gabriela Maria ROMAN, Ioana MOLDOVAN, Sándor RÓZSA, Tincuța-Marta GOCAN, Orsolya BORSAI** 70
9. THE IMPACT OF CLIMATE CHANGES ON THE PHENOLOGICAL DYNAMICS OF SOME PEAR VARIETIES IN THE MĂRĂCINENI AREA, ROMANIA - **Eugenia GHERGHINA (MAREȘI), Mădălina MILITARU, Mădălina BUTAC, Dorel HOZA** 81
10. MULTIVARIATE ANALYSIS OF GENOTYPE BY YEAR INTERACTION FOR SOME FRUIT TRAITS IN ROSEHIP (*ROSA CANINA* L.) - **Adina Gabriela MARIȘ, Sorin CIULCA** 87
11. THE REACTION OF SOME APRICOT CULTIVARS AND SELECTIONS TO THE ATTACK OF SOME PATHOGENS AND FROST IN THE CONTEXT OF CLIMATE CHANGE - **Cristina MOALE, Vlăduț Alexandru OPRIȚA, Leinar SEPTAR, Ion CAPLAN, Ioan STOLI** 98

12. COMPARISON OF QUALITY CHARACTERISTICS OF FRESH AND PROCESSED STRAWBERRIES - **Ioana MOLDOVAN, Lehel LUKÁCS, Gabriela Maria ROMAN, Sándor RÓZSA, Tincuța-Marta GOCAN, Orsolya BORSAI** 105
13. *IN SITU* SELECTION OF LOCAL SOUR CHERRY VARIETIES RESISTANT TO THERMOHYDRIC STRESS - **Silvia NICOLAE, Corina GAVĂȚ, Mihai CHIVU, Adelina Ioana STAN** 114
14. THE ACTION OF PRODUCTS BASED ON NAD, ANA AND BA ON THE FRUIT LOAD CONTROL IN GOLDEN REINDERS APPLE TREES - **Ananie PEȘTEANU, Oleg CALESTRU** 121
15. THE GROWTH SEASON FOLLOWING BBCH SCALE AND THE GDD REQUIREMENT FOR *RUBUS IDAEUS* VAR. 'PROMYK'. DURING THE ADAPTATION PERIOD, PLACED IN THE URBAN GARDEN - **Alin Laviniu POPA, Anamaria VÂTCĂ, Valentina STOIAN, Mădălina TRUȘCĂ, Ștefania GÂDEA, Sorin VÂTCĂ** 129
16. *IN VITRO* PROPAGATION OF THREE CULTIVARS OF BLUE HONEYSUCKLE (*LONICERA CAERULEA* L.) **- Ana-Maria RADOMIR, Ramona STAN, Dorin Ioan SUMEDREA** 141
17. THE EFFECT OF ALTERNATIVE TREATMENT METHODS ON THE EVOLUTION OF THE *PHILAENUS SPUMARIUS* POPULATION IN GOOSEBERRY CULTIVATION - **Liliana-Aurora RĂDULESCU (NICHIFOR), Dorel HOZA** 153
18. VARIABILITY OF BIOMETRIC AND BIOCHEMICAL CHARACTERISTICS IN SOME APPLE VARIETIES - **Daniela Nicoleta SCEDEI, Olimpia IORDANESCU, Casiana MIHUȚ, Renata SUMĂLAN, Ramona ȘTEF, Carmen DURĂU, Antonela COZMA, Vlad Dragoslav MIRCOV, Maria Mihaela MOATAR, Dacian LALESCU** 158
19. BLOSSOMING PHENOPHASE AT SOME APRICOT AND PEACH CULTIVARS DEPENDING ON AIR TEMPERATURE IN SOUTH-EASTERN AREA OF ROMANIA - **Leinar SEPTAR, Corina GAVĂȚ, Vlăduț Alexandru OPRIȚA, Cristina MOALE, Ion CAPLAN, Ioan STOLI** 166
20. NITROGEN CONTENT IN APPLE LEAVES UNDER VARIOUS FERTIGATION TREATMENTS IN YOUNG, SEMI-YOUNG AND OLD LEAVES - **Antun ŠOKEC, Goran FRUK, Tomislav KARAŽIJA, Martina SKENDROVIĆ BABOJELIĆ, Mihaela ŠATVAR VRBANČIĆ, Kristijan KONOPKA, Boris LAZAREVIĆ, Marko PETEK** 172
21. FLORAL BIOLOGY OF SOME AUTOCHTHONOUS SOUR CHERRY GENOTYPES FROM THE GERMPLASM FUND - **Ioana Adelina STAN (ZOICAN), Mădălina BUTAC, Dorel HOZA** 178
22. REPRODUCTIVE MANIFESTATIONS OF APPLES BY CONVENTIONAL FARMING - **Vera STEFANOVA, Galya DOBREVSKA, Manol DALLEV**..... 184
23. PRELIMINARY ASSESSMENT OF SOME MORPHOLOGICAL TRAITS OF JUJUBE FRUITS PRODUCED IN SOUTHERN OLTENIA - **Ana-Maria STOENESCU, Florin STĂNICĂ** 191
24. DAMAGES ON FRAGARIA ANANASSA VAR. 'SNOW WHITE' CAUSED BY *ERWINIA AMYLOVORA* AND POTENTIAL OF *CHAENOMELES* EXTRACT FOR DISEASE CONTROL **- Mariya STOYANOVA, Elena GRANCHAROVA, Miroslava VALKOVA, Teodora MIHOVA** 199
25. TOWARDS *IN VITRO* CONSERVATION OF SEVERAL PLUM CULTIVARS (*PRUNUS DOMESTICA* L.) - **Larisa-Daniela STRÎMBU (VLAȘIN), Luminița-Antonela ZAGRAI, Zsolt JAKAB-ILYEFALVI, Ioan ZAGRAI, Georgeta Maria GUZU, Mirela Irina CORDEA** 207

26. EVALUATION OF SOME QUALITY PARAMETERS OF 'ENROSADIRA' RASPBERRY VARIETY UPON DIFFERENT GROWING SYSTEMS - **Monica STURZEANU, Oana HERA, Loredana VÎJAN, Adrian ASĂNICĂ** 215
27. THE EFFECT OF PHOTOVOLTAIC SYSTEMS ON BERRIES PRODUCTION. A REVIEW - **Lavinia-Mihaela UDREA (ILIESCU), Mirela Florina CĂLINESCU, Ivona Cristina MAZILU, Emil CHIȚU, Florin PLĂIAȘU** 223
28. PROXIMATE COMPOSITION AS A FORM OF NUTRITIONAL QUALITY ASSESSMENT OF BANANA PEEL - **Ariana-Bianca VELCIOV, Antoanela COZMA, Georgeta-Sofia POPESCU, Virgil-Dacian LALESCU, Daniela STOIN, Laura RĂDULESCU, Maria RADA** 236
29. GRAFTING EFFICIENCY OF APPLE TREES IN THE NURSERY BY APPLYING DIFFERENT TECHNOLOGY ELEMENTS - **Adelina VENIG, Florin STĂNICĂ, Adrian PETICILĂ** 246
30. INVESTIGATING THE RELATIONSHIP BETWEEN WATER/NUTRIENT AVAILABILITY AND YIELD EFFICIENCY IN GRAFTED CHERRY TREES - **Adelina VENIG, Florin STĂNICĂ, Adrian PETICILĂ** 255

VITICULTURE AND OENOLOGY

1. STUDIES ON THE EVALUATION OF THE PHENOLIC POTENTIAL OF GRAPE POMACE FOR RED WINES - **Victoria ARTEM, Cristina CIOBANU, Arina Oana ANTOCE, Ancuta NECHITA, Georgeta TUDOR** 265
2. INVESTIGATING THE IMPACT OF CLUSTER LOAD ON THE CHARACTERISTICS OF TWO WHITE WINE GRAPE VARIETIES ('KIRÁLYLEÁNYKA'/'FETEASCĂ REGALĂ' AND 'KERNER') - **Géza BALLA, Zsolt SZEKELY-VARGA, Attila BANDI, Csaba MOLDOVAN, Endre KENTELKY** 272
3. ELICITORS IN VITICULTURE AND THEIR EFFECT IN WINEMAKING: A SUSTAINABLE APPROACH TO CLIMATE RESILIENCE - **Traian Ștefan COSMA, Aurora RANCA, Anamaria TĂNASE, Iulia Cristina CIOBANU, Sergiu Ayar ENE, Arina Oana ANTOCE** 279
4. RESEARCH ON THE REACTION OF THE CABERNET SAUVIGNON GRAPEVINE VARIETY TO THE VARIABILITY OF CLIMATIC CONDITIONS - **Dorin Constantin COSTEA, Daniela Doloris CICHI, Constantin BĂDUCĂ-CÎMPEANU** 291
5. EVALUATION OF SOME WHITE WINES' DYNAMIC VISCOSITY AND CORRELATION WITH OTHER PHYSICAL PARAMETERS - **Iuliana CREȚESCU, Giancarla VELICEVICI, Mihaela MĂLĂESCU, Oana MUNTEANU, Raluca HORHAT** 298
6. THE INFLUENCE OF CLIMATE VARIABILITY ON GRAPE RIPENING AND QUALITY IN A VINEYARD LOCATED IN MINIȘ-MĂDERAT AREA - **Alina Georgeta DOBREI, Eleonora NISTOR, Daniela DOBROMIR, Simion ALDA, Marcel DANCI, Daniela SCEDEI, George Dragoș CONSTANTINESCU, Alin DOBREI** 306
7. OPTIMIZING PRODUCTIVITY AND FRUIT QUALITY OF 'MUSCAT HAMBURG' CULTIVAR THROUGH PRUNING INTENSITY - **Sergiu-Ayar ENE, Ionica DINA, Anamaria TĂNASE, Traian-Ștefan COSMA, Aurora RANCA, Răzvan-Ionuț TEODORESCU** 318
8. VALIDATION OF *GRAPEVINE PINOT GRIS VIRUS* ELIMINATION IN GRAPEVINE BY *IN VITRO* CHEMOTHERAPY - **Ionela-Cătălina GUȚĂ, Elena-Cocuța BUCIUMEANU** …. 326

9. INVESTIGATION OF THE PHENOLOGICAL PHASES OF THE PINOT NOIR CLONE 777 UNDER THE INFLUENCE OF CLIMATE CHANGE IN THE SOUTHERN VINE-GROWING REGION IN BULGARIA - **Marko MIHAYLOV, Boyan STALEV** 334
10. STUDY ON THE OENOLOGICAL POTENTIAL OF SOME NATIVE YEASTS STRAINS ISOLATED FROM WINE CENTER ȘTEFĂNEȘTI - **Petronela Anca ONACHE, Andreea Elena MANOLESCU, Dorin Ioan SUMEDREA, Andrei TĂNASE, Stan BONTEA** 339
11. EVALUATION OF THE TECHNOLOGICAL POTENTIAL OF WINE GRAPE VARIETIES IN THE CONTEXT OF CLIMATE CHANGE IN THE DEALU MARE VINEYARD - **Liliana PÎRCĂLABU, Steliana Paula BARBU, Marian ION, Georgeta TUDOR, Alexandra BUCUR** 345
12. RESEARCH ON THE AGROBIOLOGICAL AND TECHNOLOGICAL VALUE OF SOME OLD ROMANIAN VINE VARIETIES IN THE CONTEXT OF CLIMATE CHANGES IN THE ODOBESTI VINEYARD, VRANCEA COUNTY - **Marioara PUȘCALĂU, Ionica BOSOI, Alina Camelia DÎRLOMAN** 351
13. INFLUENCE OF SOME SOIL HERBICIDES ON THE VEGETATIVE MANIFESTATIONS OF GRAFTED CUTTINGS OF SIYANA VINE VARIETY - **Svetlana STOYANOVA, Galina DYAKOVA, Ralitsa MINCHEVA, Evgeniya ZHEKOVA** 360
14. RESEARCH ON THE TECHNOLOGICAL CHARACTERISTICS OF SELECTED CLONAL ELITES FROM TWO POPULATIONS OF TABLE GRAPE VARIETIES UNDER THE CONDITIONS OF THE MURFATLAR VINEYARD - **Anamaria TĂNASE, Aurora RANCA, Ana NEGRARU, Traian Ștefan COSMA, Ayar-Sergiu ENE, Ionica DINA, Traian Ciprian STROE** 368
15. CHEMICAL WEED CONTROL IN FRUIT-BEARING TABLE GRAPE VINEYARDS (*VITIS VINIFERA* L.) - **Iliyan ZHELYAZKOV** 374

VEGETABLE GROWING

1. RESEARCH ON FEED BACK REACTIONS OF PLANTS SUBJECTED TO EXTERNAL FREQUENCY FIELDS FROM AN EPIGENETICAL POINT OF VIEW - **Maria ADOCHIȚEI, Adrian CHIRA, Daniel NAGY, Constantin PĂUN, Ligia ION** ……..... 385
2. EVALUATION OF THE INTEGRATED EFFECTS OF SOIL MANAGEMENT AND FERTILIZATION STRATEGIES ON THE GROWTH, YIELD AND QUALITY OF SWEET PEPPER (*CAPSICUM ANNUUM* L.) CULTIVATED IN POLYETHYLENE GREENHOUSES - **Dan-Ioan AVASILOAIEI, Claudia BĂLĂIȚĂ, Carmina BENCHEA, Gabriel-Alin IOSOB, Emilian MIREA, Mariana CALARA** 391
3. MATHEMATICAL MODELING IN THE APPLICATION OF BIOFERTILIZERS IN BELL PEPPER CROPS - **Claudia BĂLĂIȚĂ, Mariana CALARA, Ionuț STOICA, Ana-Maria GEORGESCU, Dumitra RĂDUCANU, Elena Maria DRĂGHICI** 401
4. THE RESPONSE OF SOME CHERRY TOMATO HYBRIDS TO FOLIAR FERTILIZATION - **Alexandra BECHERESCU, Gheorghița HOZA, Florin SALA** 409
5. ASSESMENT OF VARIOUS CONCENTRATION OF SALICYLIC ACID IN TISSUE CULTURE *IN VITRO* SYSTEMS FOR THEIR EFFECT ON MODULATING ABIOTIC STRESS TOLERANCE MECHANISMS IN PEPPER (*CAPSICUM ANNUUM* L.) PLANTS - **Tina Oana CRISTEA, Gabriel-Alin IOSOB, Creola BREZEANU, Petre Marian BREZEANU, Claudia BĂLĂIȚĂ, Mariana CALARA, Dan Ioan AVASILOAIEI** 417

6. CARBON FOOTPRINT OF LETTUCE GROWED WITH MINERAL AND BIOLOGICAL FERTILIZATION IN GREENHOUSES. - **Dimitar Dimitrov, Kostadin KOSTADINOV, Stoyan FILIPOV** 423
7. THE INFLUENCE OF MICROORGANISMS ON THE QUANTITATIVE AND QUALITATIVE CHARACTERISTICS OF TOMATO FRUITS - **Claudia-Loredana DRAGOMIR, Gheorghita HOZA, Alexandra BECHERESCU, Mihaela PARASCHIV, Claudia NICOLA, Mihai FRÎNCU, Liliana BĂDULESCU, Dorel HOZA** 433
8. PHYSICO-CHEMICAL CHARACTERISTICS OF WATERMELON FRUITS (*CITRULLUS LANATUS*) OF GENOTYPES CULTIVATED ON SANDY SOILS IN SOUTHWESTERN ROMANIA - **Felicia-Constantina FRĂTUȚU, Ștefan NANU, Cristina BÎRSOGHE, Alina-Nicoleta PARASCHIV, Loredana-Mirela SFÎRLOAGĂ, Maria-Diana ILINA** 444
9. CONVENTIONAL AND BIOLOGICAL CONTROL OF PESTS IN PEPPERS AND EGGPLANTS CROPS IN THE GREENHOUSE. A REVIEW - **Simona HOGEA, Gabriela Șovărel** 450
10. PARAMETRIC EVALUATION OF POD YIELD STABILITY IN BUSHING SNAP BEAN (*PHASEOLUS VULGARIS* L.) - **Corneliu IACOB, Sorin CIULCA** 458
11. CORRELATION BETWEEN CLIMATIC FACTORS AND DYNAMICS OF PATHOGENS AND PESTS: A CASE STUDY IN CABBAGE CROPS - **Gabriel-Alin IOSOB, Tina Oana CRISTEA, Petre Marian BREZEANU, Claudia BĂLĂIȚĂ, Alexandru BUTE, Dan Ioan AVASILOAIEI** 466
12. RESPONSE OF TARRAGON (*ARTEMISIA DRACUNCULUS* L.) TO THE APPLICATION OF ZINC SULFATE AND GLYCINE BETAINE UNDER WATER STRESS CONDITIONS - **Soheil KHANCHI, Asad ROKHZADI, Sirwan MOHAMMDIAZAR, Elena Maria DRĂGHICI** 473
13. PRODUCTION OF POLYPHENOLIC COMPOUNDS WITH HIGH ANTIOXIDANT ACTIVITY IN *MELISSA OFFICINALIS* L. CALLUS LINE - **Elena Monica MITOI, Alexandra Gabriela CIOCAN, Florentina ALDEA, Irina HOLOBIUC** 479
14. SCREENING OF GENE EXPRESSION ASSOCIATED WITH SALINITY AND UVB TOLERANCE IN LOCAL TOMATO LANDRACES - **Lia ȘERBAN (MLADIN), Oana SICORA, Tunde JAKO, Cosmin SICORA, Monica HÂRȚA, Mirela Irina CORDEA** 488
15. EFFECTS OF MONTMORILLONITE-ENRICHED HYDROGELS ON LETTUCE MORPHOLOGY UNDER DROUGHT STRESS - **Ion NIȚU, Elisabeta Elena POPA, Amalia Carmen MITELUȚ** 498
16. INFLUENCE OF CONVENTIONAL AND BIOLOGICAL CONTROL ON POSTHARVEST QUALITY OF TOMATOES - **Mihaela PARASCHIV, Gabriela ȘOVĂREL** 505
17. *ALLIUM CEPA* SEEDLINGS RESPONSE TO STRESS GENERATED BY CHEMICALS - **Andreea Adriana PETCOV, Florin SALA** 517
18. CONTENT OF MICROELEMENTS IN FRENCH BEAN PODS FROM DIFFERENT CULTIVATIONS - **Marko PETEK, Ema KLARIĆ, Sanja FABEK UHER, Antun ŠOKEC, Szilvia VERES, Šic Žlabur JANA** 525
19. THE INFLUENCE OF GENOTYPE AND ENVIRONMENTAL FACTORS ON THE NUTRITIONAL COMPOSITION OF *ROSA CANINA* L. FRUITS - **Rodica SOARE, Cristina BĂBEANU, Maria DINU** 532
20. APPLICATION OF WEB-BASED TOOLS FOR DOCUMENTATION OF HORTICULTURE LOCAL VARIETIES - BULGARIAN AND SLOVAKIAN EXAMPLE - **Nikolaya VELCHEVA, Asya STOYANOVA-DOYCHEVA, Pencho MALINOV, Sebiha MADANSKA, Ľubomír MENDEL, René HAUPTVOGEL** 538

21. INNOVATIVE TECHNIQUES FOR MONITORING AND EFFICIENT MANAGEMENT OF THE BULGARIAN GENEBANK - **Nikolaya VELCHEVA, Gergana DESHEVA, Katya UZUNDZHALIEVA, Asya STOYANOVA-DOYCHEVA, Lyubka DOUKOVSKA, Valko NESHEV** 544
22. EVALUATION OF THE COMBINING ABILITY AND HETEROSIS IN EGGPLANT PARENTS FROM BRGV BUZĂU FOR OBTAINING VALUABLE F1 HYBRIDS - **Costel VÎNĂTORU, Adrian PETICILĂ, Bianca MUȘAT, Camelia BRATU, Geanina NEGOȘANU, Matilda POPESCU** 551
23. GLOBAL DEVELOPMENT TRENDS AND STRATEGIC OUTLOOK OF VERTICAL AGRICULTURE - **Qichang YANG, Florin STĂNICĂ, Yuxin TONG, Bateer BAIYIN** 558

FLORICULTURE, ORNAMENTAL PLANTS, DESIGN AND LANDSCAPE ARCHITECTURE

1. THE BEHAVIOR OF *ARUM ITALICUM* MILL. IN IAȘI ECOLOGICAL CONDITIONS (NORTHEASTERN ROMANIA) - **Lucia DRAGHIA, Maria CANTOR, Maria APOSTOL, Roberto-Renato BERNARDIS, Iulia MINEAȚĂ** 565
2. MORE THAN JUST AESTHETICS: QUANTIFYING THE MONETARY VALUE OF TREE ALIGNMENTS IN "CATACUZIONO" PARK FROM FLOREȘTI – PRAHOVA COUNTY - **Cristina DRĂGHICI, Diana Lavinia CULESCU** 573
3. CENOLOGY, ECOLOGY AND DISTRIBUTION OF POPULATIONS OF *VIOLA HYMETTIA* BOISS & HELDR. (VIOLA, SECT. MELANIUM) ON THE SANDS OF SOUTHERN OLTENIA, ROMANIA - **Paula IANCU, Mariana NICULESCU** 583
4. THE ROLE OF RESIDENTIAL GREEN SPACES IN ENHANCING WELL-BEING, BIODIVERSITY, AND SUSTAINABILITY: A CASE STUDY OF IAȘI, ROMANIA - **Ana-Maria-Roxana ISTRATE, Clara-Teodora BALAN, Mirela COJOCARIU** 590
5. *DENDROBIUM ANTENNATUM* LINDL. MICROPROPAGATED UNDER THE INFLUENCE OF VARIOUS CONCENTRATIONS OF FERBANAT L® - **Endre KENTELKY, Zsolt SZEKELY-VARGA, Csanád Tas SZABÓ, Andrea TILLYNÉ-MÁNDY** 599
6. DESIGNING A PRIVATE GARDEN WITH AN ORNAMENTAL AND UTILITARIAN CHARACTER - **Manuela MANDA, Carmen NICU, Ruxandra BĂLUȚĂ, Andreea TRIF** 611
7. RESILIENCE OF URBAN TREES TO EXTREME WEATHER IN BUCHAREST, ROMANIA - **Cristina Rodica MĂNESCU, Elisabeta DOBRESCU, Ana VIȚA** 617
8. LAVENDER GROWTH AND FLOWERING IN COMPARATIVE PLANTING METHODS - **Cristina Rodica MĂNESCU, Elisabeta DOBRESCU, Ilona LICSANDRU** 625
9. THE AUTUMN LEAF CHROMATICS OF FRUIT TREE SPECIES AND THEIR AESTHETIC EFFECT - **Andreea MELINESCU, Sina COSMULESCU** 632
10. *TULIPA HUNGARICA* BORBÁS – CONSERVATION STATUS, SPECIES DISTRIBUTION AND POTENTIAL THREATS - **Luiza Silvia MIHAI, Marilena ONETE, Tiberiu SAHLEAN, Minodora MANU, Simona MIHĂILESCU** 639
11. INFLUENCE OF SOME FERTILIZERS ON THE GROWTH AND FLOWERING OF *CROSSANDRA INFUNDIBULIFORMIS* (L.) NEES PLANTS - **Carmen NICU, Manuela MANDA** 647

12. THE INFLUENCE OF SUBSTRATE TYPE ON THE MORPHOLOGICAL AND ORNAMENTAL CHARACTERS OF SOME VARIETIES OF *IPOMOEA BATATAS* GROWN IN CONTAINERS - **Alina-Stefana OZARCHEVICI, Irina CARA, Gerard JITĂREANU, Bogdan-Ionel CIOROIU, Maria APOSTOL, Lucia DRAGHIA** 654
13. SPREAD AND STABILITY OF ORNAMENTAL ALIEN PLANT SPECIES ON URBAN AND PERI-URBAN RIVERBANKS OF BUCHAREST, ROMANIA - **Maria RAICU, Mihaela URZICEANU, Petronela CAMEN-COMĂNESCU, Florin TOMA** 665
14. THE MORPHOLOGICAL STRUCTURE OF THE RENEWAL BUD IN *KNIPHOFIA NELSONII* MAST. - **Irina SFECLĂ** 674
15. RESEARCH ON THE IMPACT OF THE GROWING MEDIUM ON PRODUCTION AND QUALITY OF *GERBERA HYBRIDA* FLOWERS IN PROTECTED CULTIVATION - **Ioana Andra VLAD, Laviniu Ioan-Nuțu BURESCU, Bartha SZILÁRD, Călin Gheorghe PĂȘCUȚ, Goji GYÖZÖ, Florin-Alexandru REBREAN** 682
16. FROM NEGLECT TO VITALITY: LANDSCAPE DESIGN APPROACHES FOR RECONVERTING LOST URBAN SPACES - **Ina VLADIMIR (ASIMINEI), Mirela COJOCARIU** 687
17. GARDENS DESIGN AS OUTDOOR EVENT SPACES - **Cristina ZLATI, Măriuca ȘTIRBAN, Alice CHIHAIA, Roxana PAȘCU** 693

MISCELLANEOUS

1. CHARACTERIZATION OF FOREST TREE SEEDS USING VIBRATIONAL SPECTROSCOPY - **Luisa ANDRONIE, Ioana POP, Florica MATEI, Iulia COROIAN, Adina TRUȚĂ, Aurelia COROIAN, Stefania ANDRONIE** 701
2. COMPARATIVE RESEARCH ON THE SITUATION, STRUCTURE AND ECOLOGICAL INDICES OF ARTHROPOD SPECIES ENCOUNTERED IN SOME FOREST AND APPLE ORCHARDS IN THE NECHIT AREA, NEAMȚ COUNTY - **Vasile BARDAN, Nela TĂLMACIU, Ionela MOCANU, Renata SIPOS, Mihai TĂLMACIU, Monica HEREA** ….. 706
3. RESEARCH ON THE DEVELOPMENT OF A LOW CARB MINI-BAGUETTE FORTIFIED WITH *SPIRULINA* POWDER, INTENDED FOR DIABETICS AND OBESE INDIVIDUALS - **Luminița CATANĂ, Monica CATANĂ, Anda-Grațiela BURNETE, Monica-Alexandra DÂRĂ, Florica CONSTANTINESCU, Gabriela CRIVEANU, Adrian ASĂNICĂ** ……. 715
4. THE CHEMICAL COMPOSITION OF THE ESSENTIAL OIL AND ASSOCIATED HYDROSOL OBTAINED FROM TWO PLANTS OF THE ASTERACEAE FAMILY, *SANTOLINA CHAMAECYPARISSUS* L. AND *TANACETUM VULGARE* L. - **Raluca CIUBOTĂRAȘU, Monica Luminița BADEA, Oana LIVADARIU, Mihaela PARASCHIV, Dorel HOZA** 725
5. EFFECTIVENESS OF META-TOPOLIN IN *IN VITRO* PROPAGATION AND GENETIC STABILITY ASSESSMENT OF MEDICINAL AND AROMATIC PLANTS - **Doina CLAPA, Monica HÂRȚA, Ana Maria RADOMIR, Adrian PETICILĂ, Dorin Ioan SUMEDREA** 734
6. STRUCTURAL CHARACTERISTICS OF TREES OBTAINED THROUGH THE TRANSFORMATION OF SILVOPASTORAL SYSTEMS - **Ghiță Cristian CRAINIC, Florin Lucian COVACI** 743
7. *OXALIS ACETOSSELA* IN FORESTS. A SYSTEMATIC BIBLIOMETRIC STUDY OVER THE LAST 47 YEARS - **Lucian DINCĂ, Vlad CRIȘAN, Gabriel MURARIU, Eliza ȚUPU** 756

8. EFFECT OF ESSENTIAL OILS ON QUALITY AND SHELF LIFE OF STRAWBERRIES DURING COLD STORAGE - **Sorina DINU, Lavinia-Diana-Nicoleta BUTURUGĂ-BARBU, Maria-Cristina LUMÎNARE, Daniel-Nicolae COJANU, Viorel FĂTU, Oana-Alina BOIU-SICUIA** 762
9. CRAFTED BARREL - AGED BEER: TRADITION AND INNOVATION IN FLAVOR EVOLUTION - REVIEW - **Mihai FRÎNCU, Corina DUMITRACHE, Diana-Iuliana BĂRBULESCU, Mihaela BEGEA, Carmen-Rodica VRÎNCEANU, Răzvan-Ionuț TEODORESCU** 769
10. *LEONTOPODIUM NIVALE* SSP. *ALPINUM* (CASS.) BIOTECHNOLOGY APPLIED FOR ECONOMIC AND CONSERVATIVE PURPOSE - **Mihaela Irina HOLOBUIC, Alexandra-Gabriela CIOOCAN, Rodica CATANĂ** *779*
11. *EX SITU* CONSERVATION OF *SERRATULA BULGARICA* ACHT. & STOJ. IN THE REPUBLIC OF MOLDOVA - **Olga IONIȚĂ, Elena TOFAN-DOROFEEV** *788*
12. VARIATION AND CORRELATION OF TEXTURAL AND FRUIT MATURITY PARAMETERS DURING STORAGE OF DALINBEL, JONAFREE, AND REAL APPLE CULTIVARS - **Ionuț ISTRATE, Florin STĂNICĂ, Adrian ASĂNICĂ, Ana-Cornelia BUTCARU, Liliana TUDOREANU** 794
13. TESTING BIOLOGICAL FERTILIZERS USEFUL FOR SUSTAINABLE AGRICULTURE - **Oana-Alexandra LUȚU, Nicolae-Alin VINTILĂ, Liliana Cristina SOARE, Codruța Mihaela DOBRESCU** 801
14. CARBON STABILIZATION AND SEQUESTRATION PROCESSES IN SOIL THROUGH SYNTHETIC MICROBIOMES – **Sorin MATEI, Gabi Mirela MATEI, Sorina DUMITRU, Victoria MOCANU, Monica DUMITRAȘCU** 808
15. CLIMATE TRENDS IN OLTENIA. CASE STUDY: DĂBULENI WINE CENTRE - **Cristian MĂRĂCINEANU, Maria BĂJENARU, Aurelia DIACONU, Nicolae GIUGEA, Ramona CĂPRUCIU** 820
16. UMBRELLA EFFECT OF EUROPEAN PROTECTED SPECIES OVER NATIONAL RED LISTED *BETULA HUMILIS* SCHRANK - "MESTECĂNAȘUL DE LA COȘNA" AS A NEW NATURA 2000 SITE - **Roxana NICOARĂ, Constanța-Mihaela ION, Gabriela TAMAS, Ana-Maria MOROȘANU, Florența-Elena HELEPCIUC, Tiberiu SAHLEAN, Constantin-Ciprian BÎRSAN, Minodora MANU, MIHAELA CIOBOATĂ, Sanda MAICAN, Viorel-Dumitru GAVRIL, Miruna-Maria ȘTEFĂNUȚ, Daniel Ioan MAFTEI, Mirela-Mădălina MOLDOVEANU, Anca MANOLE, Sorin ȘTEFĂNUȚ** 828
17. ECOLOGY AND PHYTOSOCIOLOGY OF THE SPECIES *CIRSIUM CANDELABRUM* GRISEB. (SECT. CIRSIUM, ASTERACEAE, CYNAREAE) IN THE UPPER BASIN OF THE GILORT RIVER, CARPATHIANS MOUNTAINS - **Mariana NICULESCU, Paula IANCU** 840
18. SOME ASPECTS OF THE MORPHO-ANATOMICAL FEATURES OF THE MEDICINAL PLANT *ARNICA MONTANA* L. - **Sorina NIȚU (NĂSTASE), Vasilica LUCHIAN, Monica TOD, Paul ZEVEDEI, Tudor ENE, Virgil NIȚU, Adrian BLAJ** 846
19. METHODS OF COMBATING AND CONTROL REGARDING FALSE INDIGO-BUSH IN CLOSE CORRELATION WITH INVASIVE CAPACITY IN THE FORESTRY PLANT COMMUNITIES IN THE JIU-DANUBE CONFLUENCE ZONE - **Silvestru Ilie NUȚĂ, Mariana NICULESCU, Călin SĂLCEANU, Paula IANCU, Marin SOARE** 854
20. MODELLING THE DISTRIBUTION OF THE RARE, ENDEMIC, TERTIARY RELICT AND VULNERABLE SPECIES *SYRINGA JOSIKAEA* JACQ. FROM APUSENI MOUNTAINS (WESTERN ROMANIAN CARPATHIANS) - **Marilena ONETE, Florian Paul BODESCU, Roxana NICOARĂ, Minodora MANU, Simona MIHAILESCU** 862

21. ANTIOXIDANT CAPACITY AND SEASONAL MINERAL COMPOSITION VARIATION IN *ARONIA MELANOCARPA* FRUITS - **Laura POP (CARPESCU), Rui COSTA, Adrian ASĂNICĂ, Stelica CRISTEA, Corina PREDESCU, Dana TĂPĂLOAGĂ, Liliana TUDOREANU** 872
22. CONTRIBUTIONS TO THE KNOWLEDGE OF THE DISTRIBUTION OF SOME RARE TAXA IN THE FLORA OF OLTENIA (ROMANIA) - **Daniel RĂDUȚOIU** 884
23. PRECISION AGRICULTURE EXTENSION MODEL BASED ON INTERDISCIPLINARY COLLABORATION - **Dragoș-Ioan SĂCĂLEANU, Adrian ASĂNICĂ, Abigail BORRON, Daniela FAUR, Horia-Nicolae CIOCAN, Gary L. HAWKINS, Ioan-Ștefan SACALĂ, Dan POPESCU, Mihai-Gabriel MATACHE** 893
24. FRUITING BIOLOGY OF *STAPHYLEA PINNATA* L. (STAPHYLACEAE) IN THE REPUBLIC OF MOLDOVA - **Irina SFECLĂ, Elena TOFAN-DOROFEEV, Victor SFECLĂ, Vasilii SLIVCA** 900
25. DEVELOPMENT OF PLANT TISSUE CULTURE AND IN DOOR FARMING SYSTEMS IN A CONTROLLED ENVIRONMENTAL CONDITIONS USING LIGHT-EMITTING DIODES (LEDs) FOR THE PRODUCTION OF HIGH VALUE CROPS - **Sreeramanan SUBRAMANIAM, Ayyagari RAMLAL** 908
26. ASSESSMENT OF *BUXBAUMIA VIRIDIS* FROM TWO NATURA 2000 SITES: RETEZAT (ROSCI0217) AND RUSCA MONTANĂ -ȚARCU - RETEZAT CORRIDOR (ROSCI0292) - **Sorin ȘTEFĂNUȚ, Ana-Maria CORPADE, Claudia DANAU, Miruna-Maria ȘTEFĂNUȚ** 914
27. STUDY ABOUT THE INFLUENCE OF GIBBERELLIC ACID AND NAPHTHYL ACETIC ACID ON *IN VITRO* DEVELOPMENT OF FOUR ROMANIAN POTATO VARIETIES - **Andreea TICAN, Mihaela CIOLOCA, Monica POPA** 920
28. STUDIES REGARDING THE CYTOTOXICITY OF A BIOPRODUCTS BASED ON POLYSACCHARIDES DERIVED FROM *PLANTAGO* SP. **- Marilena-Gabriela OLTEANU ZAHARIE, Nicoleta RADU, Narcisa Elena BĂBEANU, Marinela BOSTAN, Viviana ROMAN** 924
29. COMPARISON OF MULTIANUAL CLIMATIC DATA IN NORTH BUCHAREST REGION, ROMANIA **- Ana Cornelia BUTCARU, Cosmin Alexandru MIHAI, Adrian ASĂNICĂ, Florin STĂNICĂ** 934


EX·TERRA·AVRVM

FRUIT GROWING


EX·TERRA·AVRVM

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

BIOCOMPATIBLE MANAGEMENT ON POSTHARVEST DISEASES

Diyana ALEKSANDROVA[1], Gabor ZSIVANOVITS[2]

[1]Agricultural Academy, Fruit Growing Institute - Plovdiv,
12 Ostromila, Plovdiv, Bulgaria
[2]Agricultural Academy, Institute of Food Preservation and Quality,
Plovdiv, Bulgaria

Corresponding author email: aleksandrova_diyana@abv.bg

Abstract

In recent years, there has been a trend to research alternative methods for controlling post-harvest fungal pathogens. Using biopolymers and plant extracts is an innovative method that includes many advantages, with human health as a priority. The research includes the efficiency of biopolymer chitosan and extract of grape seed and a combination of them used to control the fungal pathogens Alternaria spp. and Penicillium spp. in vitro conditions. Grape seed extract has antimicrobial activity, manifested by phenolic compounds that cause oxidation of the cell membranes of pathogens without affecting the plants. As a biological agent, chitosan is a biopolymer natural polysaccharide that has several amino groups, determining its activity against plant pathogens. The best percentage of mycelial inhibition varied according to the two pathogens. The pathogen Alternaria spp. was influenced by the combination of chitosan (1%) and grape seed extract (1.5%), the experiment has significant differences between different variants in research. The mycelia of Penicillium spp. showed the best result with inhibition by applying chitosan (1%) with a 1 % solution of grape seed extract. The highest percentage of mycelia growth inhibition was achieved on the sixth day of measurement 20.18% in the pathogen Alternaria spp. According to the control variant, Penicillium spp. has been highest suppressed on the fourth day 15.21% of cultivation of fungal pathogen.

Key words*: biopolymers, chitosan, grape seed extract, antimicrobial activity.*

INTRODUCTION

The used of synthetic fungicides for the control of phytopathogenic fungi has traditionally been associated with a number of harmful environmental consequences. These consequences include toxicity to non-target organisms, the development of resistance in pathogen populations, and environmental pollution. In the context of these challenges, the importance of sustainable alternatives such as biocides, which support the transition to more environmentally friendly agricultural practices, is growing (Kumar et al., 2022).

Chitosan, a unique natural biopolymer, is derived from chitin through deacetylation. It is the second most abundant natural polysaccharide, after cellulose, and the most abundant biopolymer of animal origin. It is a non-toxic, biocompatible, biodegradable polymer (Hisham et al., 2024). Chitin's sources are diverse, ranging from terrestrial arthropods (e.g., spiders, scorpions, beetles, cockroaches, and brachiopods) and marine crustaceans (e.g., crab, lobster, prawn, and krill) to Mollusca (e.g., squid) and microorganisms (e.g., fungi cell walls - Alishahi & Aïder, 2012). Currently, the primary industrial source of chitin is the waste generated by marine food production, particularly the exoskeletons of crabs, lobsters, and shrimps (Leceta et al., 2013). Based on the exciting literature it is also the most environment friendly raw material of the chitosan (Kou et al., 2021).

The management of phytopathogenic fungal *Alternaria alternata* and *Fusarium* spp. diseases through, chitosan nanoparticles (NCTs), showed antifungal properties but lower efficacy, requiring 1000 ppm for complete inhibition (Gharieb et al., 2025).

The potential of incorporating grape seed extract (GSE) into biopolymer coatings to enhance food preservation, particularly focusing on its antioxidant properties and suitability for combating lipid oxidation. Biopolymers offer a compelling alternative to synthetic polymers in food packaging due to their edibility,

biocompatibility, and biodegradability (Ezati & Rhim., 2020; Riahi et al., 2021).

Crucially, biopolymers can serve as carriers for functional compounds, enabling the development of active packaging films with enhanced properties (Roy & Rhim, 2020).

The release rate of bioactive materials from biopolymers often surpasses that of synthetic counterparts, making them ideal for delivering active ingredients. Various active compounds can be incorporated into biopolymers to impart specific functionalities. These include nanoparticles, natural plant extracts and essential oils, and antibiotic drugs (Priyadarshi & Rhim, 2020.

The review of many studies focuses on using natural plant extracts, specifically GSE, for their antioxidant potential. Lipid oxidation is a significant factor contributing to the deterioration of many foods, including peanuts. Therefore, incorporating antioxidant properties into coating formulations is paramount for preserving food quality and extending shelf life. Recent research has explored numerous natural antioxidants derived from agricultural or natural waste streams, highlighting their safety, cost-effectiveness, and potential for value-added utilization (Jiang et al., 2021; Zhang et al., 2020).

GSE has emerged as a promising natural antioxidant for enriching biopolymer coatings (Priyadarshi et al., 2021; Wu et al., 2019).

Grape seeds, a significant byproduct of the wine and grape juice industry, are abundant in polyphenolic compounds, including catechins, epicatechins, procyanidins, and gallic acid (Krishnaswamy et al., 2013; Sogut & Seydim, 2018; Wu et al., 2019).

These compounds contribute to GSE's potent antioxidant activity. Furthermore, GSE established safety and edibility make it an attractive candidate for use in food packaging applications (Krishnaswamy et al., 2013).

The positive effect of biopolymers application on fungal pathogens was confirmed in our previous research (Aleksandrova et al., 2021; Aleksandrova et al., 2019; Zsivanovits, et al., 2022).

This study aims to investigate the potential of biopolymer coatings and plant extracts for controlling post-harvest diseases. Specifically, it will evaluate the inhibitory effects of chitosan combined with grape seed extract against the fungal pathogens *Penicillium* spp. and *Alternaria* spp.

MATERIALS AND METHODS

Monitoring of infected peach fruit was collected for the experiment in the phytopathology laboratory of Fruit Growing Institute - Plovdiv. The strain of fungal pathogens *Alternaria* spp. and *Penicillium* spp. were isolated from infected peach fruit in the storage period. Pathogens were isolated by standard phytopathological methods of the infected fruit placed on potato dextrose agar. The single spore of the pathogens was cultured on potato dextrose agar (PDA) for two weeks at 25 ± 1°C for sporulation.

Seven variants of the antifungal action of 1% chitosan and grape seed extract have been studied. The grape seed extract (GSE) was used alone or in combination with chitosan. For the control variant, a Petri dish was used only with PDA and fungal pathogens. The media application includes:

Chitosan–potato dextrose agar (PDA), application 1% of chitosan and pathogen;

GSE (1%) – PDA, GSE 1%, and pathogen;

GSE (1.5%) – PDA, GSE 1.5%, and pathogen;

GSE (2%) – PDA, GSE 2%, and pathogen;

ChGSE (1%) – PDA, 1% of chitosan solution, GSE 1%, and pathogen;

ChGSE (1.5%) – PDA, 1% of chitosan solution, 1.5% GSE, and pathogen;

ChGSE (2%) – PDA, 1% of chitosan solution, GSE 2%, and pathogen.

The development of fungal cultures on the medium was studied by measuring the colony diameter every two days. The percentage inhibition of mycelial growth was calculated using the formula:

$$\text{Inhibition } \% = \frac{(dc-dt)}{dc} \cdot 100, \text{ where:}$$

dc = the average diameter of the fungal colony in the control variant;

dt = the average diameter of the fungal colony of the treatment variant.

Each variant was replaced in five repetitions.

The data obtained was analysed statistically using the SPSS 19.0 program. The data was subjected to Duncan's Multiple Range Test (MRT) at $P \leq 0.05$.

RESULTS AND DISCUSSIONS

The fungal pathogens (*Alternaria* spp. and *Penicillium* spp.) were isolated from infected peach fruits during the storage period. The pathogens were cultured on potato dextrose agar (PDA) for two weeks at 25 ± 1°C for sporulation. A mycelium disc was transferred on PDA media with additional chitosan 1% and extracted from GSE in different concentrations (1%, 1.5% and 2%) and a combination of them.

The dynamic lane in the development of *Alternaria* spp. was measured on each two days (Table 1). During the first report, the control variant which was only media showed the highest value (53.0 mm), indicating the largest colony diameter and the weakest inhibition.

Singal application of chitosan 1% was values 52.5 mm. In general, chitosan shows better inhibition than the control variant, but the differences are not statistically significant in all cases (ab).

Table 1. The development of *Alternaria* spp. pathogen on media with additional biopolymers

Variant	After two days (mm)	Inhibition (%)	After four days (mm)	Inhibition (%)	After six days (mm)	Inhibition (%)	After eight days (mm)	Inhibition (%)
Control	53.0±0.6 a	-	65.2±0.3 ab	-	77.3±0.4 ab	-	90.0±0.0 a	-
Chitosan	52.5±0.3 ab	0.94	69.2 ±0.2 a	-6.14	78.2±0.3 ab	-1.17	90.0±0.0 a	0
GSE (1%)	51.2±0.3 ab	3.40	64.7 ±0.5 ab	0.77	74.2±0.4 b	4.01	88.3±0.4 ab	1.89
GSE (1.5%)	45.0±0.4 b	15.09	55.7 ±0.2 b	14.57	61.7 ±0.3 b	20.18	78.7±0.2 b	12.56
GSE (2%)	51.5±0.1 ab	2.83	67.3±0.5 a	-3,22	80.0±0.5 a	-3.49	90.0±0.0 a	0
ChGSE (1%)	51.2±0.5 ab	3.40	65.2±0.4 ab	0	77.3±0.2 ab	0	90.0±0.0 a	0
ChGSE (1.5%)	48.8±0.2 ab	8.68	63.2±0.2 ab	3.07	68.0±0.2b	12.03	77.5±0.5 b	14.00
ChGSE (2%)	51.7 ±0.2ab	2.45	71.2±0.5 a	-9.20	85.5±0.5 a	-10.61	90.0 ± 0.0 a	0

[a–c]Different letters in the same column indicate significant differences ($p < 0.05$).

The variant with GSE showed values ranging from 45.0 mm to 51.5 mm. The concentrations of 1.5% extract from grape seed were 45.0 mm which showed good inhibition of the pathogen. The variants with combinations of chitosan and GSE (512 mm and 517 mm) show inhibition similar to chitosan alone. Significant differences were found between the control variant and the variant with GSE (1.5%).

No inhibition was observed at the control variant with 65.2 mm, compared to variants with chitosan, the highest value was 69.2 mm, after four days. The values of different concentrations of grape seed extract range from 55.7 mm to 67.3 mm. The 1.5% concentration (55.7 mm) continues to show the best inhibition.

After six days, the best inhibition was reported in the extract of grape seed 1.5% (61.7mm) and the combination variant with chitosan (68.0 mm). The both variants have significant differences between GSE (2%) and ChGSE (2%) variants.

After eight days the results indicate that ChGSE (1.5%) were the most effective in suppressing fungal colony, with colony diameters of 77.5mm and 78.7mm.

The highest percentage of inhibition was achieved with GSE (1.5%), reaching 20.18% on the 6th day of the experiment. This combination showed consistently high percentages of inhibition throughout the measurement period, indicating its effectiveness. The media with added only grape seed extract in 1.5% solution also showed good results from the second day, with 15.09% inhibition.

The results of the study on the growth of the fungal pathogen *Alternaria* spp. showed variations in the standard deviation of the measured colony diameters, reflecting the degree of data dispersion. After two days, the standard deviation ranged from ±0.1 to ±0.6, with the highest value observed in the control sample (±0.6), while the lowest (±0.1) was recorded in GSE (2%). This suggested relatively stable growth of *Alternaria* spp. in most media, with minimal differences in standard deviation.

After four days, the standard deviation varied between ±0.2 and ±0.5, with the lowest (±0.2) were observed in GSE (1.5%), Chitosan and ChGSE (1.5%).

On six days, the standard deviation again ranged from ±0.2 to ±0.5, with the lowest values (±0.2) recorded in ChGSE (1%), GSE (1.5%), and

ChGSE (1.5%). After eight days, the fungal colonies in most variants reached the maximum Petri dish diameter (90 mm).

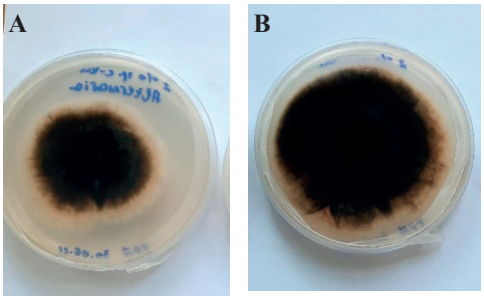


Figure 1. *Alternaria* spp culture on PDA: A - chitosan and 1.5% GSE; B - 1% chitosan

The chitosan inhibits the development of *Alternaria* spp. and also induces phytoalexin production in the host. Such interactions of chitosan with the pathogen and the host suggest that chitosan could be a useful natural material for the postharvest preservation of fruit and vegetables (Reddy et al., 2000). The results of another study Kara et. al., (2018) showed that the lonely application of GSE wasn't induce inhibition of the mycelial growth of *A. alternata,* this position was also confirmed by our results.

The management of the fungal pathogen *Penicillium* spp. through the used biopolymers in the control variant was measured with the highest values in all columns. The values of chitosan were close to those of the control, suggesting that chitosan alone haven't been showed significant antimicrobial activity against *Penicillium* spp. under these conditions.

The different concentrations of GSE there was some reduction in values in some of the columns, but the differences were statistically non-significant. With GSE (1.5%), there was a trend towards lower values but also without statistical differences. This suggests that GSE may have been inhibitory effect, but its effect was weak or depends on the concentration and time to application.

Combination of chitosan and GSE, this combination the most pronounced inhibitory effect was observed. Especially with ChGSE (1%) and ChGSE (2%), there was a significant decrease in values, denoted by "b", which indicates a statistically significant difference from the control. This suggests that the combination of chitosan and GSE has a synergistic effect and leads to better suppression of *Penicillium* spp. growth than each component separately.

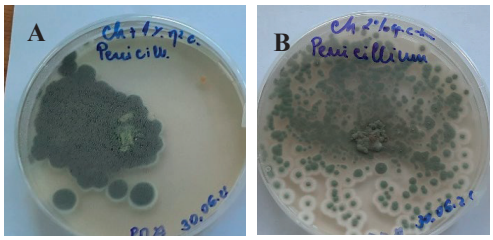


Figure 2. *Penicillium* spp culture on PDA: A - 1% chitosan and GSE 1%; B - 1% chitosan and 2% GSE

Among all the tested variants, chitosan with combination with GSE 1% solution stands out as the most effective inhibitor of *Penicillium* spp. mycelia growth.

Unlike the other variants, ChGSE (1%) demonstrates stable and high levels of inhibition throughout the measurement period, suggesting a sustained and long-lasting effect. This result contrasts with the observed rapid decrease in effectiveness in other variants, particularly GSE (2%), which showed high initial inhibition but quickly lost its activity.

The standard deviation of colony diameters of the fungal pathogen *Penicillium* spp. demonstrated variations. After two days, the standard deviation ranged from ±0.4 to ±1.2, with the highest variability observed in GSE 2% (±1.2) and the lowest in GSE 1% (±0.4). This indicated that the growth of *Penicillium* spp. was more uniform in certain media. On the four days, the standard deviation values varied between ±0.3 and ±0.9, with the lowest recorded in GSE 1.5% (±0.3) and ChGSE (1%) (±0.3). This suggested that fungal growth in GSE (1.5%) was more consistent compared to ChGSE (2%), where larger variations were observed. The standard deviation on six days ranged from ±0.3 to ±0.9, with the lowest values in media with chitosan (±0.3) and GSE 1.5% (±0.4), while the highest (±0.9) was recorded in the control sample. This showed that *Penicillium* spp. developed more uniformly in media containing chitosan and GSE 1.5%, whereas in the control, growth varied more significantly (Table 2).

Table 2 The development of *Penicillium* spp. pathogen on media with additional biopolymers

Variant	After two days (mm)	Inhibition (%)	After four days (mm)	Inhibition (%)	After six days (mm)	Inhibition (%)	After eight days (mm)	Inhibition (%)
Control	84.5±0.6 a	-	88.1±0.8 a	-	88.7±0.9 a	-	90.0±0.0 a	-
Chitosan	77.7±0.7 a	8.05	88.0±0.4 a	0.11	88.7±0.3 a	0	90.0 ±0.0 a	0
GSE (1%)	76.8±0.4 a	9.11	85.8 ±0.6 a	2.61	86.3±0.5ab	2.7	90.0±0.0 a	0
GSE (1.5%)	79.2±0.6 a	6.27	82.3±0.3 a	6.58	88.3±0.4a	0.45	88.7±0.3 a	1.44
GSE (2%)	73.7±1.2 a	12.78	85.0±0.4 a	3.52	88.3±0.7ab	0.45	88.8±0.5a	1.33
ChGSE (1%)	74.2±0.4a	12.19	74.7±0.3 a	15.21	77.5±0.4b	12.63	79.2±0.5b	11.99
ChGSE (1.5%)	82.3±0.7 a	2.60	85.0±0.4 a	3.52	85.2±0.4ab	3.95	90.0±0.0 a	0
ChGSE (2%)	82.7±0.8 a	2.13	84.7±0.9 a	3.86	87.5±0.6a	1.35	88.8±0.2 a	1.33

[a–b]Different letters in the same column indicate significant differences ($p < 0.05$).

After eight days of incubation most samples reached the maximum diameter of 90 mm. The biopolymers added to the media in variant ChGSE 1% and GSE 1.5% the fungal growth was inhibited, the colony diameters were smaller, leading to nonzero standard deviations (±0.2 to ±0.5). This indicated that these components effectively slowed or restricted fungal growth.

The chitosan demonstrates the highest percentage of inhibition in the first period, but its effectiveness quickly decreases to minimal or zero values thereafter. This suggests that chitosan has a short-term effect on *Penicillium* spp.The extract of grape seeds showed a greater inhibitory effect than chitosan, but its effect was weaker compared to the combination of them. The different concentrations of grape seeds weren't dependent on the inhibitory effect on the concentration of GSE within the studied limits. In the combination variants, the data has a significantly better inhibitory effect than each of the components (chitosan and GSE) separately. They confirmed the synergism effect between the two biopolymers. Grape seed oils and emulsions contain compounds that exhibit an inhibitory effect against fruit rot diseases, including aldehydes, phenols, and ketones. Thymol, carvacrol, and p-anisaldehyde have demonstrated fungicidal activity. Grape seeds are rich in these components, showing the highest inhibitory activity against many post-harvest pathogens (Daferera et al, 2000).

CONCLUSIONS

The present study examines the effect of chitosan and various percentages of grape seed extract on the inhibition of *Penicillium* and *Alternaria* growth. The results reveal significant variations in the effectiveness of the tested compounds, highlighting the importance of selecting appropriate biopolymers and their concentrations for controlling post-harvest pathogens.

The results of Duncan's Multiple Range Test show that the most effective was the combination of chitosan and GSE. The pathogen *Alternaria* spp. was inhibited with 1 per cent of solution chitosan and 1.5% GSE. The best combination of suppression of *Penicillium* spp. was achieved by containing media 1% chitosan and 1% GSE.

ACKNOWLEDGEMENTS

This research is supported by the Bulgarian Ministry of Education and Science under the National Program "Young Scientists and Postdoctoral Students – 2".

REFERENCES

Aleksandrova, D., Zsivanovits, G., Rankova, Z., Malchev, S., & Gandev, S. (2021). Influence of biopolymer coatings on post-harvest cherry diseases. *Journal of Mountain Agriculture on the Balkans*, 2021, Vol. 24, No. 6, 401-413.

Aleksandrova, D., Zsivanovits, G., Rankova, Z., Malchev, S., & Gandev, S. (2019). Effect of chitosan edible coating on sweet cherry post-harvest storage pathogens. 3rd International *Conference on Biosystems and Food Engineering*.

Alishahi, A., & Aïder, M. (2012). Applications of chitosan in the seafood industry and aquaculture: a review. *Food and Bioprocess Technology*, *5*, 817-830.

Daferera, D. J., Ziogas, B. N., & Polissiou, M. G. (2000). GC-MS analysis of essential oils from some Greek aromatic plants and their fungitoxicity on *Penicillium digitatum*. *Journal of agricultural and food chemistry*, 48(6), 2576-2581.

Ezati, P., & Rhim, J. W. (2020). pH-responsive chitosan-based film incorporated with alizarin for intelligent packaging applications. *Food Hydrocolloids*, 102, 105629.

Hisham, F., Akmal, M. M., Ahmad, F., Ahmad, K., & Samat, N. (2024). Biopolymer chitosan: Potential sources, extraction methods, and emerging applications. *Ain Shams Engineering Journal*, *15*(2), 102424.

Jiang, L., Luo, Z., Liu, H., Wang, F., Li, H., Gao, H., & Zhang, H. (2021). Preparation and characterization of chitosan films containing lychee (*Litchi chinensis* Sonn.) pericarp powder and their application as active food packaging. *Foods*, 10(11), 2834.

Kara, Z., Baykan, M., Doğan, M., & Ege, D. (2018). Effectiveness of grape (*Vitis vinifera* L.) seed extracts on fungi and bacteria management. Selcuk *Journal of Agriculture and Food Sciences*, 32(3), 366-372.

Krishnaswamy, K., Orsat, V., Gariépy, Y., & Thangavel, K. (2013). Optimization of microwave-assisted extraction of phenolic antioxidants from grape seeds (*Vitis vinifera*). *Food and Bioprocess Technology*, 6, 441-455.

Kou, S. G., Peters, L. M., & Mucalo, M. R. (2021). Chitosan: A review of sources and preparation methods. *International Journal of Biological Macromolecules*, *169*, 85-94.

Kumar, A., Choudhary, A., Kaur, H., Guha, S., Mehta, S., & Husen, A. (2022). Potential applications of engineered nanoparticles in plant disease management: a critical update. *Chemosphere*, *295*, 133798.

Gharieb, M. M., Omara, M. S. & Soliman, A. (2025). Antifungal activity of mycogenic selenium nanoparticles conjugated with fungal nano chitosan against three important phytopathogenic fungi. *BioNanoScience*, 15(2), 1-18.

Leceta, I., Guerrero, P., Ibarburu, I., Dueñas, M. T. & De La Caba, K. (2013). Characterization and antimicrobial analysis of chitosan-based films. *Journal of Food Engineering*, 116(4), 889-899.

Priyadarshi, R. & Rhim, J. W. (2020). Chitosan-based biodegradable functional films for food packaging applications. *Innovative Food Science & Emerging Technologies*, 62, 102346.

Priyadarshi, R., Ezati, P. & Rhim, J. W. (2021). Recent advances in intelligent food packaging applications using natural food colorants. *ACS Food Science & Technology*, 1(2), 124-138.

Reddy, M. B., Angers, P., Castaigne, F. & Arul, J. (2000). Chitosan effects on blackmold rot and pathogenic factors produced by *Alternaria alternata* in postharvest tomatoes. *Journal of the American Society for Horticultural Science*, 125(6), 742-747.

Riahi, Z., Priyadarshi, R., Rhim, J. W. & Bagheri, R. (2021). Gelatin-based functional films integrated with grapefruit seed extract and TiO2 for active food packaging applications. *Food Hydrocolloids*, 112, 106314.

Roy, S. & Rhim, J. W. (2020). Preparation of carbohydrate-based functional composite films incorporated with curcumin. *Food Hydrocolloids*, 98, 105302.

Sogut, E. & Seydim, A. C. (2018). Development of Chitosan and Polycaprolactone based active bilayer films enhanced with nanocellulose and grape seed extract. *Carbohydrate Polymers*, 195(April) 180–188. https:// doi.org/10.1016/j.carbpol.2018.04.071.

Wu, Z., Deng, W., Luo, J. & Deng, D. (2019). Multifunctional nano-cellulose composite films with grape seed extracts and immobilized silver nanoparticles. *Carbohydrate polymers*, 205, 447-455.

Zhang, L., Chen, J., Liao, H., Li, C. & Chen, M. (2020). Anti-inflammatory effect of lipophilic grape seed proanthocyanidin in RAW 264.7 cells and a zebrafish model. *Journal of Functional Foods*, 75, 104217. https://doi.org/10.1016/j.jff.2020.104217

Zsivanovits, G. I., Sabeva, P. G., Petrova, T. V., Momchilova, M. M., Zhelyazkov, S. P., Iserliyska, D. Z. & Aleksandrova, D. V. (2022). Improving the shelf-life of the sweet cherry by multicomponent edible coatings. *Agricultural Sciences*/Agrarni Nauki, 14(33)

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

EFFECTS OF BAGGING ON FRUIT DEVELOPMENT AND QUALITY IN 'KANZI' APPLES

Orsolya BORSAI[1], Andrei CRISTEA[1], Sándor RÓZSA[1], Tincuța-Marta GOCAN[1], Cosmin Alexandru MIHAI[2], Viorel MITRE[3], Lehel LUKÁCS[4]

[1]University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, 3-5 Calea Mănăștur Street, Cluj-Napoca, Romania
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăști Blvd, District 1, Bucharest, Romania
[3]Academy of Agricultural and Forestry Sciences "Gheorghe Ionescu-Șișești", 61 Mărăști Blvd, District 1, Bucharest, Romania
[4]Horticultural Research Station Cluj-Napoca, 5 Horticultorilor Street, Cluj-Napoca, Romania

Corresponding author email: lehel.lukacs@usamvcluj.ro

Abstract

Bagging is a widely used horticultural practice to modify the microenvironment surrounding developing fruit, with the goal of improving fruit quality and reducing external damage. This study investigates the effects of bagging on the growth, development, and quality of 'Kanzi' apples (Malus domestica Borkh.). Waxed paper bags were applied at early developmental stages, and their impact on fruit size, weight, skin coloration, firmness, and biochemical composition were assessed at harvest. The findings indicate that bagging significantly influenced fruit development, leading to reduced exposure to sunlight and enhanced protection against pests and environmental stressors. While bagging improved skin coloration uniformity it slightly delayed fruit maturity. Non-bagged fruits developed an attractive red colour while bagging suppressed the yellow/red colour development leading to a lower content of total phenolic and flavonoid content. Fruit damage caused by cracking was increased in bagged fruits.

Key words*: firmness, flavonoids, fruit cracking, phenolic compounds TSS.*

INTRODUCTION

The Nicoter Kanzi apple (*Malus domestica* Borkh. 'Kanzi') is a premium apple cultivar renowned for its unique blend of sweetness and tangy flavor, achieved through the hybridization of two well-established apple varieties, such as 'Gala' and 'Braeburn' (Brown & Maloney, 2009). Developed in Belgium in 2004, the 'Kanzi' apple has rapidly gained popularity in global markets due to its exceptional taste, appealing appearance, and extended shelf life (Schouten et al., 2016). 'Kanzi' apples are distinguished by their vibrant red-over-yellow skin, offering an eye-catching glossy finish. The fruit boasts a firm, crunchy texture with a well-balanced sweetness and acidity, making it ideal for both fresh consumption and culinary applications. Its dense flesh retains moisture and crispness longer than many other varieties, contributing to its commercial value (Gwanpua et al., 2016; Damarla et al., 2022). Beyond its sensory appeal, the 'Kanzi' apple holds significant economic importance in the global fruit market (Sestras & Sestras, 2003). Its robust post-harvest qualities, including long storage potential and resistance to bruising, make it a preferred choice for international distribution (Tijskens et al., 2018; Wood et al., 2025). Additionally, 'Kanzi' apples contain essential nutrients, such as dietary fiber, vitamins (particularly vitamin C), and antioxidants, which contribute to consumer health (Serni et al., 2020).

Research into the 'Kanzi' apple continues to explore its agronomic performance, pest and disease resistance, and adaptability to different climatic conditions.

Despite their commercial success, 'Kanzi' apples face several cultivation challenges, particularly in the context of climate change. Fluctuations in temperature, irregular rainfall, and increased pest pressure have led to

concerns about fruit quality and yield consistency (Bacelar et al., 2024). Extreme weather conditions can result in sunburn, skin cracking, and delayed maturation, all of which negatively impact market value. One potential solution to mitigate these challenges is fruit bagging, a practice where individual fruits are enclosed in protective bags during growth.

The bagging technique for various fruits was first introduced in Japan in the 20th century for pears and grapes. Nowadays, fruit bagging technique is widely used especially in Asian countries (Japan, China, and Korea), as well as in Australia and the USA. This method helps to protect fruits from environmental factors, primarily light and pathogens, along with stresses related to temperature, humidity, and air movement, by creating a physical barrier around the fruit.

Essentially, bagging involves enclosing young fruits in a food bag, secured to the fruit stalk with a ribbon or clamp. Fruit bagging has been shown to reduce sunburn, protect against pests, and improve skin finish in other apple varieties (Sharma et al., 2014a). In addition, bagging can create a microclimate around the fruit, helping to maintain consistent temperature and humidity levels, which is crucial under changing climatic conditions (Rajan et al., 2020). There are no studies of bagging effects on 'Kanzi' apples. Therefore, the main purpose of this research was to investigate the effects of fruit bagging in 'Kanzi' apples to improve fruit quality and reduce external damage.

MATERIALS AND METHODS

The current research has been carried out in a private high-density apple orchard located in Iaz, Sălaj County (47°06′48″N; 22°38′33″E) during 2023-2024. The trees were grafted onto M9 rootstocks, and the canopies were trained as spindle (Hrotkó et al., 2013). The apple trees were planted in 2011 at a distance of 3.5 m between the rows and 1 m between the trees within a row. Ten trees were randomly selected and monitored over a full annual growth cycle (n = 10). To determine the effect of fruit bagging on fruit development and quality, ten fruits from each tree at different positions were selected and bagged with brown paper bags (BPB) on the 22nd of May 2023, at 21 days after anthesis (DAA). Ten unbagged fruits from each tree were also selected and served as the control (Figure 1).

The fruits were harvested at commercial maturity stage in the 13th of November 2023.

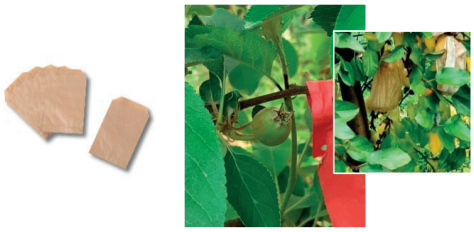


Figure 1. Brown paper bags used for fruit bagging

At harvest, the ten bagged and ten non-bagged fruits/tree were individually weighed and average fruit weight was calculated. Furthermore, other physical traits such as skin colour, fruit density, fruit firmness (Orosz-Tóth & Kincses, 2019; Sala et al., 2017) were determined and fruit cracking rate was calculated (Yang et al., 2009). Among the fruits' chemical properties, titratable acidity (TA) was assessed by titration (Nour et al., 2010) while total soluble solid content (TSS) was determined by using a hand-held refractometer. In addition, the pH and water content of the fruits were also analysed. The total phenolic content was determined according to the Folin-Ciocalteu colorimetric method and expressed as mg gallic acid equivalents per 100g of sample (Bunea et al., 2011). Total flavonoids were determined according to the aluminium chloride colorimetric assay and expressed as expressed on a fresh weight basis as mg catechin equivalents (CE) per 100 g of sample (Miliauskas et al., 2004).

The antioxidant activity of the apple extracts was determined through two methods: DPPH - the evaluation of the free radical-scavenging effect on the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical according to the protocol described by Miliauskas et al. (2004) and De Ancos et al. (2002). An aliquot (0.1 ml) of apple extract was mixed with 3.9 ml of 100 mM DPPH methanol solution. The mixture was thoroughly vortexed and kept in the dark for 30 min. The absorbance was measured at 515 nm.

A blank sample containing the same amount of methanol and DPPH solution was prepared for the blank. A calibration curve was prepared using a standard solution of Trolox (ranging from 2 to 10 µM). The results were expressed on a fresh weight basis as mmol Trolox equivalents (TE)/100 g of sample. The measurements were carried out in triplicates. The ability of the apple samples against the radical anion ABTS+ was determined according to Arnao et al. (2001). ABTS radical cation (ABTS·+) solution was prepared by the reaction of 7 mM ABTS and 2.45 mM potassium persulphate, after incubation at 23°C in the dark for 12 h. The ABTS·+ solution was then diluted with 80% ethanol to obtain an absorbance of 0.700 ± 0.020 at 734 nm. Solution of ABTS·+ (3.9 ml; absorbance of 0.700 ± 0.005) was added to 0.1 ml of the test sample and mixed thoroughly. The reaction mixture was kept at 23ºC for 6 min and the absorbance was immediately measured at 734 nm. The samples were diluted with 80% ethanol. The blank consisted of 0.1 ml of 80% ethanol. The standard curve was obtained by using Trolox standard solution. The total antioxidant activity of apples was expressed as mmol Trolox equivalents (TE) per 100 g fresh weight (Surveswaran et al., 2007). The experiment was carried out in triplicate.

The data, collected have been subjected to statistical analyses using the ANOVA procedure of SPSS V.19 Statistical package. Differences between the means of bagged and control fruits through all the physical and chemical parameters studied were ascertained by Tukey's multiple range test at $p<0.05$.

RESULTS AND DISCUSSIONS

The results showed that fruit bagging affected significantly the colour of the fruit skin. The peel colour of apples plays a crucial role in consumer acceptance, as it serves as a key indicator of fruit quality, ripeness, and overall appeal. Apple peel colour is primarily determined by the concentration and distribution of pigments such as chlorophylls, carotenoids, and anthocyanins. These pigments not only influence aesthetic appeal but also provide insights into the fruit nutritional value and physiological state of the (Chen et al., 2019). The present study found that in comparison to non-bagged fruits, those covered with BPBs resulted in an intense yellow colour exhibiting almost a complete lack of red colouration of the apple peel. Non-bagged apples developed redder peel with more red colour intensity (Figure 2).

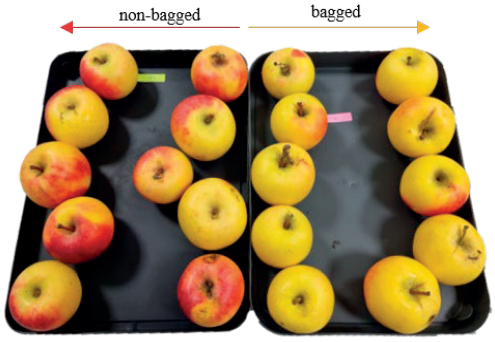


Figure 2. Peel coloration of non-bagged and bagged 'Kanzi' apples

It is assumable that the effects of bagging are associated with solar radiation and temperature regarding fruit pigment production (Saure, 1990). However, the current findings indicate that bagging induced light deprivation which led to limited pigment production resulting in paler fruit (Sharma et al., 2014b). Furthermore, bagging increased humidity and altered temperature conditions compared to non-bagged fruits. These environmental changes can further suppress pigment formation, affecting the overall intensity and uniformity of apple peel colour (Feng et al., 2014).

Regarding fruit size, the results suggest that fruit bagging slightly impacted the size of 'Kanzi' apples as well. The average fruit length of bagged apples was slightly bigger (66.16±1.78 mm) as compared to the control (60.47±2.96 mm), but the average size of the apples was not significantly different between the treatments (Table 1).

Table 1. Effects of fruit bagging on 'Kanzi' apple fruit size

	Fruit length (mm)	Fruit width (mm)	Fruit thickness (mm)	Average
Non-bagged	60.47±2.96	78.07±1.19	75.00±1.23	71.18±4.79 a
Bagged	66.16±1.78	75.86±1.02	74.17±0.89	72.06±3.41 a

*same lowercase letters indicate no significant differences between the bagging treatments at $p < 0.05$ according to Tukey's multiple range test.

These differences in fruit size can be explained by the altered light exposure, temperature, humidity and gas exchange, due bagging which can modify cell expansion and overall fruit growth (Sharma et al., 2014a).
These results are consistent with the findings reported by Tran et al. (2015) in pitaya fruits. Other reports proved that bagging had no effect on fruit size in other fruits either such as pears (Amarante et al., 2002), peach (Jia et al., 2005) and mangoes (Hofman et al., 1997).
The influence of fruit bagging on fruit weight, fruit density, fruit firmness and fruit cracking rate are displayed in Table 2.

Table 2. Effects of fruit bagging on fruit weight, density, firmness and cracking rate in 'Kanzi' apple

	Fruit weight (g)	Fruit density (g/cm^3)	Fruit firmness (kgf/cm^2)	Fruit cracking rate (%)
Non-bagged	192.03±6.66 a	0.92±0.05 a	2.98±0.06 a	11.20±2.89 a
Bagged	200.74±8.82 a	0.87±0.05 a	2.83±0.09 a	43.8±9.33 b

*different lowercase letters indicate significant differences between the bagging treatments within the same fruit characteristic at $p < 0.05$ according to Tukey's multiple range test.

After fruit colour, fruit firmness is another important quality parameter which indicates external fruit quality and post-harvest handling (Shewa et al., 2022). In the present study, there were no significant differences detected in terms of fruit firmness, fruit weight and fruit density among the treatments (Table 2). Previous research results obtained due fruit bagging show inconsistent effects of fruit firmness. For example, fruit bagging amplified fruit softening in apples 'Red Fuji' appled (Chen et al., 2017), red pitaya (Tran et al., 2015), pears (Hudina et al., 2011; Amarante et al., 2002) and pomegranate (Asrey et al., 2020), but reduced slightly in guava (Abbasi et al., 2014) and 'Fuyu' persimmon (Katagiri et al., 2000). In the case of 'Kanzi' apples, less firm fruits might be the results of lower light penetration, higher light absorbance and temperature that resulted from the BPB having darker colour and no holes (Yang et al., 2009). Fruit cracking is a physiological disorder that affects the quality and marketability of apples. A few studies demonstrated that incidences of fruit cracking can be reduced by bagging (Son & Lee, 2008; Oosthuizen, 1989). However, our study showed that the fruit cracking rate was more than 3-fold higher (43.8±9.33%) in the bagged apples than those without cover (11.20±2.89%). This phenomenon might be explained by type of the bagging material which has different physicochemical characteristics such as light transmittance, vapour permeability and heat conductance, and consequently generate differential effects on microenvironment and quality of fruit. The formation of cracks or fissures on the fruit surface are caused in general by imbalances in fruit water uptake, cuticle elasticity, and cell wall integrity (Knoche & Lang, 2017). In recent years, fruit bagging has been employed as a horticultural practice for various reasons. However, bagging can inadvertently influence the incidence of fruit cracking due to microclimatic changes around the developing fruit (Amarante et al., 2002). Bagging alters the fruit's immediate environment by increasing relative humidity and reducing temperature fluctuations. Elevated humidity can enhance cuticle hydration, reducing its mechanical strength and increasing susceptibility to cracking (Knoche & Grimm, 2008; Legay et al., 2015). Furthermore, during peak growth stages, rapid cell enlargement can exert mechanical stress on the fruit surface. In bagged apples, limited transpiration may

promote faster growth, amplifying the risk of cracking (Schrader et al., 2003).

With respect to the chemical characteristics of the 'Kanzi' apples under study, the results revealed that fruit bagging increased slightly the TSS content of the fruits and reduced acidity and water content (Table 3). While fruit bagging is commonly associated with a reduction in TSS due to light deprivation (Ma et al., 2018a), several studies report cases where TSS levels increase in bagged apples. This paradoxical outcome is influenced by specific physiological and environment. With lower water content of the fruits, the concentration of sugars increases leading to a higher TSS content (Wang et al., 2024; Srivastava et al., 2023). In addition, fruit bagging alters carbon partitioning by reducing photooxidative stress, allowing more efficient sugar metabolism (Sharma et al., 2014a). Furthermore, bagging may delay fruit ripening slightly, allowing the fruit to mature longer on the tree. Extended maturation increases sugar accumulation, contributing to higher TSS (Wang et al., 2013).

Table 3. Effects of fruit bagging on 'Kanzi' apple chemical characteristics

	TSS (°Brix)	TA (%)	TSS/TA	pH	Water content (%)
Non-bagged	15.2±1.15 a	0.48±0.02 a	31.67±13.21 a	3.39±0.76 a	84.66±11.01 a
Bagged	16.2±0.89 a	0.54±0.03 b	30.00±10.09 a	3.82±0.83 b	83.83±9.86 a

*different lowercase letters indicate significant differences between the bagging treatments within the same fruit characteristic at $p < 0.05$ according to Tukey's multiple range test.

The colour of apple peel is not only a visual attribute; it is a multifaceted quality parameter linked to consumer perception, ripeness but most importantly nutritional content (Pontesegger et al., 2023). However, a rich, vibrant peel colour may subconsciously influence consumers' decisions suggesting that the fruit is healthier and richer in nutrients (Boyer & Liu, 2004). The pigments responsible for apple peel colour, such as anthocyanins (red) and carotenoids (yellow-orange), are not only aesthetic but are also closely associated with the phenolic compounds and flavonoid content of the apple fruits, which are linked further to the fruit's antioxidant activity (Zhang et al., 2012; Wang et al., 2022).

The results of this study proved that the colour intensity of fruits was strongly related to their total phenolic and flavonoid content. Therefore, the results showed that fruit bagging had a negative impact on fruit coloration and reduced the accumulation of phenolic and flavonoid content. The phenolic content of the non-bagged 'Kanzi' apple fruits was significantly higher (83.5±2.1 mg GAE/100 g FW) than those covered with BPBs (53.21±1.99 mg GAE/100 g FW) as presented in Figure 3. However, there are no previous studies which investigate the influence of bagging on the quality parameters of 'Kanzi' apples, a few previous studies reported similar values for the total phenolic content. For example, Preti & Tarola (2021) investigated both ancient and commercial apple varieties and reported a polyphenol content of 12.38 mg/100 g in 'Kanzi' apple pulp, and 43.45 mg/100 g FW in the apple peel. Furthermore, Mitić et al. (2013) in their study analyzed 15 apple cultivars which exhibited considerable differences regarding the total phenolic content ranging from 72.80 mg GAE/100 g FW ('Braeburn') to 217.37 mg GAE/100 g FW in 'Kozara' apples. Other previous findings also indicate the suppressive effect of bagging on total phenolic and flavonoid content in fruits, such as loquat (Xu et al., 2010) and red Chinese sand pear (Huang et al., 2009).

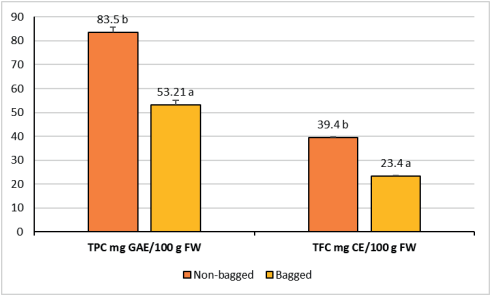


Figure 3. Effects of fruit bagging on total phenolic and flavonoid content of 'Kanzi' apples

Other researchers have found that apples with intense red peel coloration have higher concentrations of anthocyanins, a class of flavonoids known for their strong antioxidant properties (Boyer & Liu, 2004).

These compounds act as scavengers of free radicals, helping to reduce oxidative stress in the human body. Other studies have also shown a positive correlation between the intensity of red colour in apple peels and the total phenolic content (González-Talice et al., 2013). Flavonoids, especially quercetin and kaempferol, are another important class of compounds found in apple peel. (Zhang et al., 2020). These flavonoids contribute to both the coloration of the fruit and its antioxidant activity. Apples with a dark red peel, are typically rich in anthocyanins, and have been shown to exhibit higher antioxidant activity, which is directly related to their flavonoid content (Kunradi et al., 2009). The results of this study are in agreement with this statement and showed a significantly higher total flavonoids content in non-bagged apples (39.4±0.33 mg CE/100 g FW) with red coloured peels than those covered with BPBs (23.4±0.22 mg CE/100 g FW). Similar results were reported by other authors suggesting similar amounts of flavonoids accumulated in apples. Mitić et al. (2013) who investigated the total phenolics, flavonoids and antioxidant activity of 15 apple cultivars, reported a total flavonoid content ranging from 37.2±0.4 mg CE/100 g FW to 112±3 mg CE/100 g FW among the cultivars, out of which 'Gala' (42.0±0.5 mg CE/100 g FW) and 'Braeburn' (37.2±0.4 mg CE/100 g FW) cultivars displayed very similar flavonoid content to the current results. These similarities might be attributed to their genetic background, and biochemical composition.

Apple consumption has been widely linked to a reduced risk of degenerative diseases, including cancer and cardiovascular diseases (Boyer & Liu, 2004; Hyson, 2011). This protective effect is primarily attributed to the high levels of polyphenols and antioxidants found in apples, which play a crucial role in mitigating oxidative stress by scavenging oxygen free radicals in the human body (Lee et al., 2003). These bioactive compounds, including flavonoids and phenolic acids, are known to contribute significantly to the overall antioxidant capacity of apples. Despite the fact that apples also contain ascorbic acid also known as vitamin C, research suggests that it accounts for less than 0.4% of the fruit's total antioxidant activity, underscoring the importance of phenolic compounds as the primary contributors to antioxidant activity (Wolfe et al., 2003). In this regard, the antioxidant activity of non-bagged and bagged 'Kanzi' apples were determined using DPPH radical scavenging activity and ABTS radical cation scavenging activity. The antioxidant activity of non-bagged and bagged apple fruits is presented in Figure 4.

The DPPH Assay involves a stable DPPH radical that accepts an electron or hydrogen atom from antioxidants and works primarily in non-polar or organic solvents (e.g., methanol or ethanol), favoring lipophilic antioxidants such as carotenoids and certain phenolic acids (Mishra et al., 2012).

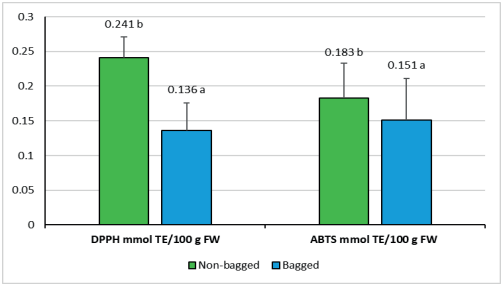


Figure 4. Effects of fruit bagging on the antioxidant activity of 'Kanzi' apples

ABTS assay uses the ABTS•+ radical cation, generated by oxidation with potassium persulfate, which reacts with both hydrophilic and lipophilic antioxidants. It operates in aqueous and organic solvents, making it more suitable for hydrophilic antioxidants like flavonoids, ascorbic acid, and phenolic acids (Re et al., 1999). The results obtained corelate fairly with previous findings reported by Mitić et al. (2013) and Preti and Tarola (2021) and further confirm the strong antioxidant activity of 'Kanzi' apples.

Due to the large dataset resulted from the analyzed fruit traits, a principal component analysis (PCA), a multivariate technique was employed to compare the bagged and non-

nagged fruits through specific fruit quality traits, including morphological characteristics, textural attributes and chemical compounds of the 'Kanzi' apples (Figure 5).

The result of the PCA analysis highlighted that fruit weight and fruit size are positively correlated and primarily contribute to Component 1. Furthermore, it was revealed that the water content of the fruits (%) is moderately associated with fruit size, indicating that larger fruits may have higher water content.

TPC and TFC contents were clustered together with a strong contribution to Component 2, indicating that phenolic and flavonoid contents behave similarly but independently of fruit size or water content. Variables further from the origin, such as TPC and fruit weight, explain more of the variance in the dataset. Higher

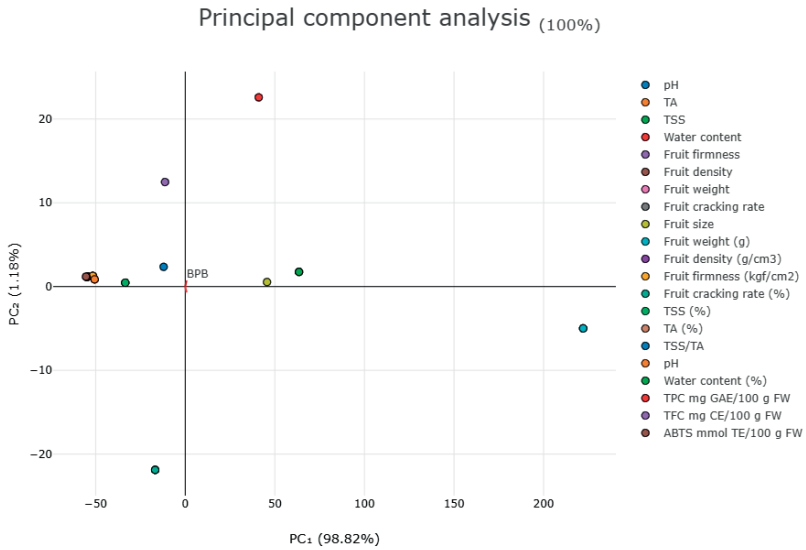


Figure 5. Principal component analysis (PCA) for the types of bagging treatment applied and the fruits traits analyzed in 'Kanzi' apples

phenolic and flavonoid content is independent of fruit size but may reflect other fruit quality traits.

TSS to TA Ratio and TA (%) are located near the origin, which suggest a minimal influence on both components.

The fruit cracking rate (%) is located in the lower left quadrant, negatively correlated with most other variables. This suggests that higher cracking rates are associated with smaller fruit size and lower water content. This relation might be explained by the fact that bagging can create a microclimate that traps heat and humidity especially when the bags do not allow air circulation (Ali et al., 2021). This promotes skin softening and weakens the epidermis, predisposing it to crack. In addition, sudden changes in temperature can exacerbate fruit cracking. During hot days, the fruit inside the bag warms quickly, promoting cell expansion. If followed by a sudden cooling (e.g., evening or rain), the rapid shrinkage can cause skin splitting (La Spada et al., 2024). Furthermore, certain apple cultivars, including 'Kanzi' and other high-turgor varieties, are more prone to cracking due to their genetic tendency toward high internal pressure. In these cases, bagging exacerbates this susceptibility by intensifying moisture retention (Knoche and Grimm, 2008)

CONCLUSIONS

In summary, the current study demonstrated that bagging can protect apples from environmental damage, but it poses a challenge by suppressing fruit coloration, particularly anthocyanin accumulation and further the phenolic and flavonoid content of the apples

and ultimately the antioxidant capacity. In order to balance protection and appearance, strategic bagging techniques, including material choice and timing, are essential. The timing of bag removal could significantly influence these outcomes. Since the exposure of fruits to light/UV accelerate colour development, the removal of the bag before fruit ripening should allow the fruits to regain red coloration of the skin. The same phenomenon of colour induction occurs also in non-red apple varieties (Ma et al., 2018b) and even peaches (Zhao et al., 2017). It has been demonstrated that red-coloration was re-acquired after bag removal and UV treatment.

Understanding the underlying mechanisms allows growers to optimize bagging practices for improved fruit quality.

Choosing breathable, UV-resistant bags with good ventilation holes could help to obtain higher fruit quality.

ACKNOWLEDGEMENTS

This research work was carried out with the support of the University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, Faculty of Horticultural and Business in Rural Development and financed also by AgroTransilvania Cluster, through the project "AgroTransilvania Cluster-Cluster specialized in bioeconomy" (Competitiveness Operational Program Axis 1. Program cofinanced by the European Regional Development Fund "Investing for your future", Section B-Competition code POCA1-A1.1.1-B-2015-Project Type-Innovation clusters. P_35_476, co-funded by European Regional Development Fund) and through the project "AgroTransilvania Cluster".

REFERENCES

Abbasi, N. A., Chaudhary, M. A., Ali, M. I., Azhar Hussain, A. H., & Irfan Ali, I. A. (2014). On tree fruit bagging influences quality of guava harvested at different maturity stages during summer.

Ali, M. M., Anwar, R., Yousef, A. F., Li, B., Luvisi, A., De Bellis, L., ... & Chen, F. (2021). Influence of Bagging on the Development and Quality of Fruits. *Plants*, *10*(2), 358.

Amarante, C., Banks, N. H., & Max, S. (2002). Effect of preharvest bagging on fruit quality and postharvest physiology of pears (*Pyrus communis*). *New Zealand Journal of Crop and Horticultural Science*, *30*(2), 99-107.

Arnao, M. B., Cano, A., Alcolea, J. F., & Acosta, M. (2001). Estimation of free radical-quenching activity of leaf pigment extracts. *Phytochemical Analysis: An International Journal of Plant Chemical and Biochemical Techniques*, *12*(2), 138-143.

Asrey, R., Kumar, K., Sharma, R. R., & Meena, N. K. (2020). Fruit bagging and bag color affects physico-chemical, nutraceutical quality and consumer acceptability of pomegranate (*Punica granatum* L.) arils. *Journal of food science and technology*, 57, 1469-1476.

Bacelar, E., Pinto, T., Anjos, R., Morais, M. C., Oliveira, I., Vilela, A., & Cosme, F. (2024). Impacts of climate change and mitigation strategies for some abiotic and biotic constraints influencing fruit growth and quality. *Plants*, *13*(14), 1942.

Bermúdez-Soto, J. M., & Tomás-Barberán, A. F. (2004). Evaluation of commercial red fruit juice concentrates as ingredients for antioxidant functional juices. *European Food Research and Technology*, *219*(2), 133-141.

Boyer, J., & Liu, R. H. (2004). Apple phytochemicals and their health benefits. *Nutrition journal*, *3*, 1-15.

Brown, S. K., & Maloney, K. E. (2009). Making sense of new apple varieties, trademarks and clubs: Current status. *New York Fruit Quarterly*, *17*(3), 9-12.

Bunea, A., Rugina, O. D., Pintea, A. M., SCONŢA, Z., Bunea, C. I., & Socaciu, C. (2011). Comparative polyphenolic content and antioxidant activities of some wild and cultivated blueberries from Romania. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 39(2), 70-76.

Chen, B., Mao, J., Huang, B., Mi, B., Liu, Y., Hu, Z., & Ma, Z. (2017). Effect of bagging and time of harvest on fruit quality of'Red Fuji'apple in high altitude area in China. *Fruits*, *72*(1), 36-46.

Chen, W., Zhang, M., Zhang, G., Li, P., & Ma, F. (2019). Differential regulation of anthocyanin synthesis in apple peel under different sunlight intensities. *International Journal of Molecular Sciences*, *20*(23), 6060.

Damarla, R. B., & Rao, K. N. (2022). Factors affecting firmness loss in apples during postharvest handling and cold/controlled atmosphere storage in India: A. *Journal of Applied Horticulture*, *24*(1), 98-104.

De Ancos, B., Sgroppo, S., Plaza, L., & Cano, M. P. (2002). Possible nutritional and health-related value promotion in orange juice preserved by high-pressure treatment. *Journal of the Science of Food and Agriculture*, *82*(8), 790-796.

Feng, F., Li, M., Ma, F., & Cheng, L. (2014). The effects of bagging and debagging on external fruit quality, metabolites, and the expression of anthocyanin biosynthetic genes in 'Jonagold' apple (*Malus domestica* Borkh.). *Scientia Horticulturae*, *165*, 123-131.

González-Talice, J., Yuri, J. A., & del Pozo, A. (2013). Relations among pigments, color and phenolic concentrations in the peel of two Gala apple strains according to canopy position and light environment. *Scientia Horticulturae*, *151*, 83-89.

Gwanpua, S. G., Verlinden, B. E., Hertog, M. L. A. T. M., Van Impe, J., Nicolai, B. M., & Geeraerd, A. H. (2013). Towards flexible management of postharvest variation in fruit firmness of three apple cultivars. *Postharvest biology and technology*, 85, 18-29.

Hofman, P. J., Smith, L. G., Joyce, D. C., Johnson, G. I., & Meiburg, G. F. (1997). Bagging of mango (*Mangifera indica* cv.Keitt') fruit influences fruit quality and mineral composition. *Postharvest biology and technology*, *12*(1), 83-91.

Huang, C., Yu, B., Teng, Y., Su, J., Shu, Q., Cheng, Z., & Zeng, L. (2009). Effects of fruit bagging on coloring and related physiology, and qualities of red Chinese sand pears during fruit maturation. *Scientia Horticulturae*, *121*(2), 149-158.

Hudina, M., & Stampar, F. (2011). Effect of fruit bagging on quality of'Conference'pear (*Pyrus communis* L.). *European Journal of Horticultural Science*, *76*(5), 176.

Hyson, D. A. (2011). A comprehensive review of apples and apple components and their relationship to human health. *Advances in nutrition*, *2*(5), 408-420.

Jia, H. J., Araki, A., & Okamoto, G. (2005). Influence of fruit bagging on aroma volatiles and skin coloration of 'Hakuho' peach (*Prunus persica* Batsch). *Postharvest Biology and Technology*, *35*(1), 61-68.

Hrotkó, K. (2013). Development In Fruit Trees Production Systems. *Agrolife Scientific Journal*, *2*(1).

Katagiri, T., Satoh, Y., Fukuda, T., & Kataoka, I. (2000). Improving marketability of'Fuyu'persimmon fruit by bagging culture. In *II International Persimmon Symposium 601* (pp. 213-217).

Knoche, M., & Grimm, E. (2008). Surface moisture induces microcracks in the cuticle of 'Golden Delicious' apple. *HortScience*, *43*(6), 1929-1931.

Knoche, M., & Lang, A. (2017). Ongoing growth challenges fruit skin integrity. *Critical Reviews in Plant Sciences*, *36*(3), 190-215.

Kunradi Vieira, F. G., da Silva Campelo Borges, G., Copetti, C., Valdemiro Gonzaga, L., da Costa Nunes, E., & Fett, R. (2009). Activity and contents of polyphenolic antioxidants in the whole fruit, flesh and peel of three apple cultivars. *Archivos Latinoamericanos de Nutrición*, *59*(1), 101-106.

La Spada, P., Dominguez, E., Continella, A., Heredia, A., & Gentile, A. (2024). Factors influencing fruit cracking: An environmental and agronomic perspective. *Frontiers in Plant Science*, *15*, 1343452.

Lee, K. W., Kim, Y. J., Kim, D. O., Lee, H. J., & Lee, C. Y. (2003). Major phenolics in apple and their contribution to the total antioxidant capacity. *Journal of agricultural and food chemistry*, *51*(22), 6516-6520.

Legay, S., Guerriero, G., Deleruelle, A., Lateur, M., Evers, D., André, C. M., & Hausman, J. F. (2015). Apple russeting as seen through the RNA-seq lens: strong alterations in the exocarp cell wall. *Plant Molecular Biology*, *88*, 21-40.

Ma, Y., Zhao, X., Ren, H., Wu, H., Guo, M., Zhang, Y., ... & Tong, R. (2018a). Significant reduction of the expression of peach (*Prunus persica* L. Batsch.) allergen-encoding genes by fruit bagging with opaque paper. *Journal of Agricultural and Food Chemistry*, *66*(16), 4051-4061.

Ma, C., Jing, C., Chang, B., Yan, J., Liang, B., Liu, L., ... & Zhao, Z. (2018b). The effect of promoter methylation on MdMYB1 expression determines the level of anthocyanin accumulation in skins of two non-red apple cultivars. *BMC plant biology*, *18*, 1-14.

Miliauskas, G., Venskutonis, P. R., & Van Beek, T. A. (2004). Screening of radical scavenging activity of some medicinal and aromatic plant extracts. *Food chemistry*, *85*(2), 231-237.

Mishra, K., Ojha, H., & Chaudhury, N. K. (2012). Estimation of antiradical properties of antioxidants using DPPH assay: A critical review and results. *Food chemistry*, *130*(4), 1036-1043.

Mitić, S. S., Stojanović, B. T., Stojković, M. B., Mitić, M. N., & Pavlović, J. L. (2013). Total phenolics, flavonoids and antioxidant activity of different apple cultivars. *Bulg. Chem. Commun*, *45*, 326-331.

Nour, V., Trandafir, I., & Ionica, M. E. (2010). Compositional Characteristics of Fruits of several Apple (Malus domestica Borkh.) Cultivars. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, *38*(3), 228–233.

Oosthuizen, J. H. (1989). Is covering of litchi clusters worthwhile? *Information Bulletin*, 199, 10-12.

Orosz-Tóth, M., & Kincses, S. (2019). The examination of flesh firmness in different apple varieties.

Pontesegger, N., Rühmer, T., & Siegmund, B. (2023). Physicochemical attributes, volatile profile and sensory quality of organic Crimson Crisp apples during on-tree Maturation. *Foods*, *12*(7), 1425.

Preti, R., & Tarola, A. M. (2021). Study of polyphenols, antioxidant capacity and minerals for the valorisation of ancient apple cultivars from Northeast Italy. *European Food Research and Technology*, *247*, 273-283.

Rajan, R., Ahmad, M. F., Pandey, K., & Solankey, S. S. (2020). Bagging of fruit crops: a low-cost sustainable technology for quality fruit production. *Singh, HK; Solankey, SS; Roy, MK Farmers' prosperity through improved agricultural Technologies. Delhi: Jaya Publishing House*, 121-140.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free radical biology and medicine*, *26*(9-10), 1231-1237.

Sala, F., Iordănescu, O., & Alin Dobrei. (2017). Fractal Analysis as a Tool for Pomology Studies: Case Study In Apple. *Agrolife Scientific Journal*, *6*(1)

Saure, M. C. (1990). External control of anthocyanin formation in apple. *Scientia horticulturae*, *42*(3), 181-218.

Schouten, R. E., Tijskens, L. M. M., Zanella, A., & Sadar, N. (2016, June). Apples from Monalisa-biological variation of firmness behaviour in storage and shelf life. In *VIII International Postharvest Symposium: Enhancing Supply Chain and Consumer Benefits-Ethical and Technological Issues 1194* (pp. 1415-1420).

Schrader, L., Zhang, J., & Sun, J. (2003). Environmental stresses that cause sunburn of apple. *Acta Hort*, *618*, 397-405.

Serni, E., Venir, E., Romano, G., Guerra, W., & Robatscher, P. (2020). Determination of major phenolics content in dried apples from three new cultivars (*Malus domestica* Borkh.) using HPLC-UV-FL with pentafluorophenyl stationary phase. *Food Analytical Methods*, *13*, 863-871.

Sestras, R. E., & Sestras, A. F. (2023). Quantitative traits of interest in apple breeding and their implications for selection. *Plants*, *12*(4), 903.

Sharma, R. R., Pal, R. K., & Asrey, R. (2014a). Influence of bagging on fruit quality and shelf life of apples. *Scientia Horticulturae*, 167, 114-121.

Sharma, R. R., Reddy, S. V. R., & Jhalegar, M. J. (2014b). Pre-harvest fruit bagging: a useful approach for plant protection and improved post-harvest fruit quality–a review. *The Journal of Horticultural Science and Biotechnology*, *89*(2), 101-113.

Shewa, A. G., Gobena, D. A., & Ali, M. K. (2022). Review on postharvest quality and handling of apple. *J. Agric. Sci. Food Technol*, *8*(1), 028-032.

Son, I. C., & Lee, C. H. (2008). The effects of bags with different light transmittance on the berry cracking of grape'Kyoho'. *Horticulture Environment and Biotechnology*, *49*(2), 98-103.

Srivastava, K. K., Soni, S. K., Kumar, D., & Dwivedi, S. K. (2023). Effect of different bagging materials on guava fruit physiology and its quality attributes. *Plant Physiology Reports*, *28*(2), 238-246.

Surveswaran, S., Cai, Y. Z., Corke, H., & Sun, M. (2007). Systematic evaluation of natural phenolic antioxidants from 133 Indian medicinal plants. *Food chemistry*, *102*(3), 938-953.

Tijskens, L. M. M., Schouten, R. E., Zanella, A., & Sadar, N. (2018). Apples from Monalisa-biological variation of firmness behaviour in storage and shelf life.

Tran, D. H., Yen, C. R., & Chen, Y. K. H. (2015). Effects of bagging on fruit characteristics and physical fruit protection in red pitaya (*Hylocereus* spp.). *Biological Agriculture & Horticulture*, *31*(3), 158-166.

Wang, L., Zhang, X., Liu, Y., Shi, X., Wang, Y., Zhang, C., & Zhao, Z. (2013). The effect of fruit bagging on the color, phenolic compounds and expression of the anthocyanin biosynthetic and regulatory genes on the 'Granny Smith'apples. *European Food Research and Technology*, *237*, 875-885.

Wang, X. Y., Wang, H. L., Zhang, G. J., Yan, A. L., Ren, J. C., Liu, Z. H., ... & Sun, L. (2022). Effects of fruit bagging treatment with different types of bags on the contents of phenolics and monoterpenes in muscat-flavored table grapes. *Horticulturae*, *8*(5), 411.

Wang, Z., Feng, Y., Wang, H., Liu, X., & Zhao, Z. (2024). Effects of Different Pre-Harvest Bagging Times on Fruit Quality of Apple. *Foods*, *13*(8), 1243.

Wolfe, K., Wu, X., & Liu, R. H. (2003). Antioxidant activity of apple peels. *Journal of agricultural and food chemistry*, *51*(3), 609-614.

Wood, R. M., Schut, D. E., Balk, P. A., Trull, A. K., Marcelis, L. F., & Schouten, R. E. (2025). Assessing the development of internal disorders in pome fruit with X-ray CT before, during and after controlled atmosphere storage and shelf life. *Food Control*, *168*, 110970.

Xu, H. X., Chen, J. W., & Xie, M. (2010). Effect of different light transmittance paper bags on fruit quality and antioxidant capacity in loquat. *Journal of the Science of Food and Agriculture*, *90*(11), 1783-1788.

Yang, W. H., Zhu, X. C., Bu, J. H., Hu, G. B., Wang, H. C., & Huang, X. M. (2009). Effects of bagging on fruit development and quality in cross-winter off-season longan. *Scientia Horticulturae*, *120*(2), 194-200.

Zhang, G. Z., Ji, B. P., & Chen, G. (2012). Antioxidant activities and phenolic composition of apple peel, core and flesh extracts on selected apple cultivars. *Advanced Materials Research*, *554*, 1103-1109.

Zhang, X., Xu, J., Xu, Z., Sun, X., Zhu, J., & Zhang, Y. (2020). Analysis of antioxidant activity and flavonoids metabolites in peel and flesh of red-fleshed apple varieties. *Molecules*, *25*(8), 1968.

Zhao, Y., Dong, W., Wang, K., Zhang, B., Allan, A. C., Lin-Wang, K., ... & Xu, C. (2017). Differential sensitivity of fruit pigmentation to ultraviolet light between two peach cultivars. *Frontiers in Plant Science*, *8*, 1552.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

DIGITAL MANAGEMENT OF ARONIA CROP: THE BENEFITS OF HUMIDITY AND TEMPERATURE MONITORING THROUGH SPECIFIC SENSORS

Anca Maria CHIOREAN[1, 2], Zsolt JAKAB- ILYEFALVI[1], Ioan ZAGRAI[1], Smaranda ROȘU- MAREȘ[1], Georgeta Maria GUZU[1], Claudiu MOLDOVAN[1], Adrian ASĂNICĂ[3]

[1]Fruit Research & Development Station Bistrita, 3 Drumul Dumitrei Nou Street, Bistrita, Romania
[2]Faculty of Horticulture and Bussines in Rural Development,
University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca,
3-5 Calea Mănăștur Street, Cluj-Napoca, Romania
[3]University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Horticulture,
59 Mărăşti Blvd, District 1, Bucharest, Romania

Corresponding author email: ancasofron09@gmail.com

Abstract

This study investigated the digitalization of chokeberry (Aronia melanocarpa) cultivation in the Bistrița region through the implementation of a cost-effective and optimized NB-IoT (Narrowband Internet of Things) sensor network, designed to monitor critical environmental parameters essential for optimal plant growth and yield. The objectives of the research were to identify critical phenological stages in chokeberry development and to validate the effectiveness of soil and air sensors for automated control, integrated via a mobile application. Climatic factors were monitored in real-time using an nMETOS 80SM agrometeorological station installed within the chokeberry experimental plot at the Fruit Research and Development Station Bistrița, with data acquisition and visualization facilitated by the FieldClimate platform. Analysis of sensor data recorded during the 2024 vegetation period highlighted intervals of pronounced soil moisture deficit, enabling timely and targeted agronomic interventions. Results emphasized that the implementation of digital technologies for crop management represents a valuable and sustainable strategy for chokeberry crop and is a great decisional support instrument with a significant potential for improving yield performance and fruit quality in general.

Key words*: precision, smart technology, sustainability, irrigation, sensors.*

INTRODUCTION

Precision agriculture is a management approach that uses technologies and principles to address the spatial and temporal variability of agricultural production, ultimately leading to better crop performance and environmental quality (Pierce & Nowak, 1999).

Farmers are increasingly adopting digital crop management, which relies on a range of sensors to optimize their operations (Queiroz et al., 2021). Soil sensors provide data on moisture, nutrients, allow precise determination of water content, leading to reduced waste in case of irrigated crops (Santos et al., 2024).

Weather stations equipped with specific air temperature and humidity sensors represents an important tool for monitoring climatic factors and provide essential information about the microclimate of the crop, allowing the anticipation and prevention of heat stress or the risk of some diseases (McBratney et al., 2005; Pradipta et al., 2022).

The valuable data gathered by these sensors, when combined with data analytics, empowers informed decision-making, leading to precision agriculture with its advantages of increased efficiency, reduced waste, and higher crop yields (Johnson et al., 2020).

Wireless sensor networks (WSNs) are used in agriculture for environmental monitoring including water and temperature levels to control plant growing conditions through data collection (Khoa et al., 2019; Shaheb et al, 2022).

The use of specific type of sensors in berry crops is a valuable investment, contributing to resource optimization, cost reduction, sustainnable and increased yields (Banđur et al.,

2019). This technology allows real-time monitoring of essential parameters, providing crucial data for informed decision-making in crop management (Weiss et al., 2020; Vera et al., 2021). Furthermore, sensors can play a crucial role in the early detection of any signs or problems targeted for rapid interventions and contributes to the increase of the productivity and fruit quality (Pereira et al., 2022; Adu-Manu et al., 2017).

With the goal of optimizing crop management, the precision in agriculture is a modern approach relying on advanced technologies to collect and analyze data, facilitating informed decisions about irrigation, fertilization and pest control (Mulla, 2013; Mgendi, 2024; Ali et al., 2023).

The aim of the study was to identify through the use of specific soil and air sensors, the critical periods within the annual development cycle of chokeberry plants and to validate a low-cost NB-IoT (Narrowband-Internet of Things) sensor network designed for efficient automated monitoring via a mobile application.

MATERIALS AND METHODS

The research was carried out in a chokeberry experimental plot, established at Fruit Research and Development Station Bistrita (FRDS Bistrita) comprising four cultivars, under the specific pedoclimatic conditions of Northern Transylvania (Figure 1).



Figure 1. Aronia plantation at FRDS Bistrita

For the purpose of this study, in 2024 a latest-generation mini weather station (nMETOS 80SM) was installed, operating on the NB-IoT (Narrowband-Internet of Things) network in order to provide real time monitoring weather data (Khoa et al., 2019; Muangprathuba et al., 2019; Munir et al. 2018) (Figure 2).

The Mini nMETOS from Pessl Instruments (Austria) is a low-cost compact weather station designed for environmental monitoring. It typically measures parameters like air temperature, relative humidity and it integrates essential sensors (PI54-D and Watermark) that continuously measure soil parameters.

The PI54-D sensor provides precise data on soil temperature and volumetric water content (VWC) expressed as a percentage of total soil volume that is occupied by water (Figure 2).



Figure 2. nMetos 80SM mini weather station

The Watermark sensor offers near real-time measurements of soil moisture (Figure 2). These units (kPa) represent the soil water tension, which is the force that a plants roots must exert to extract water from the soil. The reading values approaching zero in watermark sensor indicate an increase in soil moisture content, meaning that water is readily available for the plants (www.specmeters.com) (Table 1).

Table 1. Analysis and interpretation of Watermark sensor reading

Watermark values	
0-10 kPa	Saturated soil
10-30 kPa	Adequately moist soil, field capacity range (except for coarse sandy soils)
30-60 kPa	Usual irrigation range (except for heavy clay soils)
60-100 kPa	Fairly dry soil, many plants begin to experience water stress
100-200 kPa	Very dry soil, significant water stress for most plants

Source: www.specmeters.com

The logger is installed completely underground (Figure 3) protecting the device from external factors, and is powered by a high-capacity primary battery, ensuring autonomy between 6 and 12 months.
The system provides detailed data on soil moisture and temperature allowing for precise identification of water stress areas, which enables rapid and efficient interventions to protect crops. All climatic parameters were monitored and recorded at 15-minute intervals.

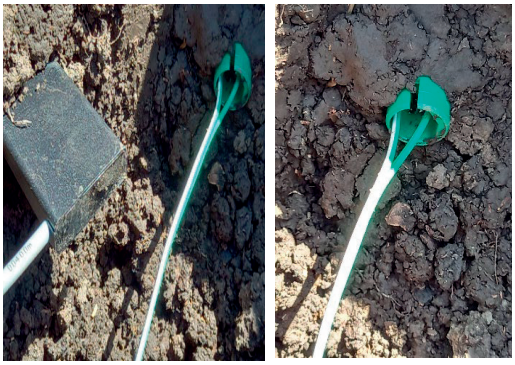


Figure 3. Temperature and humidity soil sensors installed in the field

The data is centralized on the Field Climate online platform, which generates graphs illustrating the daily evolution of the recorded values that are stored, enabling remote access and analysis, facilitating the identification of climatic trends
The interpretation of sensor data was carried out in accordance with the manufacturer's guidelines, considering the soil type specific to the experimental plot and the specie requirements with related to soil moisture.

RESULTS AND DISCUSSIONS

The meteorological statistics for Bistrița region over the past decade indicate a significant trend of increasing average temperatures (approximately 1.1°C), accompanied by the occurrence of severe seasonal droughts and irregular rainfall distribution in certain periods of the vegetation cycle.
Considered a dry year due to a combination of high temperatures and decreased precipitation, 2024 particularly fulfilled these conditions during its growing season, notably the fruit development phenophase, when the water requirements of chokeberry plants are at their highest (Figure 4).
During the vegetation period, fluctuations in both temperatures and relative air humidity can be observed, which indicates the dynamic and often unpredictable nature of the environmental conditions influencing plant development (Figure 4).



Figure 4. Climatic data extracted from the meteorological online platform

Periods of high temperature coupled with low humidity can lead to increased water stress, potentially hindering growth and development of the Aronia plants.
The beginning of the growing season was promising given that sufficient water accumulated in the soil during the dormant period, ensuring optimal conditions for vegetative growth and a uniform start of plant development.
The recorded temperature data showed high average and maximum temperatures starting from April, which accelerated the progression

of the phenophases in the studied Aronia cultivars (Figure 5).

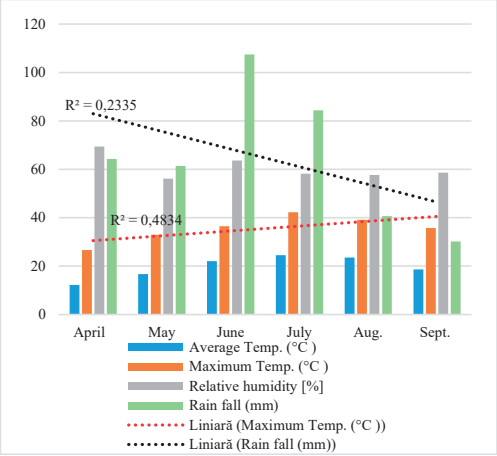


Figure 5. Evolution of temperatures, relative humidity and rain fall during the vegetation stage of Aronia

At the time when the digital monitoring system was installed, the plants from the experimental plot underwent the phenophases of blossoming corresponding to BBCH 56/73.

Aronia is known for its limited water requirements and resistance to short-time drought. The optimal development of the specie is associated with moderately humid environments and a rainfall requirement of 400-500 mm throughout the growing season (Roșca et al., 2022). These needs regarding water consumption may increase depending on each phenophase the plant goes through it and also is influenced by the high temperatures and soil type.

Once the weather station installed, we were able to closely monitor soil and atmospheric parameters dynamics, with the high-precision sensors. This system enables to quickly detect any significant changes, such as drought, excessive moisture, sudden temperature fluctuations, allowing for a prompt appropriate technological interventions to maintain optimal growing conditions for the plants in the field.

The rising temperatures that occurred during May, synchronized with a reduction in precipitation levels, which lead to a slight drying of the soil, indicated by the watermark sensor (Table 2). High watermark values (above 60-80 kPa) means that the soil slowly become drier and that the Aronia plants faced difficulty in absorbing water (Alahmad et al., 2023).

Both the data provided by the watermark sensor (over 60kPa) and the PI54D volumetric water content sensor (53.57%), in May, indicated that the soil currently holds sufficient water, which means the irrigation is not required (Table 2).

Table 2. Monthly average of soil parameters

Month	VPD (%)	PI54D (%)	Watermark (kPa)	PI54D temp (°C)
May	0.98	53.57	66.89	16.92
June	1.19	67.95	25.21	22.42
July	1.64	62.04	102.72	24.74
August	1.55	49.09	178.86	23.46
Sept	1.16	47.70	165.37	9.78

The data from the first decade of June revealed a total of precipitation of 58.4 mm, which was reflected in the readings transmitted by the Watermark (28.80 kPa) and PI54D (57.7%), both indicating high level of soil moisture. (Figure 5).

This situation persisted throughout the second and third decades of June, as the soil became saturated in water due to the 48.4 mm of total precipitation recorded during this period.

The second decade of July was characterized by practically no rainfall, and a significant increase in temperature, climatic conditions that caused a pedological drought. This is reflected in the rising values recorded by the Watermark sensor, indicating a high level of soil dryness, which suggest that irrigation of the crop is requested (Figure 6).

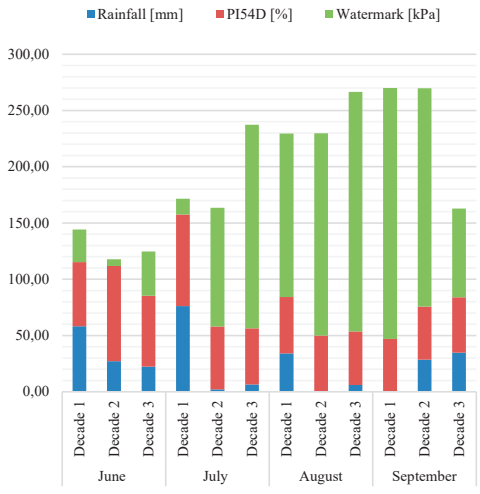


Figure 6. Dynamic of indicators regarding soil moisture content during June to September in Aronia crop

The graph above clearly illustrates that between the second decade of July and the last decade of

September the Aronia plants had to put much more energy to extract the water from the soil. This increased water stress were further combined with the heat stress caused by the high atmospheric temperatures in that period (Figure 4).
Despite the fact that some amounts of rain were recorded in the subsequent period (August-September) it was not enough to effectively restore the water in the soil. The effects of the drought have been observed in fruits, which showed signs of the water stress (Figure 7).



Figure 7. Aronia cluster fruits developed in a water stress condition

Considering the spread of computer-assisted irrigation in berry crops, we believe that the data provided by digital monitoring systems such as these one tested in this experiment can contribute to a digital automation of production. Thus, the data received through the mobile application can help the farmer in real-time to fast decide on the intervention by starting irrigation in case of under-optimal values of water content, preventing drought in the roots area.
Maintaining optimal water content in the soil has beneficial effects on the absorption of nutrients from the soil steadily throughout the growing season.
In addition, the detection of atmospheric humidity levels exceeding certain limits, when coupled with optimal temperatures for the development of pathogens, could serve as a basis for a timely intervention with appropriate phytosanitary treatments.

CONCLUSIONS

This research contributes to the advancement of smart horticulture by demonstrating the importance, feasibility and benefits of integrating IoT-based sensor networks for sustainable and efficient farming practices in a Aronia experimental plantation.
The implementation of high-precision sensors for continuous monitoring of soil and atmospheric parameters lead to an early detection of environmental shifts like drought, excessive moisture, or rapid temperature changes.
This proactive approach empowers timely technological interventions to sustain optimal growing conditions for the Aronia crop.
The research indicate that the period of July to September 2024 proved to be a critical phase for Aronia fruits development in Bistrita region. Characterized by insufficient rain and high temperatures, this period was accurately reflected in the values recorded by the soil (PI54-D, Watermark) and air sensors.
Developing a strategy based on soil and air parameters, data retrieved from the sensors can help to mitigate drought, to secure healthy yields and to optimize water use through precise irrigation interventions.
To ensure sustainable and high-quality Aronia production, future research is required to optimize technological practices that mitigate the negative impacts of abiotic stress factors.

ACKNOWLEDGEMENTS

This research work was carried out with the support of Ministry of Agriculture and Rural Development, Project ADER 6.1.3/2023.

REFERENCES

Adu-Manu, K. S., Tapparello, C., Heinzelman, W., Katsriku, F. A., & Abdulai, J. D. (2017). Water quality monitoring using wireless sensor networks: Current trends and future research directions. *ACM Transactions on Sensor Networks* (TOSN), *13*(1), 1-41.

Ali, A., Hussain, T., Tantashutikun, N., Hussain, N., & Cocetta, G. (2023). Application of smart techniques, internet of things and data mining for resource use

efficient and sustainable crop production. *Agriculture*, *13*(2), 397.

Alahmad, T., Neményi, M., & Nyéki, A. (2023). Applying IoT sensors and big data to improve precision crop production: a review. *Agronomy*, *13*(10), 2603.

Banđur, Đ., Jakšić, B., Banđur, M., & Jović, S. (2019). An analysis of energy efficiency in Wireless Sensor Networks (WSNs) applied in smart agriculture. *Computers and electronics in agriculture*, *156*, 500-507.

Johnson, N., Kumar, M. S., & Dhannia, T. (2020). A study on the significance of smart IoT sensors and Data science in Digital agriculture. *Advanced Computing and Communication Technologies for High Performance Applications (ACCTHPA)*, 80-88.

Khoa, T. A., Man, M. M., Nguyen, T. Y., Nguyen, V., & Nam, N. H. (2019). Smart agriculture using IoT multi-sensors: A novel watering management system. *Journal of Sensor and Actuator Networks*, *8*(3), 45.

McBratney, A., Whelan, B., Ancev, T., & Bouma, J. (2005). Future directions of precision agriculture *Precision agriculture*, *6*, 7-23.

Mgendi, G. (2024). Unlocking the potential of precision agriculture for sustainable farming. *Discover Agriculture*, *2*(1), 87.

Muangprathub, J., Boonnam, N., Kajornkasirat, S., Lekbangpong, N., Wanichsombat, A., & Nillaor, P. (2019). IoT and agriculture data analysis for smart farm. *Computers and electronics in agriculture*, *156*, 467-474.

Munir, M. S., Bajwa, I. S., Naeem, M. A., & Ramzan, B. (2018). Design and implementation of an IoT system for smart energy consumption and smart irrigation in tunnel farming. *Energies*, *11*(12), 3427.

Mulla, D. J. (2013). Twenty five years of remote sensing in precision agriculture: Progress and prospects. *Precision Agriculture*, *14*(1), 1-17.

Pradipta, A., Soupios, P., Kourgialas, N., Doula, M., Dokou, Z., Makkawi, M., ... & Yassin, M. (2022). Remote sensing, geophysics, and modeling to support precision agriculture - Part 1: Soil applications. *Water*, *14*(7), 1158.

Pierce, F. J., & Nowak, P. (1999). Aspects of precision agriculture. *Advances in agronomy*, *67*, 1-85.

Pereira, L.S., Oweis, T., Zairi, A. (2002). Irrigation management under water scarcity. *Agricultural water management, 57*(3), 175-206.

Queiroz, D. M. D., Coelho, A. L. D. F., Valente, D. S. M., & Schueller, J. K. (2021). Sensors applied to Digital Agriculture: A review. *Revista Ciência Agronômica,* 51*,* e20207751.

Shaheb, M. R., Sarker, A., & Shearer, S. A. (2022). Precision agriculture for sustainable soil and crop management. In *Soil Science-Emerging Technologies, Global Perspectives and Applications*. IntechOpen.

Santos, M. M. D., Sousa, O. N. D., Guerra Filho, P. A., Dias, N. D. S., Aragão, A. R. D., Souza, Y. C. D. S., ... & Moreno-Pizani, M. A. (2024). Intelligent system for automated soil moisture monitoring. *Revista Brasileira de Engenharia Agrícola e Ambiental*, *29*(2), e279856.

Vera, J.; Conejero, W.; Mira-García, A. B.; Conesa, M. R.; Ruiz-Sánchez, M. C. Towards irrigation automation based on dielectric soil sensors. *The Journal of Horticultural Science and Biotechnology*, v. *96*, p. 696-707, 2021. https://doi.org/10.1080/14620316.2021.1906761

Weiss, M.; Jacob, F.; Duveiller, G. (2020). Remote sensing for agricultural applications: A meta-review. *Remote sensing of environment*, v. *236*, e111402. https://doi.org/10.1016/j.rse.2019.111402

Yin, H., Cao, Y., Marelli, B., Zeng, X., Mason, A. J., & Cao, C. (2021). Soil sensors and plant wearables for smart and precision agriculture. *Advanced Materials*, *33*(20), 2007764

**https://metos.global/ro/

**https://www.specmeters.com

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

FRUIT CHARACTERISTICS OF DIFFERENT AUTOCHTHONOUS CHERRY GENOTYPES SELECTED FOR THE INTERACTION WITH THE PLUM POX VIRUS

Florentina DOBRINESCU[1], Viorel Cătălin OLTENACU[2], Ligia ION[1]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest,
59 Mărăşti Blvd, District 1, Bucharest, Romania
[2]Research and Development Station for Fruit Growing Băneasa,
4 Ion Ionescu de la Brad Blvd, District 1, Bucharest, Romania

Corresponding author email: florentina_dobrinescu@yahoo.com

Abstract

The Plum pox virus (PPV), , is the one that greatly affects stone fruit crops, considered one of the most destructive viruses worldwide, as a quarantine pathogen. Over time, numerous researches have been carried out because the virus affects particularly the productivity and quality of the fruits, but, unfortunately, no treatment has been discovered, and as a last measure taken for a short time, at the present moment, being to eradicate the infected trees and plant new virus-free trees. The highlighting and selection of valuable genes, of economic importance in cherry, through modern techniques, and their transmission to new cherry cultivars, the production of agro-productive and superior quality planting material and the launch on the market of the most valuable cultivars, is a major objective of all programs of cherry breeding both worldwide and in Romania. In this work, the plant material was made up of different F1 hybrid combinations from the specie Cerasus avium. The hybrids studied were obtained in Mihai Bravu locality, Giurgiu County.

Key words*: genetic improvement, tolerance or resistance to pathogens, fruit parameters.*

INTRODUCTION

In the improvement of horticultural plants, the history of the development of knowledge and achievements in the field were specific to the level of knowledge and the concrete possibilities of each era (Sestraş, 2004).

On a global level, there is an intense and continuous concern for the genetic improvement of cherry varieties as the main way to improve agricultural performance, the quality of the harvest, to increase the commercial value of the fruit and the only possibility of increasing disease resistance, with implications in reducing production costs and the level of pollution of the agroecosystem (Cepoiu, 2001).

The main aim of the work was to study the behavior of some autochthonous varieties of cherry when grafted onto three rootstocks, in order to find variants that are resistant or tolerant to plum-pox, in the context of climate changes that have left their mark on the growth and cherry development.

The evaluation of the differences in growth and fruiting of combinations of cherry varieties grafted on different rootstocks will allow the identification and evaluation of the agribiological potential of the studied material, in relation to the ecological factors (Ion, 2007).

In the relationship between grafts and rootstocks, a series of factors intervene with immediate or delayed influence regarding the phenotypic reaction of the grafted plant. Compliance with the general principles for establishing cherry orchards as well as with current culture technologies does not ensure and cannot guarantee the maximum percentage absence of delayed incompatibility phenomena in the young orchard.

The grafting of cherry varieties from the species *Cerasus avium* L. on rootstocks other than frank, referring in particular to interspecific rootstocks, creates the premises for the appearance of these delayed incompatibility phenomena. The causes are generated on the one hand by genetic, biochemical, morpho-anatomical or physiological factors and on the other hand by external factors such as climate (temperature, water), soil, pathogens that, above a certain threshold of intensity in a

negative sense, trigger irreversible imbalances in the plant (Asănică, 2011). In some situations, for the appearance of symptoms of incompatibility, it is enough that only one of the factors is involved, but most of the time, the decline of the tree is caused by a combination of several factors in a greater or lesser weight.
The success of grafting depends to a large extent on placing the graft cambium and the rootstock as close as possible to make the weld as fast as possible. Environmental conditions during the grafting period are very important (Mazilu and Duțu, 2014).
The grafting of a variety belonging to one species can be done on a rootstock of the same species, on a rootstock belonging to another species, or on a hybrid rootstock between two or more species.
In practice in our country, cherry varieties are grafted on cherry rootstocks, sour cherry rootstocks, mahaleb rootstocks and interspecific rootstocks with vegetative propagation.

MATERIALS AND METHODS

The plant material consists of different F1 hybrid combinations from the specie *Cerasus avium* L. The hybrids studied were obtained at Mihai Bravu, Giurgiu County.
The plant material consisted of:
1. Pollen obtained from the varieties 'Boambe de Cotnari', 'Daria', 'Superb', 'Vlad Țepeș', 'Grădiștea 3', 'Copaciu 5', 'Coteni' and 'Dărăști' as sources of resistance to PPV used as male parent.
2. The varieties used in hybridization, 'Timpurii de Comana', 'Samurcășești', 'Fălăștoaca', 'Budeni 1' and 'Câmpurelu', varieties used as female parent.
3. F1 hybrids considered valuable from the specie *Cerasus avium* L.
The pollen obtained from the varieties 'Daria', 'Superb' and 'Dărăști' as a source of resistance to PPV used as the male parent was harvested in the spring of 2022 in the SCDP Băneasa farm, but also from the bordering area of the Mihai Bravu locality, Giurgiu County.
For statistical data processing, we used the IBM SPSS Statistics 20 software. The variance of fruit firmness is calculated and plotted as a function of weight and soluble dry matter content.

RESULTS AND DISCUSSIONS

In order to have a broad picture of the quality of the fruit in the studied variety and hybrid, Table 1 shows the average values for a sample of 10 fruits from the 'Timpurii de Comana' x 'Daria' hybrid with an average weight of 7.53 g, in which the stone has an average weight of 0.44 g. For the 'Timpurii de Comana' variety, the average weight of the fruit was 7.35 g, and the weight of the stone in this variety was 0.54 g.
The average value of soluble dry matter for the hybrid combination was 14.21% and for the 'Timpurii de Comana' variety, the average value of soluble dry matter was 13.96%.
In summary, from Table 1 it can be seen that the 'Timpurii de Comana' x 'Daria' hybrid cross has larger fruits and smaller pits, compared to the mother variety studied 'Timpurii de Comana', but also the ratio of pulp to pulp in favor of the pulp. It is therefore observed how the exogenous pollen partner in the pollination process positively influences the characteristics of the hybrid fruit, not being identical to the varieties used as parents.
The results in Table 1 clearly illustrate the average fruit weight of the 'Timpurii de Comana' x 'Daria ' hybrid combination with a value of 7.53 g and the average fruit weight of the 'Timpurii de Comana' variety with a value of 7.35 g. Between the hybrid fruits and the fruits of the variety there are differences that are not visible, but the hybrid fruits from the hybrid combination 'Timpurii de Comana' x 'Daria' are larger than the fruits of the 'Timpurii de Comana' variety. Also the percentage of the average value of the weight of the seeds in the hybrid combination 'Timpurii de Comana' x 'Daria' which is 0.44 g and the variety 'Timpurii de Comana' 0.54 g, helps us to conclude that the smaller seeds from the hybrid combination actually mean a higher percentage of pulp, which is of most interest when we talk about what is consumed in a fruit, i.e. the pulp.
Also in Table 1, the results on the soluble dry matter of the hybrid combination 'Timpurii de Comana' x 'Daria', demonstrate that the hybrid 'Timpurii de Comana' x 'Daria' has a higher soluble dry matter content of 14.21% compared to the mother variety 'Timpurii de Comana' 13.96%. As soluble dry matter is associated

with sugar content, this would demonstrate that the hybrid is sweeter than the parent variety.

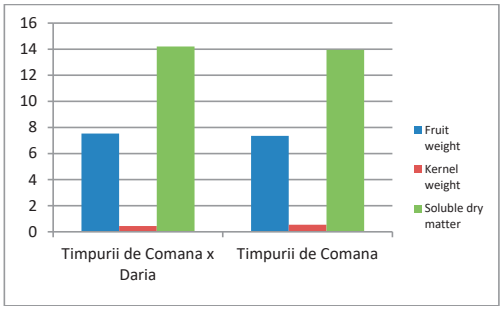


Figure 1. The average of the results regarding the internal characters of the fruits of the hybrid combination 'Timpurii de Comana' x 'Daria'

Table 2 shows the results regarding the firmness of the fruits of the hybrid combination 'Timpurii de Comana' x 'Daria', and we can see that the average of the results of the firmness of the hybrid fruits is 0.168 kgf/cm^2, instead the average of the firmness of the fruits of the mother variety is 0.157 kgf/cm^2.
In statistical interpretation, we can see that the differences are not significant, but they are significant which means that the hybrid is superior to the variety.

Table 1. Results regarding the internal characters of the hybrid fruits and the fruits of the variety from the hybrid combination 'Timpurii de Comana' x 'Daria'

No. crt.	Fruit weight of the hybrid combination Timpurii de Comana x Daria (g)	Fruit weight Timpurii de Comana variety (g)	Kernel weight of the hybrid combination Timpurii de Comana x Daria (g)	Kernel weight Timpurii de Comana variety (g)	Soluble dry matter in the hybrid combination Timpurii de Comana x Daria (%)	Soluble dry matter Timpurii de Comana variety (%)
1	8.10	8.73	0.42	0.52	14.25	13.20
2	6.45	7.67	0.48	0.49	13.15	14.45
3	7.78	7.12	0.45	0.57	13.55	13.50
4	7.89	7.25	0.43	0.53	13.70	12.90
5	8.74	6.91	0.55	0.65	14.72	13.80
6	6.36	7.45	0.39	0.49	14.93	14.15
7	7.02	7.59	0.36	0.56	14.10	14.40
8	8.67	7.22	0.38	0.43	13.80	14.75
9	6.20	6.92	0.48	0.58	14.80	14.55
10	8.16	6.62	0.52	0.55	15.15	13.92
Total	**75.37**	**73.48**	**4.46**	**5.37**	**142.15**	**139.62**
Average	**7.53**	**7.35**	**0.44**	**0.54**	**14.21**	**13.96**

Table 2. Results regarding the fruit firmness of the 'Timpurii de Comana' x 'Daria' hybrid combination

Nr. crt.	Fruit firmness	
	The hybrid combination Timpurii de Comana x Daria	Maternal variety Timpurii de Comana
	kgf/cm^2	kgf/cm^2
1	0.17	0.17
2	0.16	0.16
3	0.17	0.15
4	0.17	0.16
5	0.16	0.16
6	0.15	0.17
7	0.18	0.16
8	0.18	0.16
9	0.17	0.12
10	0.17	0.16
Total	**1.68**	**1.57**
Average	**0.168**	**0.157**

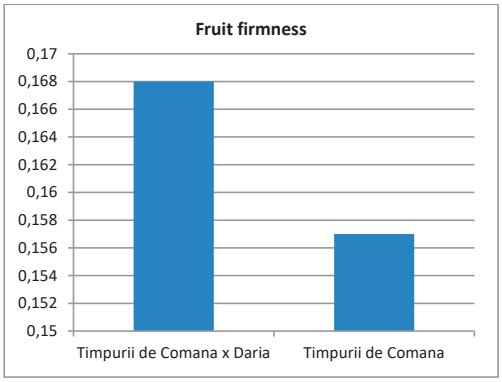


Figure 2. Average fruit firmness results for the hybrid combination 'Timpurii de Comana' x 'Daria'

According to Figure 3, the values obtained according to the Brix analyzes can be observed for the hybrid combination 'Timpurii de Comana' x 'Daria' with an average of 28.44% compared to the maternal variety with an average value of 27.87%.
The fruits of the 'Timpurii de Comana' x 'Daria' hybrid combination are significantly sweeter compared to the fruits of the native variety 'Timpurii de Comana', obtained on the same plant.
Of course, this does not reflect the intrinsic values of the hybrid until after the seeds have germinated and the actual hybrid plants will result, which will flower and bear fruit.

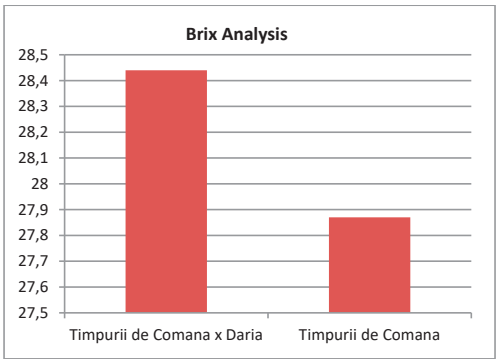


Figure 3. Average Brix analysis results for the hybrid combination 'Timpurii de Comana' x 'Daria'

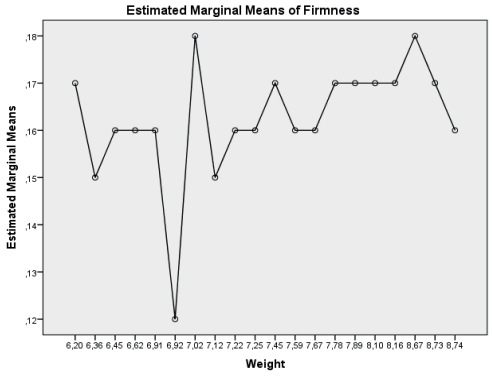


Figure 4. Variation of fruit firmness depending on weight

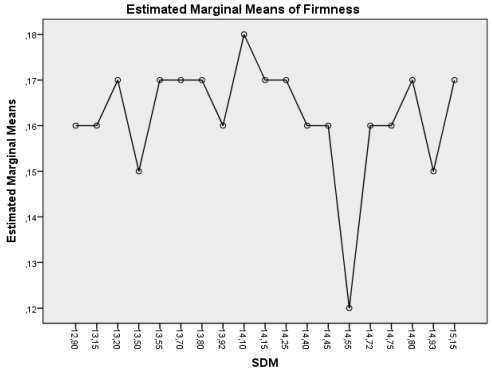


Figure 5. Variation of fruit firmness depending on soluble dry matter

As can be seen from Figure 6, at low values of soluble dry matter, the firmness of the fruits of the Timpurii de Comana variety is higher than that of the hybrid combination, on the other hand at medium and high values within the specific range of SDM, the hybrid combination 'Timpurii de Comana' x 'Daria' presents a much higher firmness than 'Timpurii de Comana'.

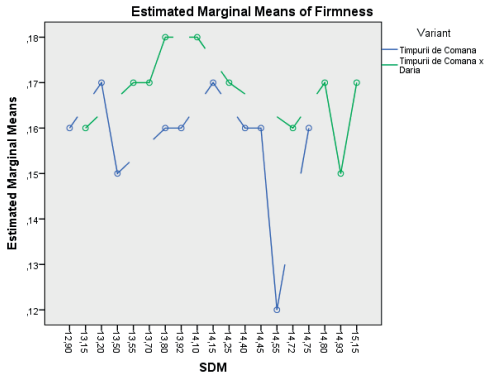


Figure 6. Comparative variation of fruit firmness according to soluble dry matter

CONCLUSIONS

The average fruit weight of the 'Timpurii de Comana' x 'Daria' hybrid combination was higher than the average fruit weight of the maternal parent ('Timpurii de Comana').

The same hierarchy was recorded in the case of the weight of soluble dry matter.

It can be seen that the 'Timpurii de Comana' x 'Daria' hybrid cross has larger fruits and smaller pits, compared to the studied mother variety 'Timpurii de Comana', but also the pulp/stone ratio in favor of the pulp. It is therefore observed how the exogenous pollen partner in the pollination process positively influences the characteristics of the hybrid fruit.

Also, the firmness of the fruits of the hybrid combination as well as the average Brix value were higher compared to the results recorded by the fruits from the 'Timpurii de Comana' variety.

REFERENCES

Asănică, A. (2011). *Integrated model for evaluation of the expression of compatibility and incompatibility of variety/rootstock for cherry in the orchard, Bucharest*, UEFISCDI Project code PD-85

Cepoiu, N. (2001). *Applied horticulture*, Bucharest, RO: Ştiinţelor Agricole Publishing House.

Chira, A. (2005). *Fruit trees. Plantation establishment and maintenance work*, Chisinau, RO: M.A.S.T. Publishing House.

Chira, L., Asănică, A. (2010). *The cherry and sour cherry*, Chișinău, RO: M.A.S.T. Publishing House.
Ion, L. (2007). *Fruit growing*, Bucharest, RO: Ceres Publishing House
Ion, L. (2008). *Micropropagation of horticultural plants and initiation into molecular biology*, Bucharest, RO: Ceres Publishing House
Ion, L., Moale, C. (2013). *The study of culture technologies and methods of virus detection in apricot and peach*, Bucharest, RO: Estfalia Publishing House.
Mazilu, C., Duțu I. (2014). *The orchardist's guide*, Bucharest, RO: Invel Multimedia Publishing House
Mitre V. (2008). *Pomology*, Cluj-Napoca, RO: Todesco Publishing House.
Mitre, V. (2001). *Special fruit growing*, Cluj-Napoca, RO: Academicpress Publishing House.
Oprea, Ș., Gavrilă, R. (2010). *General fruit growing*, Cluj-Napoca, RO: Academicpress Publishing House.
Popescu, M. (1992). *General and special fruit growing*, Bucharest, RO: Didactică şi Pedagogica Publishing House.
Roman, I., Gavrilă, R. (2009) *Orchard technologies*, Cluj-Napoca, RO: Academicpress Publishing House.
Sestras, R. (2004). *Plant improvement*, Cluj-Napoca, RO: Academicpress Publishing House
Stănică, F., Braniște, N. (2011). *Guide for fruit growers*, Bucharest, RO: Ceres Publishing House.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

STUDY ON THE IMPACT OF SEMI-TRANSPARENT PHOTOVOLTAIC PANELS IN HORTIVOLTAIC SYSTEMS ON MICROCLIMATE AND STRAWBERRY PRODUCTION IN ROMANIA

Damian DRAGOMIR[1], Cătălin-Viorel OLTENACU[1], Dragoș Vasile OFRIM[2], Cristian CĂLINIȚĂ[1], Bogdan Alexandru OFRIM[3], Dragoș Mihai OFRIM[4]

[1]Fruit Research and Development Station Băneasa, 4 Ion Ionescu de la Brad Blvd, District 1, Bucharest, Romania
[2]InterNET Ltd, 21 Comana Street, District 1, 011273, Bucharest, Romania
[3]Flash Software Ltd, 27 Banul Nicolae Street, District 2, Bucharest, Romania
[4]Sunshine Breeze Tech Solutions Ltd, 36 Mircea Vulcanescu Street District 1, 010812, Bucharest, Romania

Corresponding author email: dragomir.damian@scdpbaneasa.ro

Abstract

Hortivoltaic systems, integrating renewable energy generation with microclimate control, offer sustainable solutions for agriculture under climate change. This study examines the effects of semi-transparent photovoltaic panels on strawberry (Fragaria x ananassa) cultivation. Conducted at the Moara Domneasca Experimental Base in Romania, the experiment used two panel types: PV1 (48.92% transparency) and PV2 (77.01% transparency), alongside a control area. Microclimate monitoring included soil moisture, soil and air temperature, and humidity. Results showed that air temperatures under PV systems were 5.30°C lower than the uncovered control, reducing heat stress. PV panels also lowered soil temperatures (e.g., 21.86°C under PV1 vs. 22.60°C in the control) and increased soil moisture due to reduced evapotranspiration. Regarding strawberry quality, PV1 panels negatively affected fruit weight and sugar content, while PV2 panels maintained comparable sugar levels (30.33% Brix) and firmness (3.27N) to the control. These findings highlight the potential of hortivoltaic systems to optimize crop productivity in hot climates, offering dual benefits of energy production and sustainable agriculture. Future research should explore long-term impacts.

Key words*: agrivoltaics, hortivoltaic systems, semi-transparent photovoltaic panels, microclimate control, strawberry cultivation, quality, fruit firmness.*

INTRODUCTION

The climate change and intensification demand for sustainable food production have generated worldwide interest in novel agricultural technologies. A potential method that is able to supply a part of these needs, is hortivoltaics, a category of agrivoltaic systems that combines photovoltaic (PV) panels with horticulture cultivation. Agrivoltaics, first proposed by (Goetzberger and Zastrow, 1982), means the simultaneous use of land for energy production and agricultural purposes. This idea has developed into several forms, such as opaque panels placed over crops, semi-transparent solar panels, and photovoltaic greenhouses (Dupraz et al., 2011; Barron-Gafford et al., 2019).

Agrivoltaic with its branch Hortivoltaic systems are more and more popular for their technical capacity to enhance light distribution, retain soil moisture, and modify crop microclimates, especially in climate-stressed environments (Weselek et al., 2021). The European Commission recognized agrivoltaics as a significant factor in meeting renewable energy objectives, predicting that the deployment of even 1% of agricultural land may provide a photovoltaic capacity of 1 TW, exceeding the 590 GW goal for 2030 (Chatzipanagi et al., 2023). However, while agrivoltaics offers environmental and economic advantages, crop performance in shaded environments varies considerably, requiring focused study on species-specific responses (Elamri et al., 2018).

Hortivoltaics and its role in sustainable horticulture

Strawberry (*Fragaria* × *ananassa*) production is particularly sensitive to microclimatic variations, including temperature extremes, excessive solar radiation, and water scarcity

(Gommers et al., 2013). Integrating semi-transparent PV panels can mitigate heat stress, reduce evapotranspiration, and improve water-use efficiency (Valle et al., 2017). Yet, shading intensity and light spectrum alterations influence critical fruit quality parameters such as sugar accumulation (Brix), fruit firmness, and overall yield (Dupraz et al., 2011). The ability of strawberries to adapt to shaded environments is an area of ongoing investigation, with some studies suggesting that moderate shading may enhance fruit quality while excessive shade reduces yield (Marrou et al., 2013; Weselek et al., 2021)

This study evaluates the impact of semi-transparent PV panels on strawberry (*Fragaria × ananassa* 'Sibilla') cultivation in Southeastern Romania, focusing on microclimatic modifications, water efficiency, and fruit quality parameters. Conducted at the Moara Domnească Experimental Base, the research compares two semi-transparent photovoltaic (PVST) panel types - PV1 (48.92% transparency) and PV2 (77.01% transparency) - against an open-field control. Microclimate monitoring encompasses air and soil temperatures, soil moisture, and relative humidity, addressing the dual role of photovoltaics as an energy source and a potential agricultural microclimate stabilizer.

Initial findings indicate that PV panels reduce air temperature fluctuations (5.30°C lower under PV vs. control), contribute to soil moisture conservation, and modify fruit development. While PV1 panels resulted in lower fruit weight and sugar content, PV2 panels maintained comparable sugar levels (30.33% Brix) and firmness (3.27N) to control conditions, suggesting that transparency level plays a pivotal role in optimizing strawberry growth.

Given the rising frequency of heatwaves and prolonged droughts, horticultural systems integrating semi-transparent photovoltaics may provide a sustainable solution to enhance crop resilience while supporting renewable energy goals. However, further research is required to establish optimal panel transparency, crop compatibility, and long-term yield implications. This study contributes to the growing body of knowledge on hortivoltaics as a climate adaptation strategy, offering insights into its potential for scaling up sustainable strawberry production in Romania and beyond.

MATERIALS AND METHODS

In this article, we focus exclusively on hortivoltaic systems that utilise semi-transparent panels installed above strawberry crop, based on practical experience acquired from the design, integration, and implementation of the first two such systems in semi-open spaces in Romania in 2023.

There are multiple factors that are to be taken in to account when we describe the materials and methods used in our experiment.

1. Experimental site description

The research site, situated at approximately 44°30'14.85" latitude and 26°14'45.60" longitude, with an altitude of around 660 meters above sea level, is located within the premises of the Moara Domnească Experimental Base, which is part of the Research and Development Station for Fruit Tree Growing Baneasa. This base is situated in the Vlăsiei Plain, a subunit of the Romanian Plain, in Ilfov County, Romania, specifically in the northeastern outskirts of Bucharest (17 km from city center).

The area around Moara Domnească is characterized by a temperate-continental climate with an average annual temperature of 10.9°C. Summers are warm with an average July temperature of 22.9°C, while winters are cold with an average January temperature of -2.8°C. Extreme temperatures can range from -30°C in January to 41.1°C in August.

Precipitation averages 580 mm annually, with June receiving the most (92 mm) and February receiving the least (31 mm). This uneven distribution can lead to periods of excess humidity throughout the year. Interestingly, winter receives about 27% (or 564.1 mm) of the total annual precipitation. Overall climatic farm characterization is humid climate with hot summers and harsh winters and powerful wind. (Dogaru and Dragomir, 2021)

The dominant air circulation is from the East and North-East in winter and from the West in the rest of the year, with a maximum wind speed of 12.6-14.4 km/h. The zonal soil type is reddish luvisol. In the depressed areas and in

the crevices, there are reddish luvisols and stagnosols.

2. *The hortivoltaic system design*

The agrivoltaic/hortivoltaic system is composed by a mobile metal structure that supports semi-transparent photovoltaic panels (STPV) from Brite Solar, optimized to allow partial light penetration. This design reduces thermal stress on the crops and enhances energy efficiency.

The system was arranged with multiple rows of photovoltaic (STPV) panels, tailored to the specific dimensions of the strawberry crops (Figure 1) and the operational needs of agricultural machinery.



Figure 1. Hortivoltaic system above the strawberry rows

This configuration has been designed in an innovative way to support both electricity generation and favourable conditions for strawberry growth, serving as a concrete example of integrating green technologies into agriculture. The hortivoltaic system has the potential to improve crop productivity and quality under controlled environmental conditions.

Installed in June 2023, the system covers a total area of 0.0125 hectares, with an installed capacity of 9 kWp. It includes two types of semi-transparent photovoltaic (STPV) panels: PV1, with 48.92% transparency (model BSG-250/49-F [BR], 250 Wp), and PV2, with 77.01% transparency (model BSG-115/77-F [BR], 115 Wp) (Figure 2). An uncovered area serves as a control plot.

Microclimate monitoring was done using a system of data acquisition and control, SDAQ, composed by a Wireless Sensor Network, WSN, based on wireless sensor node, which includes different type of sensors, a microcontroller, a communication module and a power source. The sensor network was designed to monitor atmospheric and soil parameters.



Figure 2. Hortivoltaic two types of semi-transparent photovoltaic panels

Soil parameters monitoring was done with Aranet sensors able to capture in real time moisture, temperature, nutrient level, plant condition, etc. Microclimate parameters were also monitored with *Aranet* sensors for air temperature and humidity at a height of 2 m above the ground.

To allow field maintenance activities using traditional land machinery, the sensors were positioned in the strawberry rows' non-processable area, level with the AV facility's steel columns. Soil sensors for the recording of soil moisture and temperature were placed next to the sampling areas and only installed during the period June to August 2023. All soil sensors were removed after October. Each parameter and treatment were averaged for statistical analysis. Data on rainfall, solar radiation, and temperature were obtained from the weather station (*Adcon Telemetry)* in order to compare the meteorological conditions.

3. *Strawberry variety 'Sybilla'*

In our experiment, the strawberry variety 'Sibilla' of *Fragaria x ananassa* was the topic of observations. This cultivar is distinguished by robust plants featuring deep green, lustrous foliage. The fruits are of medium to large size, with a conical or truncated conical form with a subtly tapering apex, having a vivid red hue, a smooth texture, sweet and robust flavour harmoniously balanced between sweetness and acidity, rendering them suitable for both fresh consumption and processing. The maturation phase starts in May-June, and the plants exhibit

significant resistance to foliar and root pathogens.

The plants were arranged in a double-row configuration per plot. Each plot measured 140 cm wide. Plant spacing within each row was 30 cm, while inter-row spacing was 70 cm. A 50 cm gap separated each plot. *Agrotextile* mulch was used for soil cover (Căliniţă et al., 2022). Irrigation was delivered via a subsurface drip irrigation system comprised of two pipes.

The experimental design incorporated randomized blocks (Figure 3) with three replicates, each including three 2-meter zones on raised beds subjected to various treatments: PV1, PV2, and an uncovered Control. Six rows of strawberry plants spanning the entire length of both photovoltaic panel variants and continued beyond the covered area control group, oriented in a north-south direction were chosen for the experiment. To reduce the potential edge effects and the influence of shading on data collection, two central rows were used for specific measurements (atmospheric and soil temperature, relative humidity, soil moisture) and fruit sampling.

Fruit samples were gathered at three distinct periods, and biometric studies were performed on 20 samples at each interval. Biometric determinations were including fruit average weight (Precisa XT 620M balance), height and diameter (digital caliper), soluble solid content (°Brix), pH (Hanna Instruments pH meter HI 700630) and firmness (computerized penetrometer).



Figure 3. Experimental plots under Hortivoltaic system

Statistical analyses were carried out using Microsoft Excel and Duncan's test with a significance level of 0.05%.

RESULTS AND DISCUSSIONS

Microclimate change under semi-transparent photovoltaic panels

Variation in microclimate factors is one of the most vital issues for horticultural practices underneath semi-transparent photovoltaic panels (STPV) array. The reduction in solar radiation is the primary altered factor under the agri photovoltaic canopy (Cossu et al., 2020). However, several other microclimate factors, such as air temperature and humidity, may also be affected.

In our experiment, we observed the average air and soil temperatures across June, July, August, September, and October, along with corresponding relative humidity levels for both air and soil.

The Table 1, these values are presented alongside data for two specific metrics related to photovoltaic (STPV) panels with varying transparency levels, respectively PV1 - 48.92% transparency and PV2 - 77.01% transparency. An uncovered area, reference, serves as a control plot.

Table 1. Microclimatic data based on variants Moara Domnească, Experimental Base

Medium/ Month/Parameter	VI	VII	VIII	IX	X
Average air temperature (2m), °C					
Reference	21.48	25.40	25.88	21.76	15.39
PV1	16.47	20.10	20.07	16.55	10.27
PV2	18.18	22.40	22.68	18.36	12.29
Average air relative humidity - % RH					
Reference	68.30	60.80	69.20	67.00	56.00
PV1	72.05	64.15	73.50	71.68	59.58
PV2	70.52	62.40	71.60	69.25	57.82
Average soil temperature (10 cm depth), °C					
Reference	22.60	25.80	26.20	20.30	16.30
PV1	21.86	25.00	25.25	19.60	15.70
PV2	22.14	25.35	25.70	19.94	16.00
Average soil relative humidity (10 cm depth), % RH					
Reference	16.47	20.44	21.41	20.20	19.80
PV1	19.20	23.70	24.75	23.30	22.90
PV2	18.69	23.10	24.21	22.84	22.35

As can be observed in the Figure 4, the ***air temperature*** profiles recorded under the influence of photovoltaic systems, showed

noticeable variations compared to the uncovered conditions (21.98°C), compared with PV1 (16.29°C) and PV2 (18.38°C). This indicates a cooling effect associated with the shading provided by the less transparent photovoltaic panels PV1. The minimum temperatures were registered also under PV1 (10.27°C), while under PV2 (12.29°C). These minimum values are much lower than the minimum reached in the reference area (15.39°C). Regarding the maximum temperatures, we have observed a direct relation between the level of transparency and recorded values, respectively: PV1 (20.10°C), PV2 (22.68°C), and the reference (25.88°C). The thermal range, representing the difference between maximum and minimum temperatures, was slightly narrower in the PV treatments (9.83°C for PV1 and 10.39°C for PV2) compared to the reference (10.49°C), suggesting a moderating effect on temperature fluctuations due to the photovoltaic structures.

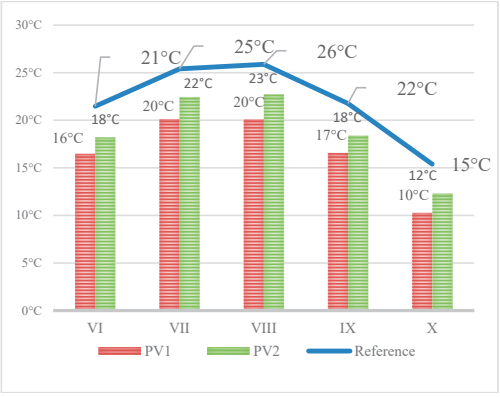


Figure 4. Average air temperature (height 2 m), °C

Regarding air relative humidity (Figure 5), it is to be taken in to consideration that the hortivoltaic system is a semi-open structure, with no barriers that can obstruct air circulation. Thus, air relative humidity may be affected. Nevertheless, under the two semi-transparent photovoltaic panels, the relative humidity values recorded notable differences compared to the reference conditions. The average relative humidity in the reference area was 64.26%, while under PV1 value was 68.19% and under PV2 66.32%. The higher humidity value indicates that the shading effect of STPV reduces evapotranspiration and helps retain moisture in the microclimate. Maximum air relative humidity was recorded under PV1 (73.5%) and PV2 (71.6%), compared to 69.2% in the reference.

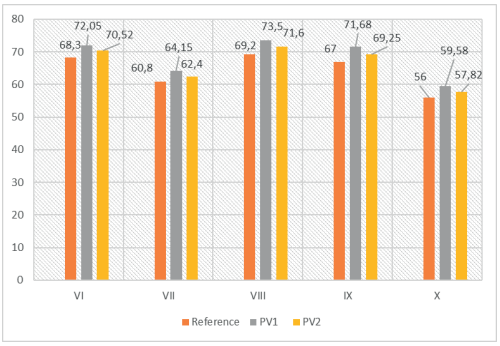


Figure 5. Average air relative humidity (%) under STPV and reference

With respect to the ***soil temperature*** influenced by PVST and measured at 10 cm depth, it has been observed similar trends to the other two factors analysed before. Under PV1 the soil temperature ranged between 15.70°C and 25.25°C, under PV2 - 16.00-25.70°C and the reference area was recording slighter higher values compared to the variants, respectively 16.30°C to 26.20°C. Regarding the maximum and minimum soil temperature, it is to be observed that the semi-transparent photovoltaic panels have an marginal influence, the STPV moderating this factor, particularly during peak temperature intervals. The warmest day, the uncovered soil has reached 26.20°C, while the soil under PV1 and PV2, the values remained slightly reduced at 25.25°C and 25.70°C, respectively. These data suggest that the the photovoltaic panels are having a buffering effect on soil temperature, with the potentially for reducing thermal plant stress and with positive effect on improving moisture conservation in the root zone. Both, soil humidity and temperature could influence various factors like plant growth, microbial activity, and overall soil health. Further research is necessary to explore these potential effects in detail.

Regarding the average soil humidity, our finding confirms the previous studies (Marrou, Dufour and Wery, 2013) that reported increased soil moisture under agrivoltaic due to

reduced evapotranspiration. This research, however, was mostly concerned with spring vegetables like cucumber, lettuce, and also maize grown in irrigated systems. Our study refers to the microclimate under hortivoltaic system, semi-transparent photovoltaic panels (STPV) with different transparency levels. When compared to uncovered control areas, our data reveals a significant drop in soil and air temperatures beneath STPV panels

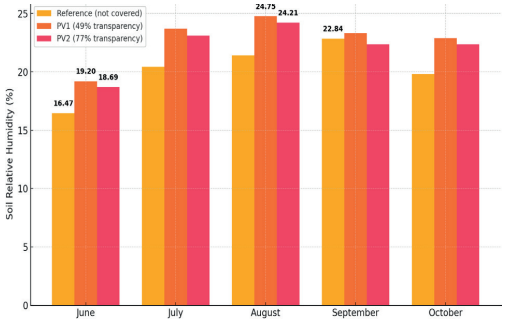


Figure 6. Average soil relative humidity (%) under STPV and reference

As shown in the graph Figure 6, higher mean soil moisture has been recorded under STPV panels compared to the reference.
The variant PV1 with a value of 22.77%, is followed by PV2 with 22.24%, while the reference plot had a lower average of 19.66%.
If we consider the maximum and minimum levels of soil moisture, the minimum values were reached in June under PV1 at 19.20%, PV2 at 18.69%, and reference plots registered a minimum of 16.47%. Concerning maximum relative humidity, the highest soil moisture content was observed in July, PV1 (24.75%) and PV2 (24.21%), but the reference plot recorded 21.41% during the same period. This peak may be attributed to seasonal factors such as increased rainfall or irrigation during the summer months.
As it can be observed in the Figure 6 there is a consistent increase in soil moisture from June to July, followed by a slight decrease towards September. The PV systems maintained higher moisture levels throughout the growing season compared to the reference plot.

Effect of STPV panels on fruit characteristics
During 2023-2024 the fruit characteristics were observed regarding biometric and qualitative parameters. The collected indicators include average weight, fruit height and diameter, soluble solid content (Brix), pH, and firmness.
The Table 2 presents the influence of semi-transparent photovoltaic panel coverage on selected biometric and qualitative traits of strawberry fruit. The dataset compares three experimental variants: PV1 (48.92% transparency), PV2 (77.01% transparency), and a reference (uncovered) control.

Table 2. Fruit characteristics under hortivoltaic system

Variant	Average fruit weight (g)	Sugar (Brix)	pH	Fruit firmness (kgf)
PV1	15.46	7.20	3.52	3.27
PV2	15.25	7.90	3.51	3.15
Reference	19.49	8.60	3.64	3.08

The collected data highlighted significant effects of light modulation on fruit development and compositional parameters.
Regarding, the **average fruit weight** it was observed that in PV1 as well as in PV2 the values were lower than the reference. So, under PV1 the average fruit weight was 15.46 g while under PV2 15.25 g, respectively, compared to 19.49 g in the reference area. Figure 7 illustrates the variations in average fruit weight across the experimental treatments, highlighting the effect of photovoltaic panel transparency on strawberry biomass accumulation.

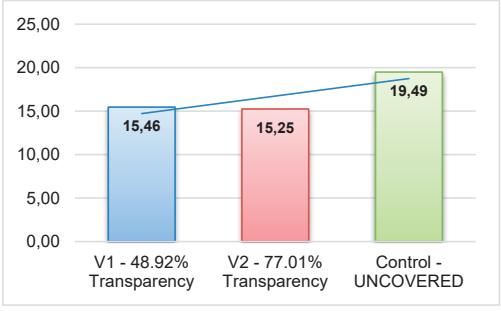


Figure 7. Average fruit weight under STPV and reference

The high differences suggest that the reduction in available solar radiation, particularly under the lower-transparency PV1 panels, had a more pronounced inhibitory effect on fruit growth.

This is likely attributable to diminished photosynthetic activity, which in turn affects carbohydrate accumulation and sink development. The shade effect had significant effect on fruit weight reducing it if we compare to the fruits from control area. This indicates that any level of artificial shading may constrain productivity in strawberry cultivation to some extent.

The soluble solids content (SSC, °Brix), a key quality index in pomological evaluation, followed a similar decreasing trend under shading, with values of 7.20 (PV1) and 7.90 (PV2) compared to 8.60 in the reference area. This reflects a possible delay in sugar accumulation processes, commonly associated with lower light intensity and cooler microclimates under photovoltaic coverage. Interestingly, fruit firmness, a determinant of postharvest behaviour and market value - was enhanced under the PV systems, reaching 3.27 kgf in PV1 and 3.15 kgf in PV2, surpassing the control (3.08 kgf). Figure 8 shows the effect of semi-transparent photovoltaic panels over the fruit firmness.

This increase may be attributed to a moderated ripening rate and reduced transpiration under shaded conditions, which contributes to denser tissue structure.

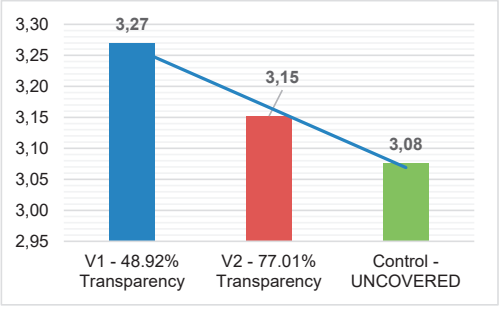


Figure 8. Average fruit firmness under STPV and reference

The **fruit pH** showed minor yet consistent reductions in PV treatments (3.52 and 3.51), suggesting a slightly higher titratable acidity under shaded environments, which may influence both organoleptic properties and shelf stability.

From a pomological perspective, the study is revealing complex interactions between light and fruit quality traits. Reduced solar radiation under PV panels may constrain certain yield-related characteristics such as fruit size and sugar concentration it can simultaneously enhance attributes like firmness, which are critical for transport and storage.

Impact of semi-transparent photovoltaic panels on microclimate and its correlation with fruit qualitative characteristics

Reduced air temperature and lower average fruit weight

The reduction in air temperature observed under semi-transparent photovoltaic (STPV) panels, particularly in the PV1 variant (16.29°C) compared to the uncovered control (21.98°C), had a direct influence on the growth dynamics and fruit development of strawberries. Cooler air conditions are known to slow down plant metabolic activity and biomass accumulation, which likely explains the lower average fruit weight recorded under PV1 (15.46 g) and PV2 (15.25 g) compared to the control (19.49 g). This outcome suggests a strong correlation between light availability, ambient temperature, and fruit biomass formation.

Decreased solar radiation and lower sugar content (Brix)

The soluble solids content (expressed in °Brix), is an indicator of fruit sweetness, The value of sugar content was significantly lower in the shaded variants - 7.20 in PV1 and 7.90 in PV2 - compared to 8.60 in the control. This drop can be attributed to a dropped photosynthetic exertion under limited solar radiation, leading to lower sugar accumulation in the fruits. therefore, the cooler and shadier microclimate beneath the STPV systems results in fruits with lower agreeableness, a quality parameter directly told by light interception.

Air humidity and fruit firmness

Interestingly, despite producing lower and lower sweet fruits, the shops grown under STPV panels yielded firmer strawberries, with values of 3.27 kgf under PV1, compared to 3.08 kgf in the control. This increase in firmness may be linked to the advanced relative air moisture observed beneath the panels (PV1 68.19, control 64.26). Humid soils can reduce plant water stress and evapotranspiration,

decelerating down fruit growing processes and thereby enhancing fruit texture and firmness.

Soil temperature and moisture – synergistic effects on quality

The cooler and more humid soil environment under the STPV panels (PV1 outside soil temperature 25.25°C vs. 26.20°C in the control; average soil humidity 22.77 in PV1 vs. 19.66 in the control) contributed to maintaining a more favourable hydric balance in the root zone. This medium supports a nonstop force of water and nutrients to the factory, which may enhance fruit firmness and post-harvest eventuality. also, increased soil humidity can stimulate salutary microbial exertion in the rhizosphere, laterally perfecting nutrient vacuity and factory health.

Table 3 shows the overall fruit characteristics in relation with microclimatic conditions. The data indicate that semi-transparent photovoltaic panels (PV1 and PV2) led to reduced air temperature and fruit weight, but increased soil moisture and fruit firmness compared to the uncovered reference.

Table 3. Combined microclimate and fruit quality indicators across STPV and reference treatments

Treatments/ parameters	Air temp. (°C)	Air RH (%)	Soil temp. (°C)	Soil RH (%)	Fruit weight (g)	Sugar (Brix)	pH	Firmness (kgf)
Reference	21.982	64.26	22.24	19.664	19.49	8.6	3.64	3.08
PV1	16.692	68.192	21.482	22.77	15.46	7.2	3.52	3.27
PV2	18.782	66.318	21.826	22.238	15.25	7.9	3.51	3.15

CONCLUSIONS

This research emphasizes the advantages and downsides of using semi-transparent photovoltaic (STPV) systems in strawberry growing. The objectification of STPV panels redounded in significant differences to the microclimatic conditions, including diminishments in air and soil temperature and elevations in relative moisture. These changes established a more softened and stable terrain, especially during peak temperature times, which might be profitable for easing factory stress and enhancing water retention in the root zone.

From fruit quality point of view, shade displayed both salutary and mischievous impacts. Reduced light vacuity identified with reduced fruit weight and sugar content, indicating a constraint in photosynthetic exertion and assimilate buildup. Again, the cooler and further sticky microclimate beneath the panels enhanced fruit firmness, a characteristic profitable for transportation, shelf life, and post-harvest quality.

The experiment identified obstacles from functional perspective, effect of semi - transparent phtovoltaic panel shadowing intensity, the need for accurate microclimate monitoring, and the difficulty in coordinating light conditions with energy generation. These factors emphasize the need of maximizing panel translucency and spatial arrangement to fulfil the physiological conditions of the crop while maintaining the system's energy effectiveness.

The study effectively established that the agricultural land can be used for fruit producing in the same time with electricity generation, having under STPV horticultural crop with high value. Also, the study has underlined the need for promotion of interdisciplinary cooperation between agrarian and energy exploration fields.

nevertheless, constraints such the limited observation duration, the attention on a single crop, and the lack of physiological assessments at the splint or factory position suggest that further exploration is necessary. unborn study need to probe colorful crop kinds, long- term goods, and profitable evaluations to enhance the assessment of the sustainability and scalability of STPV systems in horticulture.

The strawberry varieties selection for cultivation in the hortivoltaic systems should consider two main factors: tolerance to partial shading and avoidance of fast-maturing varieties. Varieties that can thrive in diffuse light conditions and develop their fruits during the summer will benefit the most from the

advantages of these innovative systems. This type of integration of agriculture with renewable energy not only optimizes the use of natural resources but also contributes to the sustainability of modern agriculture

Hortivoltaic systems can offer several advantages, including:

- increased crop yield, particularly for shade-tolerant or high-value horticultural crops;
- improved land-use efficiency through dual-purpose utilization - simultaneous food production and solar energy generation on the same surface;
- reduced water consumption, due to lower evapotranspiration rates, contributing to better water-use efficiency;
- dual use of water resources, with irrigation water also serving for PV panel maintenance and cleaning, enhancing operational sustainability;
- integration of sensors and smart technologies on PV structures to enable real-time crop and environmental monitoring, supporting precision horticulture;
- partial protection of crops against abiotic stressors such as UV-b radiation, extreme temperatures, wind, or hail, improving overall plant resilience and reducing losses.

ACKNOWLEDGEMENTS

The authors are grateful to the colleagues who were actively involved and supported the field experiment, including those responsible for data loggers and rain gauges.
This work was carried out within the ADER project 6.3.23 and received funding from the Romanian Ministry of Agriculture.

REFERENCES

Barron-Gafford, G.A., Pavao-Zuckerman, M.A., Minor, R.L., et al. (2019). Agrivoltaics provide mutual benefits across the food–energy–water nexus in drylands. *Nature Sustainability*, *2*, 848–855.

Chatzipanagi, A., Taylor, N. and Jaeger-Waldau, A. (2023). Overview of the potential and challenges for Agri-Photovoltaics in the European Union., EUR 31482 EN, Publications Office of the European Union, Luxembourg, ISBN 978-92-68-02431-7, doi:10.2760/208702, JRC132879

Călinică C., Dragomir D., Oltenacu C.V. (2022). Study of some strawberry varieties in the organic system in the pedo-climatic conditions of the Vlăsia Plain. *Scientific Papers. Series B, Horticulture*, *Vol. LXVI*, Issue 1, Print ISSN 2285-5653, 34-39.

Dinesh, H., Pearce, J.M. (2016). The potential of agrivoltaic systems. *Renewable and Sustainable Energy Reviews*, *54*, 299–308, *36*(10), 2725–2732.

Dogaru, M., and Dragomir, D., 2021. Research on soil fertility in the experimental field at the Moara Domnească Experimental Station. *Scientific Papers, Series A, Agronomy*, *64*(2): 34-39.

Dupraz, C., Talbot, G., Querné, A., Dufour, L. (2010). What explanations for the surprising productivity of temperate agroforestry systems as measured by their Land Equivalent Ratio? *European Society of Agronomy, Agro2010 Proceedings*, 271–272.

Dupraz, C., Marrou, H., Talbot, G., Dufour, L., Nogier, A., Ferard, Y. (2011). Combining solar photovoltaic panels and food crops for optimising land use: Towards new agrivoltaic schemes. *Renewable Energy*.

Elamri, Y., Cheviron, B., Mange, A., Dejean, C., Liron, F., et al. (2018). Rain concentration and sheltering effect of solar panels on cultivated plots. *Hydrology and Earth System Sciences*, *22*(2), 1285–1298.

Goetzberger, A., Zastrow, A. (1982). On the coexistence of solar-energy conversion and plant cultivation. *International Journal of Solar Energy*, *1*(1), 55–69.

Gommers C.M., Visser E.J., St Onge K.R., Voesenek L.A., Pierik R. (2013). Shade tolerance: when growing tall is not an option. *Trends Plant Sci*., *18*(2):65-71. doi: 10.1016/j.tplants.2012.09.008. Epub 2012 Oct 17. PMID: 23084466.

Guerin, T.F. (2019). Impacts and opportunities from large-scale solar photovoltaic (PV) electricity generation on agricultural production. *Environmental Quality Management*, *28*, 7–14.

Marrou, H., Wery, J., Dufour, L., Dupraz, C. (2013). Productivity and radiation use efficiency of lettuces grown in the partial shade of photovoltaic panels. *European Journal of Agronomy*, *44*, 54–66.

Mavani, D.D., Chauhan, P.M., Joshi, V. (2019). Beauty of agrivoltaic system regarding double utilization of same piece of land for generation of electricity & food production. *International Journal of Scientific and Engineering Research*, *10*, 118–148.

Mead, R., Willey, R.W. (1980). The concept of 'land equivalent ratio' and advantages in yields from intercropping. *Experimental Agriculture*, *16*, 217–228.

Pascaris, A.S., Schelly, C., Burnham, L., Pearce, J.M. (2021). Integrating solar energy with agriculture: Industry perspectives on the market, community, and socio-political dimensions of agrivoltaics. *Energy Research & Social Science*, *75*, 102023.

Santra, P., Pande, P.C., Kumar, S., Mishra, D., Singh, R.K. (2017). Agri-voltaics or solar farming. The concept of integrating solar PV-based electricity generation and crop production in a single land use system. *International Journal of Renewable Energy Research*, *7*, 694–699.

Sekiyama, T., Nagashima, A. (2019). Solar sharing for both food and clean energy production: Performance of agrivoltaic systems for corn, a typical shade-intolerant crop. *Environments*, *6*(6), 65.

Trommsdorff, M., Schindele, S., Vorast, M., Durga, N., Patwardhan, S., Baltins, K., Söthe-Garnier, A., Grifi, G. (2019). Feasibility and economic viability of horticulture photovoltaics in Paras, Maharashtra, India. Fraunhofer Institute for Solar Energy Systems (ISE).

Valle, Benoît & Simonneau, Thierry & Sourd, Francis & Pechier, Philippe & Hamard, Philippe & Frisson, Thibault & Ryckewaert, Maxime & Christophe, Angélique. (2017). Increasing the total productivity of a land by combining mobile photovoltaic panels and food crops. *Applied Energy*. 206. 10.1016/j.apenergy.2017.09.113.

Vollprecht, J., Trommsdorff, M., Hermann, C. (2021). Legal framework of agrivoltaics in Germany. *AIP Conference Proceedings*, 2361, 020002.

Weselek, A., Bauerle, A., Zikeli, S., Lewandowski, I., Högy, P. (2021). Effects on crop development, yields and chemical composition of celeriac (*Apium graveolens* L. var. *rapaceum*) cultivated underneath an agrivoltaic system. *Agronomy*, *11*(4).

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

PLANT MATERIAL IN SOME STONE FRUIT SPECIES ROOTSTOCKS

Elena Andreea VOCHIN (DUMITRESCU)[1, 2], Valentina ISAC[2], Florin STĂNICĂ[1]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăşti Blvd, District 1, Bucharest, Romania
[2]Research Institute for Fruit Growing Piteşti - Mărăcineni, 402 Mărului Street, Mărăcineni, Piteşti, Romania

Corresponding author email: andreeaelenadumitrescu00@gmail.com

Abstract

This paper present results regarding the sterilization effectiveness of plant material applied to peach and plum rootstocks explants necessary for the initiation of the in vitro culture. For peach and plum rootstocks tissue culture initiation, two pre-sterilization agents (sanitary alcohol and ethanol) and two sterilization agents (sodium hypochlorite and calcium hypochlorite) where tested in 16 different variants. The Mirobolan dwarf explants were cultured in Murashige and Skoog, 1962 basal medium and the Adaptabil explants were cultured in Querin and Lepoivre, 1977 basal macronutrients and micronutrients and Linsmaier and Skoog, 1965 vitamins. The growth chamber for the in vitro culture had 22±2˚C temperature, with a photoperiod of 16h day light and 8h dark. For Mirobolan dwarf rootstock a good survival rate of explants was obtained both in sterilization variant 15 (56.67%) and in sterilization variant 16 (63.33%).With the Adaptabil rootstock, good sterilization results were also obtained in variant 16 (70%). A good percentage of survival with a lower contamination rate was obtained also in variant 1 (69.67%). Both for Mirobolan dwarf and for Adaptabil rootstocks, the sterilizing agents from variant 16 can be used to sterilize the plant material.

Key words*: rootstock, in vitro culture, sterilization, explants, sodium hypochlorite, calcium hypochlorite.*

INTRODUCTION

The interest in the use of rootstocks grew along with the development of fruit growing as a commercial activity, imposed by the need to produce a large number of trees in specialized nurseries, from the varieties demanded by the consumer market. Vegetative rootstocks propagated on a commercial scale appeared at the beginning of the 20th century, in England, for seed species. In Romania, the interest in rootstocks necessary for the propagation of fruit tree varieties appeared with the establishment of commercial nurseries, but after 1990, both the number of research units in the field of fruit growing and nurseries decreased (Ştefan et al., 2018). The traditional propagation methods allow the clonal multiplication of varieties of interest but at relatively low propagation rates, which explains why the introduction of a new genotype into agricultural practices may take a number of years. Tissue cultures allow the rapid production of a large number of plants, even where normally the species has low multiplication rate. At the same time, the space requirement for such multiplication is considerably smaller. Therefore, *in vitro* propagation techniques provide an efficient and effective method of creating quality-planting material that is true to type in less time and space (Thorpe, 2007). Propagation by seed can generate a large amount of genetic variation so efforts have been made to propagate plum and peach rootstocks *in vitro*. There are several rootstocks for peach, like Nemaguard, which imparts excellent scion vigour and productivity (Handoo et al., 2004), but is sensitive to fungal root rots, iron chlorosis and root waterlogging (Nyczepir et al., 1983; Zehr et al., 1976). The seedlings of Nemared rootstock have few lateral branches, but it is highly susceptible to bacterial crakers (Reighard et al., 2008). Other rootstocks are Guardian, Lovell and Halford, Bailey, Flordaguard, GF-677, which are sensitive to root waterlogging (Loreti et al., 2006). Adaptabil is a interspecific hybrid (Prunus besseyi x open pollination) obtained at

RIFG Maracineni - Pitesti that is recommended as rootstock for peach, nectarine and some plum and apricot variety (Duţu et al., 2002; Duţu et al., 2004; Mazilu et al., 2008). Also there are several rootstocks for plum, such as Brompton (from England), BNKR, Mirobolan C5, Saint Julien A and Pixy. Mirobolan Dwarf it was obtained at RIFG Maracineni - Pitesti and is characterized by low vigour (Duţu et al., 2002). Microbial contamination is one of the most serious problems in micropropagation (Leifert and Cassells, 2001). Successful tissue culture of all plant species depends on the removal of exogenous and endogenous contaminating microorganisms (Constantine, 1986; Buckley and Reed, 1994). The disinfectants usually used are sodium hypochlorite, calcium hypochlorite, ethanol, mercuric chloride, hydrogen peroxide and silver nitrate. Since these sterilization agents are toxic to the plant tissue, contaminants must be removed without killing the plant cells (Pranjić, 2013; Olew et al., 2014). Hypochlorite is known to be a very effective killer of bacteria. When diluted in water, the hypochlorite salts (NaOCl and $CaOCl_2$) lead to the formation of HOCl whose concentration is correlated with bactericidal activity (Nakagarwara et al., 1998). Determination of an effective explant sterilization procedure is essential to avoid contamination during in vitro culture, therefore, the objective of this work was to establish a protocol that is efficient and cost effective and has the highest grade of explants survival.

MATERIALS AND METHODS

Healthy and vigorous shoots of approximately 20-25 cm long, containing 10-15 axillary buds from the mother plantation of RIFG were collected for Mirobolan dwarf and Adaptabil rootstocks. The leaves were cut leaving 0.5 cm of the petiole and were washed under tap water. The shoots were cut into pieces of 1.5-2 cm each including an axillary bud, half of the explants was washed with tap water and the other half was washed with tap water and surfactant Tween 20 for 30 minutes to physically remove most of the microorganisms. Two pre-sterilization agents: sanitary alcohol and ethanol for 10 minutes and two sterilization agents: sodium hypochlorite - commercial bleach Ace with 5.25% active chlorine ingredient and calcium hypochlorite with 6% active chlorine were tested in 16 different variants. Two sterilization times were used: 10 and 20 minutes, and then the biological material was rinsed with distilled water, three times (Table 1).

Cultivation was done in test tubes containing 2 ml of Murashige and Skoog, 1962 (MS) basic medium for the Mirobolan dwarf rootstock, and for Adapatabil rootstock Querin and Lepoivre, 1977 (QL) basic medium with Linsmaier and Skoog, 1965 (LS) vitamins, without growth hormones, supplemented with 9 g agar/l and 20 g sucrose/l. In the experiment were used three repetitions on the variant with ten explants for each repetition. Results were taken after 28 days of in vitro culture and the following data were recorded: the number of survived and contaminated explants (fungus and bacteria). The results were presented as a percentage. Data were analyze using Microsoft Excel 2010 facility.

RESULTS AND DISCUSSIONS

The study showed that for Mirobolan dwarf rootstock, the most effective treatments with 63.33% survival rate was in the case of using Tween 20, pre-sterilizing with ethanol and sterilizing with 6% calcium hypochlorite for 20 minutes and with 56.67% survival rate in the same conditions for 10 minutes. These treatments exceeded those with Ace as source of sodium hypochlorite (Table 2).

These results are similar to the studies of Vujovic et al., 2012, which reference that bleach, as a source of sodium hypochlorite, proved ineffective in disinfecting explants derived from greenhouse-grown plants of Fereley Jaspi and Gisela 6.

The results obtained with Adaptabil rootstock showed that 6% calcium hypochlorite for 20 minutes with Tween 20 and ethanol as pre-sterilizing agent is the most effective treatment with 70% survival rate. For Adaptabil rootstock good results (66.67% survival rate) were also observed in the case of treatment with bleach as the source of sodium hypochlorite for 10 minutes and sanitary alcohol as pre-sterilizing agent without Tween 20 (Table 2).

Gertlowski and Petersen, 1993, reported the efficiency of sodium hypochlorite for superficial sterilization of explant. This method was efficient and did not injure the explants. Also, Al. Ghasheem et al., 2018, worked on peach explants and they found that sodium hypochlorite was the most effective treatment with 50% survival rate at 15% NaOCl for 5 min and 10% NaOCl for 10 min. This result was an important one because the costs in the case of *in vitro* cultures are high, and the use of sanitary alcohol, instead of ethanol, would contribute to reducing the costs of mass production of plant material in vitro.

Regarding the Mirobolan dwarf rootstock, the least effective sterilization protocols with a survival rate of 10% are found in the cases of washing without Tween 20, pre-sterilization with sanitary alcohol, sterilization with 6% calcium hypochlorite, for 10 minutes and in the case of washing without tween, pre-sterilization with ethanol and sterilization with Ace as a source of sodium hypochlorite, for 20 minutes.

Regarding the Adaptabil rootstock, the least effective sterilization protocols with a survival rate of 30% are found in the cases of washing with tween 20, pre-sterilization with sanitary alcohol, sterilization with 6% calcium hypochlorite, for 10 minutes and in the case of washing without tween, pre-sterilization with ethanol and sterilization with calcium hypochlorite, for 10 minutes (Table 2).

For the Mirobolan dwarf rootstock the most effective sterilization treatments with the lowest contamination rate of 10% were in the case of washing with Tween 20, pre-sterilization with ethanol, sterilization with 6% calcium hypochlorite for both 10 minutes and 20 minutes.

Another variant with a contamination rate of 10% is washing without Tween 20, pre-sterilisation with ethanol, sterilization with 6% calcium hypochlorite for 20 minutes. And in the case of washing without Tween 20, pre-sterilization with sanitary alcohol, sterilization with 6% calcium hypochlorite, a contamination rate of 10% was also obtained (Table 2).

For Adaptabil rootstock, the most effective disinfection protocol with a 10% contamination rate was in the case of washing without Tween 20, pre-sterilization with ethanol, sterilization with Ace for 10 minutes (Table 2).

For the Mirobolan dwarf rootstock the least effective sterilization protocols with the highest contamination rate of 60% were for variants 5, 8, and 12. For the Adaptabil rootstock, the least effective sterilization protocol with a 50% contamination rate was for variant 3 (Table 2).

In the case of the two studied rootstocks, Mirobolan dwarf and Adaptabil, in the sterilization variant that offered the highest percentage of survival and growth of the explants (V16), a low degree of contamination was also recorded.

The decontamination process of the explants is not dependent only on the sterilization agents or the method used, a fact proven by the results obtained in this experiment.

It turned out that the results were uneven, in this sense the human factor could contribute to the increase in the degree of contamination.

On total experiment regardless of the pre-sterilization agent or the sterilization agent used or the exposure time, the average survival rate in the case of the Adaptabil rootstock is higher than the average survival rate in the case of the Mirobolan dwarf rootstock (Figure 1).

Regarding the contamination, we notice that the average rate of contamination in the Adaptabil rootstock is lower than in the case of the average rate of contamination in the Mirobolan dwarf rootstock (Figure 1).

The behavior during pre-sterilization and sterilization showed that the Adaptabil rootstock has a higher survival and a lower and distinctly significant contamination ($r = 0.4883$), in other words the percentage of survival decreases with the increase of the percentage of contamination (Figure 2).

Table 1. Types of sterilizing agents used in different concentrations with varying time of sterilizing on peach and plum rootstocks

Variants	Tween 20	Pre-sterilization substance disinfectans	Concentration (%)	Exposure time (min)	Surface sterilizer	Concentration (%)	Exposure time (min)
V1	-	Sanitary alcohol	70	10	Sodium hypochlorite	5.25	10
V2	-	Sanitary alcohol	70	10	Sodium hypochlorite	5.25	20
V3	-	Sanitary alcohol	70	10	Calcium hypochlorite	6	10
V4	-	Sanitary alcohol	70	10	Calcium hypochlorite	6	20
V5	+	Sanitary alcohol	70	10	Sodium hypochlorite	5.25	10
V6	+	Sanitary alcohol	70	10	Sodium hypochlorite	5.25	20
V7	+	Sanitary alcohol	70	10	Calcium hypochlorite	6	10
V8	+	Sanitary alcohol	70	10	Calcium hypochlorite	6	20
V9	-	Ethanol	70	10	Sodium hypochlorite	5.25	10
V10	-	Ethanol	70	10	Sodium hypochlorite	5.25	20
V11	-	Ethanol	70	10	Calcium hypochlorite	6	10
V12	-	Ethanol	70	10	Calcium hypochlorite	6	20
V13	+	Ethanol	70	10	Sodium hypochlorite	5.25	10
V14	+	Ethanol	70	10	Sodium hypochlorite	5.25	20
V15	+	Ethanol	70	10	Calcium hypochlorite	6	10
V16	+	Ethanol	70	10	Calcium hypochlorite	6	20

Table 2. Effect of surface sterilizer, various concentrations and exposure time on survival and contamination of Mirobolan Dwarf explants and Adaptabil explants (%)

Variants	Surface sterilizer	Concentration (%)	Exposure time (min)	Mirobolan dwarf		Adaptabil	
				Survived (%)	Contamination (%)	Survived (%)	Contamination (%)
V1	Sodium hypochlorite	10	10	16.67	13.33	69.67	13.33
V2	Sodium hypochlorite	10	20	53.33	10	50	30
V3	Calcium hypochlorite	6	10	10	33.33	40	50
V4	Calcium hypochlorite	6	20	46.67	26.67	33.33	23.33
V5	Sodium hypochlorite	10	10	13.33	60	43.33	23.33
V6	Sodium hypochlorite	10	20	46.67	33.33	56.67	26.67
V7	Calcium hypochlorite	6	10	26.67	40	30	30
V8	Calcium hypochlorite	6	20	16.67	60	43.33	26.67
V9	Sodium hypochlorite	10	10	36.67	33.33	42.33	10
V10	Sodium hypochlorite	10	20	10	60	56.67	23.33
V11	Calcium hypochlorite	6	10	13.33	40	30	23.33
V12	Calcium hypochlorite	6	20	26.67	10	46.67	16.67
V13	Sodium hypochlorite	10	10	40	33.33	36.67	30
V14	Sodium hypochlorite	10	20	6.67	53.33	53.33	26.67
V15	Calcium hypochlorite	6	10	56.67	10	40	13.33
V16	Calcium hypochlorite	6	20	63.33	10	70	20

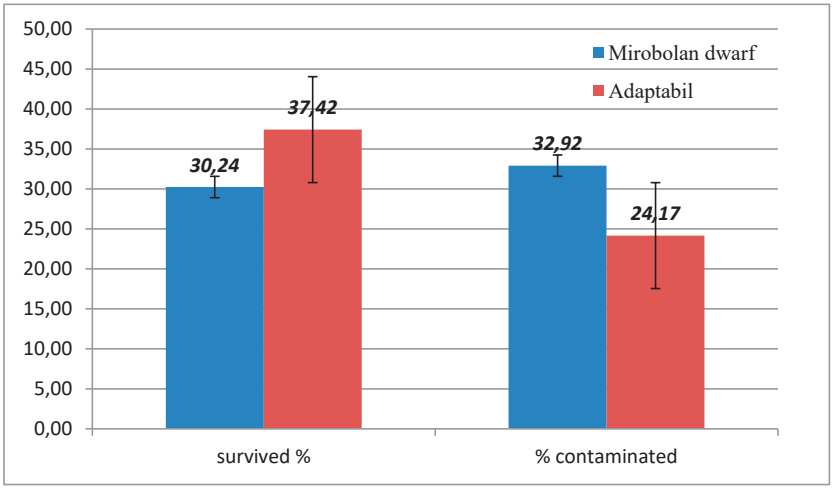


Figure 1. Average survival rate and average contaminated rate for Mirobolan dwarf and Adaptabil roostocks

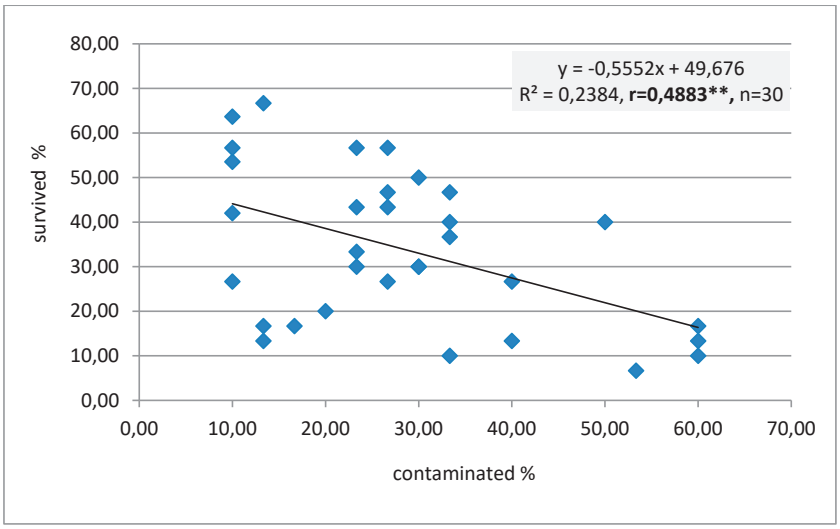


Figure 2. The correlation between the contamination and the survival of the explants in the case of the explants of Mirobolan dwarf and Adaptabil rootstocks

CONCLUSIONS

The establishment of aseptic culture is the first important step in in vitro propagation process. This study presents an effective protocol for surface sterilization of Mirobolan dwarf and Adaptabil rootstocks with promising results for large-scale propagation.

For the two rootstocks studied, Mirobolan dwarf and Adaptabil, the best sterilization results were obtained using the same sterilization protocol. The effective sterilization protocol consists of washing in the presence of Tween 20, presterilization with ethanol and sterilization with 6% calcium hypochlorite, for 20 minutes. The survival percentages were 63.33% in the case of Mirobolan dwarf rootstock and 70% in the case of Adaptabil rootstock.

For Adaptabil rootstock, the combination of sanitary alcohol, commercial bleach Ace as a source of sodium hypochlorite, without Tween, can also be used with good results. This is useful for the practice as it leads to cost savings.

ACKNOWLEDGEMENTS

The authors thank all collegues and researchers in micropropagation Laboratory of Fruit Plants Propagation and Virology within the Research Institute for Fruit Growing for their assistance in this work.

REFERENCES

AL Ghasheem N., Stănică F., Peticilă A.G., Venat O. (2018). In vitro effect of various sterilization techniques on peach (*Prunus persica* (L.) Batsch.) explants. *Scientific Papers. Series B, Horticulture, Volume LXII*, Print ISSN 2285-5653, 227-234.

Buckley, P. M. & Reed, B. M. (1994). Antibiotic susceptibility of plant-associated bacteria. *Hort. Sci.* 29, 434.

Constantine, D.R., (1986). Micropropagation in the commercial environment. In: Withers, L., Alderson, P.G. (Eds), *Plant tissue culture and its agricultural applications*. Butterworth, London, 176-186.

Duţu, I., Viscol, I. (2002). "Adaptabil" – portaltoi vegetativ pentru piersic. *OFERTA cercetării ştiinţifice pentru transfer tehnologic în agricultură, industria alimentară şi silvicultură*: 75. Ed. Tehnică, Bucureşti, ISBN 973-31-2115-0.

Duţu, I., Mazilu, Cr., Ancu, S. (2004). "Adaptabil", portaltoi vegetativ performant, pentru soiurile de piersic şi nectarin. *Hortinform*, 7/143: 22-26.

Gertlowski, C., Petersen, M. (1993). Influence of the carbon source on growth and rosmarinic acid production in suspension cultures of Coleus blumei . *Plant Cell Tiss Organ Cult*, 34, 183–190. https://doi.org/10.1007/BF00036100

Handoo, Z.A.; Nyczepir, A.P.; Esmenjaud, D.; Van Der Beek, J.G.; Castagnone-Sereno, P.; Carta, L.K.; Skantar, A.M.; Higgins, J.A.(2004). Morphological, Molecular, and Differential-Host Characterization of Meloidogyne floridensis n. sp. (Nematoda: Meloidogynidae), a Root-Knot Nematode Parasitizing Peach in Florida. *J. Nematol*, 36, 20–35.

Leifert, C. & Cassells, A. C.(2001). Microbial hazards in plant tissue and cell culture. *In Vitro Cell. Dev. Biol. Plant*, 37, 133–138.

Loreti, F.; Massai, R. (2006). 'Castore' and 'Polluce': Two new hybrid rootstocks for peach. *Acta Hortic*, 713, 275–278.

Mazilu, C.,Duţu I., Ancu, S., Posedaru, A., (2008). Adaptabil (*Prunus* besseyi x) – a clonally romanian rootstock with high efficiency to nursery propagation. *Fruit Growing Research*, vol, XXIV, 71-75.

Murashige, T. & Skoog, F. (1962). A revised medium for rapid growth and bio-assays with tobacco tissue cultures. *Physiol. Plant.* 15, 473–497.

Nakagarwara, S., Goto T., Nara, M., Ozawa, Y., Hotta, K., Arata, Y.(1998) . Spectroscopic characterization and the pH dependence of bacterial activity of the aqueous chlorine solution. *Anal. Sci.* 14, 691–698.

Nyczepir, A.P.; Zehr, E.I.; Lewis, S.A.; Harshman, D.C. (1983). Short life of peach trees induced by *Criconemella xenoplax*. *Plant Dis*.67, 507–508.

Olew, O., Adesoye, A., Ojobo, O., Amusa, O. & Liamngee, S. (2014) Effects of sterilization and phytohormones on shoot tip culture of *Telfairia occidentalis*. *J. Nat. Sci. Res.* 4, 53–58.

Pranjić, A., Čmelik, Z., Puškar, B. & Jurković, Z.(2013) In vitro sterilization procedures for micropropagation of 'Oblačinska' sour cherry. *J. Agric. Sci.* **58**(2), 117–126. https://doi.org/10.2298/JAS1302117M.

Reighard, G.L.; Loreti, F. (2008). Rootstock development. In The Peach: Botany, Production and Uses; Layne, D., Bassi, D., Eds.; CAB International: Cambridge, MA, USA. 193–215.

Ştefan, N., Glăman, Gh., Branişte , N.,Stănică F., Duţu, I., Coman, M. (2018). *Pomologia României, X, Soiuri noi de piersic, nectarin, nucifere, specii pomicole noi, arbuşti fructiferi, căpşun şi portaltoi creaţi în România.* Editura Ceres, Bucureşti.

Thorpe, T. (2007). History of plant tissue culture. *Molecular Biotechnology*, 37:169-180.

Vujović, T., Ružić, D., & Cerović, R. (2012). *In vitro* shoot multiplication as influenced by repeated subculturing of shoots of contemporary fruit rootstocks. *Horticultural Science*, *39*(3), 101-107. doi: 10.17221/208/2011-HORTSCI

Zehr, E.I.; Miller, R.W.; Smith, F.H. (1976). Soil fumigation and peach rootstocks for protection against Peach Tree Short Life. *Phytopathology*, 66, 689–694

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

EFFECTS OF DWARFING ROOTSTOCKS ON THE VEGETATIVE AND GENERATIVE DEVELOPMENT OF SEVERAL CHERRY CULTIVARS IN PEDOCLIMATIC CONDITIONS OF BISTRIŢA FRUIT REGION

Zsolt JAKAB-ILYEFALVI, Daniela-Larisa VLASIN, Anca Maria CHIOREAN, Georgeta-Maria GUZU, Claudiu MOLDOVAN, Smaranda ROȘU-MAREȘ

Fruit Research & Development Station Bistriţa, 3 Drumul Dumitrei Nou, Bistriţa, Romania

Corresponding author email: zsolt.jakab@yahoo.com

Abstract

The field trial was conducted in the Bistrita Fruit Region, Romania, to assess the vegetative growth and generative characteristics of `Lapins`, `Tamara`, and `Kordia` sweet cherry cultivars (Prunus avium L.) grafted on 'GiSelA 5', 'GiSelA 6', and 'GiSelA 3' rootstocks. Trees were drip-irrigated and trained as spindle bushes at a density of 1250 trees/ha on molic eutricambosoil. Parameters such as trunk cross-sectional area, canopy volume, leaf area, shoot length and number, tree height, leaf area/fruit ratio, crop load, yield, fruit number per tree, average fruit size, and fruit quality were evaluated. Data showed that trees grafted on 'GiSelA 5' and 'GiSelA 6' induced the most intensive growth characteristics, they proved to be more vigorous when considering trunk cross-sectional area and canopy volume when compared with 'GiSelA 3' rootstock. Higher fruit calibers were obtained at 'Tamara' in comparison with 'Kordia' and 'Lapins' cherry cultivars. Conversely, a higher number of fruits/trees was observed at 'Lapins' cultivar, showing a very intense bearing capacity.

Key words*: rootstocks, high-density orchard, drip irrigation.*

INTRODUCTION

Sweet cherry cultivation is increasingly popular nowadays both internationally and also in Romania, especially with the implementation of the PNDR programme 4.1a Investments in the fruit orchards. Cherry fruits are highly valued fruits due to nutritional qualities (Szpadzik et al., 2022) and other bioactive parameters such as carotenoids, quercetin (Martini et al., 2019), simple sugars (Nawirska-Olszanszka et al., 2017; Boskov et. al, 2023; Dziadzek et al., 2019), organic acids, vitamins.

Sweet cherry cultivar and rootstock combinations have been recently studied for their effectiveness in controlling tree sizes and maximizing yield efficiency and other technical and economical parameters.

Several breeding programs from Germany (Giessen) aimed to produce dwarfing rootstocks, targeting key orchard production indicators which describe optimum development and bearing processes like, growth, yield, precocity, fruit quality and others (Gyeviki, 2008; Whiting et al., 2005; Martins et al., 2021).

Several dwarf rootstocks suitable for high density orchards were bred recently from the 'GiSelA'series ('GiSelA 3', '5', '12', '13', '17') with different characteristics and different adaptability to distinct pedo-climatical conditions (Aglar et al., 2014; Hrotko et al., 2009; Milic et al., 2019). 'GiSelA 5', 'GiSelA 6' rootstocks were used more recently in Europe and are considered the most productive rootstocks for modern cherry growing (Gjamowski et al., 2016). 'GiSelA' rootstocks derived from the crosses from *Prunus cerasus* and *Prunus canascens* were patented as different selections, they induced precocity, allow tree size control and are very productive (Franken Bambenek, 2010). There are a series of other rootstocks available on the market, such as 'Weigi' ('1', '2', '3', '4') with different size control ability and different adaptation for cherry cultivation (Sjaak, 2024).

Adaptability of 'GiSelA' rootstocks, the relationship with evapotranspiration and irrigation were previously studied in Romania (Jakab et al., 2022; Călinescu et al., 2024) with good results, several other researches revealed the nutritional status, relationship of yield and

canopy training systems (Balan, 2024; Bujdosó et al., 2012; Jakab et. al., 2024). The objective of the researches conducted at FRDS Bistrita was to assess the eco-pedological adaptability, vegetative growth and generative characteristics of 'Lapins', 'Tamara', and 'Kordia' sweet cherry cultivars (*Prunus avium* L.) grafted on 'GiSelA 5', 'GiSelA 6', and 'GiSelA 3' scion/rootstock combinations.

MATERIALS AND METHODS

The study was carried out during 2020-2024 in a 5 year old, drip irrigated sweet cherry experimental orchard (*Prunus avium* L.) planted with 'Lapins' on 'GiSelA 5' and 'GiSelA 6', 'Kordia' on 'GiSelA 5' and 'GiSelA 3', respectively 'Kordia' on 'GiSelA 5' and 'GiSelA 3' (knip trees), in a high density plot, planted at 4 x 2 m distance, the adopted training system was the spindle bush. The research orchard is located in Northern Transylvania, Romania at Fruit Research & Development Station Bistrita (FRDS Bistrita). The soil is a deep eutricambosoil with medium NPK and organic matter content. During the study, standard orchard management techniques were applied regarding weed management with herbicide sprays, appropriate fertilization and phytosanitary treatments.

Biometrical measurements were made regarding the height of tree, trunk cross-sectional area 15 cm above grafting point, volume of trees, length of shoot growth, and the yield per tree (kg/tree). The measurements were done in September at the end of vegetation period, on 14 trees/combination. The trees were subjected to standard irrigation treatments, drippers 2 L h^{-1} flow rate, placed in line, 1m apart. Watermark Spectrum transducers were installed at 20-40 cm deep in the direction of the row.

The trunk diameter was measured with a digital caliper and the height of trees and length of the branches was measured using a measure tape. The statistical analyses were performed using XLSTAT software version 2018.1 (Addinsoft, Paris, France).

RESULTS AND DISCUSSIONS

Meteorological data recorded at FRDS Bistrita showed that the temperatures recorded in the last five years fluctuated with an increasing tendency (Table 1). Annual average temperatures were between 9.5°C (2021), to 11.63°C (2024) with absolute maximum temperatures between 33.6°C (2021) ranging 36.9°C (2024). Regarding the rainfall, data showed that it ranged between 678.9 mm to 784.5 mm. The average value of annual rainfall in Bistrita region is around 758.8 mm (Table 1). Researches showed that in the summer period, is mandatory to implement the drip irrigation, due to the uneven distribution of rainfall. The necessary water amount required was calculated using the FAOSTAT ETP Calculator, which employed the Pennmann-Monteth ETr formula.

Table 1. Climate indicators 2020-2024

Climate indicators	2020	2021	2022	2023	2024
Average annual temp. (°C)	10.1	9.5	11.2	10.9	11.63
Absolute maximum temp.(°C)	33.7	33.6	35.6	36.40	36.9
Rainfall (mm)	678.9	784.5	759.3	765.7	743.2

Research data for the evapotranspiration water quantity during the vegetative season in Bistrita area, showed that the value ranged between 623 to 694 mm with an increasing tendency also, in the last years. Total water deficit calculated showed that in 2022 it was a -249 mm, in 2023 was -220.50 mm and in the last year of experiment even lower: -268.9 mm (Table 2).

Table 2. Evapotranspiration, rainfall and water deficit (2022) in the experimental plot

Months	2022		
	Eto	Rainfall	Difference
March	71.3	0.7	-70.6
April	67.3	102.3	35
May	107.8	70.5	-37.3
Iune	132.9	18	-114.9
Iuly	131.3	35.4	-95.9
August	112.4	78.2	34.2
TOTAL	**623**	**305.1**	**-249.5**

Due to higher monthly temperatures there were higher evapotranspiration also, thus, the calculated Eto values are higher. In 2023 water deficit was more pronounced, in the summer periods (Table 3).

Table 3. Evapotranspiration, rainfall and water deficit (2023) in the experimental plot

Months	2023		
	Eto	Rainfall	Difference
March	67.5	38.20	-29.30
April	75.5	80.2	4.70
May	116.4	24.8	-91.60
Iune	111.5	86.8	-24.70
Iuly	121.2	108	-13.20
August	138	71.6	-66.40
TOTAL	**630.1**	**409.6**	**-220.50**

In the summer period we can observe that there is constantly a water deficit starting with the month of June, but especially in 2024 in the months July (-57.70 mm) and August (-88.90 mm) (Table 4).

Table 4. Evapotranspiration, rainfall and water deficit (2024) in the experimental plot

Months	2024		
	Eto	Rainfall	Difference
March	48.90	50.0	1.1
April	101.80	64.80	-37.00
May	127.40	62.00	-65.40
Iune	149.00	128.00	-20.80
Iuly	148.50	90.80	-57.70
August	119.30	30.40	-88.90
TOTAL	**694.9**	**426.00**	**-268.9**

In 2024 a hail event was recorded and affected the leaves and tree branches and also the fruits. Observations regarding plant physiology showed that the implementation of drip irrigation systems in cherry plantations with cultivars grafted on 'GiSelA 3' and 'GiSelA 5' is mandatory.

Research data showed that the most intensive vegetative growth (Table 5) of the cherry cultivars in shoot length parameter, was observed at 'Lapins'/'GiSelA 5' cultivar in 2022 (65.5 cm) with close values at 'Lapins'/'GiSelA 5' cultivar (65.8 cm) being in the same statistical class as 'Kordia' cultivar on both rootstocks; in 2023 data showed that the growth of shoots was quite similar, 'Lapins' cultivar had higher growth parameters (Table 6), on both rootstocks than the other cultivars.

Regarding the height of trees in the period 2022-2023, trees grew almost in the same growth pattern, higher values being registered for 'Lapins' cultivar grafted on both rootstocks ('GiSelA 5', '6') ranged between 291-317 cm. In 2024 data showed again that the highest values were registered again for 'Lapins' cultivar on both rootstocks, with significant difference for 'Lapins' cultivar grafted on 'GiSelA 6' (347 cm); for the other cultivars 'Tamara' and 'Kordia' on 'GiSelA 5' and 'GiSelA 3', the registered values were close (247-272 cm). The statistical analysis showed that also in 2023 there were significant differences between 'GiSelA 5' and 'GiSelA 6' rootstocks, and there were significant differences regarding 'GiSelA 3' also. In our researches, data showed that the smallest vegetative growth was observed at 'Tamara' grafted on 'GiSelA 3' rootstock combination, having the smallest vigor.

Trunk cross-sectional area (TCSA) of the fruit trees is a useful index for the estimation of fruit yield and other vegetative growth parameters. In 2022 results showed (Table 5) that the lowest vigor was registered on 'GiSelA 3' rootstock with slight differences between 'Tamara' (10.6 cm^2) and 'Kordia' cultivars (12.2 cm^2). Higher values were recorded from the beginning in the other rootstock combinations 'Lapins' on 'GiSelA 5' and 'GiSelA 6', respectively 'Tamara' cultivar, ranging 15.63-16.05 cm^2.

Table 5. Vegetative parameters measured (2022) in the cherry plot

Cultivar/ rootstock	2022		
	Shoot length	Height of trees (cm)	TCSA (cm^2)
'Tamara' / Gi. 3	54.5 ^{bc}	214.5 ^{a}	10.6 ^{a}
'Tamara' / Gi. 5	63.0 ^{c}	240.1 ^{b}	15.9 ^{b}
'Lapins' / Gi. 5	65.8 ^{c}	238.8 ^{a}	16.0 ^{b}
'Lapins' / Gi. 6	65.5 ^{c}	308.2 ^{b}	16.2 ^{b}
'Kordia' / Gi. 5	48.5 ^{b}	247.7 ^{a}	15.6 ^{b}
'Kordia' / Gi. 3	32.9 ^{a}	229.8 ^{a}	12.2 ^{ab}

*Duncan's multiple ranges test. Mean values followed by the same letter within a column are not significantly different ($P \leq 0.05$)

Significant differences were observed at 'Lapins' cultivars on both rootstocks in 2023, showing the growth superiority of this rootstock combination. The characteristics of vigor showed that 'Tamara' cultivar with both rootstocks ('GiSelA 3', 'GiSelA 5') achieved the same growth as 'Kordia' grafted on 'GiSelA 5' (Table 6). Distinct growth pattern was observed at 'Lapins' on 'GiSelA 5' and more pronounced growth was observed at 'GiSelA 6'. In the third year of development the vigor differences were observable between variants.

Table 6. Vegetative parameters measured (2023) in the cherry plot

Cultivar/ rootstock	2023		
	Shoot length	Height of trees (cm)	TCSA (cm^2)
'Tamara' / Gi. 3	23.3[a]	225.4[a]	16.90[a]
'Tamara' / Gi. 5	18.4[a]	248.6[ab]	18.60[a]
'Lapins' / Gi. 5	44.1[b]	291.2[cd]	34.94[b]
'Lapins' / Gi. 6	43.0[b]	317.9[d]	46.30[c]
'Kordia' / Gi. 5	19.2[a]	276.0[bc]	20.60[a]
'Kordia' / Gi. 3	23.6[a]	270.3[bc]	31.90[b]

*Duncan's multiple ranges test. Mean values followed by the same letter within a column are not significantly different (P≤0.05)

In 2024 data showed that the highest values regarding trunk cross sectional area were registered for 'GiSelA 6' rootstock with 'Lapins' cultivar (46.32 cm^2), followed by 'GiSelA 5' cultivar with 'Lapins' (35.59 cm^2) being in the same statistical class (Table 7).

Table 7. Vegetative parameters measured (2024) in the cherry plot

Cultivar/rootstock	2024		
	Shoot length	Height of trees (cm)	Trunk cross-sectional area TCSA (cm^2)
'Tamara' / Gi. 3	40.89[a]	247,6[a]	17,56[a]
'Tamara' / Gi. 5	43.53[a]	250,7[a]	19,89[a]
'Lapins' / Gi. 5	51.47[c]	320,9[b]	35.59[c]
'Lapins' / Gi. 6	60.14[c]	343,4[b]	46.32[c]
'Kordia' / Gi. 5	49.52[c]	272,2[a]	22,71[a]
'Kordia' / Gi. 3	44.02[c]	270,3[a]	32.10[b]

*Duncan's multiple ranges test. Mean values followed by the same letter within a column are not significantly different (P≤0.05)

Among the low vigor rootstock combinations grafted on 'GiSelA 3', 'Kordia' (32.10 cm^2) grew more vigorously when compared with 'Tamara' (17.56 cm^2). Results showed that the weakest growth was registered in 'Tamara'/ 'GiSelA 3' combination.

A certain tree die-back was observed as shown in Tables 8-10, sporadically. In 2022 a minor tree die-back was observed at 'Tamara' cultivar (Table 8) on 'GiSelA 3' and 'GiSelA 5'.

Regular conventional phytosanitary treatment was applied. Research data showed that no suckering was observed at the studied rootstocks (Tables 8-10).

Table 8. Tree die-back, suckering and canopy volume (2022) in the cherry plot

Cultivar/ rootstock	2022		
	Tree die-back %	Suckering (points)	Canopy volume (m^3)
'Tamara' / Gi. 3	7,14	0	0.30[a]
'Tamara' / Gi. 5	7,14	0	0.70[b]
'Lapins' / Gi. 5	0	0	0.75[a]
'Lapins' / Gi. 6	0	0	0.85[a]
'Kordia' / Gi. 5	7.14	0	0.95[a]
'Kordia' / Gi. 3	0	0	0.35[a]

*Duncan's multiple ranges test. Mean values followed by the same letter within a column are not significantly different (P≤0.05)

For 'Kordia' grafted on 'GiSelA 5' in 2023 the phenomena was repeated (Table 9).

Table 9. Tree die-back, suckering and canopy volume of tree crown (2023) in the cherry plot

Cultivar/rootstock	2023		
	Tree die-back %	Suckering (points)	Canopy volume (m^3)
'Tamara' / Gi. 3	14,28	0	1,51[a]
'Tamara' / Gi. 5	7,14	0	2,46[d]
'Lapins' / Gi. 5	0	0	1,91[c]
'Lapins' / Gi. 6	0	0	2.52[d]
'Kordia' / Gi. 5	0	0	2.02[c]
'Kordia' / Gi. 3	0	0	1.65[b]

*Duncan's multiple ranges test. Mean values followed by the same letter within a column are not significantly different (P≤0.05)

In 2024 just a single case of tree die-back was observed in 'Kordia'/'GiSelA 5' (Table 10).

Table 10. Tree die-back, suckering and canopy volume of tree crown (2024) in the cherry plot

Cultivar/ rootstock	2024		
	Tree die-back %	Suckering (points)	Canopy volume (m^3)
'Tamara' / Gi.3	0	0	1.78[a]
'Tamara' / Gi.5	0	0	2.62[cd]
'Lapins' / Gi.5	0	0	2.42[bc]
'Lapins' / Gi.6	0	0	2.72[d]
'Kordia' / Gi.5	7.14	0	1.72[b]
'Kordia' / Gi.3	0	0	1.82[a]

*Duncan's multiple ranges test. Mean values followed by the same letter within a column are not significantly different (P≤0.05)

Regarding canopy volume of tree crown, results showed that in 2022 the smallest values were achieved on 'GiSelA 3' rootstocks, on 'Tamara' and 'Kordia'. On 'Lapins' cultivar results showed values ranged 0.75-0.85 m^3 (Table 8). In 2023 canopy volume registered higher values in

'Lapins'/'GiSelA 6' and 'Tamara' on 'GiSelA5' being in the same statistical class followed by 'Lapins' and 'Kordia' both grafted on 'GiSelA 5' (Table 9). In 2024 canopy volume registered slightly higher values than in 2023 and differences between cultivar/rootstock combinations were more evident, highest values were registered again on 'Lapins' cultivar grafted on 'GiSelA6' cultivar followed by slight differrences of 'Tamara' and 'Lapins' on 'GiSelA 5'. 'Tamara' and 'Kordia' on 'GiSelA 3' had the lowest canopy volume.

Regarding the vegetative growth (Table 11) characterized by the indicator number of shoots showed that in 2022 the cultivar 'Lapins'/ 'GiSelA 6' gave the most intensive growth in terms of number of shoots followed by 'Tamara', 'Lapins', 'Kordia' grafted on 'GiSelA 5'.

In 2023 it was observed that 'Lapins' cultivar grafted on 'GiSelA 6' and '5' rootstocks had a higher number of shoots than the other combinations.

In terms of shoot growth in 2024 one can observe that 'Kordia' grafted on both rootstocks gave quite the same growth characteristics as 'Tamara' grafted on 'GiSelA 3', presenting the lowest values and conversely in the 'Tamara'/'GiSelA 5' rootstock combination the results showed higher values being close to 'Lapins'/'GiSelA 5' combination.

Results regarding leaf area (m^2) showed that in 2022 the most intensive leaf development was specific at 'Lapins' cultivar grafted on 'GiSelA 5' and 'GiSelA 6' rootstocks.

In 2022 leaf development ranged between 0.89-1.65 m^2 with significant differences between cultivars (Table 11).

Table 11. The number of shoots and leaf area parameters in the cherry plot during 2022

Cultivar/ rootstock	2022	
	Number of shoots (no)	Leaf area (m^2)
'Tamara' / Gi. 3	32.64[a]	0.89[a]
'Tamara' / Gi. 5	45.64[c]	1.11[b]
'Lapins' / Gi. 5	45.84[c]	1.35[d]
'Lapins' / Gi. 6	53.64[d]	1.65[e]
'Kordia' / Gi. 5	47.84[c]	1.28[c]
'Kordia' / Gi. 3	38.07[b]	1.15[bc]

*Duncan's multiple ranges test. Mean values followed by the same letter within a column are not significantly different (P≤0.05)

In 2023 leaf area surface indicator showed a higher development phase as trees developed in terms of canopy volume, registering a leaf development ranged between 1.74-2.52 m^2 (Table 12).

As in 2022, also in 2023 it was observed that the highest development was specific to 'Lapins' cultivar (2.22-2.52 m^2), followed by 'Kordia' cultivar on 'GiSelA 5' and 'GiSelA 3' (2.18-2.32 m^2) and finally, 'Tamara' cultivar (1.74-1.87 m^2).

Table 12. The number of shoots and leaf area parameters in the cherry plot during 2023

Cultivar/rootstock	2023	
	Number of shoots (no)	Leaf area (m^2)
'Tamara' / Gi. 3	35[a]	1.74[a]
'Tamara' / Gi. 5	44[b]	1.87[b]
'Lapins' / Gi. 5	50[c]	2.22[c]
'Lapins' / Gi. 6	58[d]	2.52[e]
'Kordia' / Gi. 5	40[ab]	2.32[d]
'Kordia' / Gi. 3	37[a]	2.18[c]

*Duncan's multiple ranges test. Mean values followed by the same letter within a column are not significantly different (P≤0.05)

Results showed the same tendency in 2024, but the registered values were even higher, ranging 2.42-2.90 m^2 (Table 13).

Table 13. The number of shoots and leaf area parameters in the cherry plot during 2024

Cultivar/rootstock	2024	
	Number of shoots (no)	Leaf area (m^2)
'Tamara' / Gi. 3	38[a]	2.42[a]
'Tamara' / Gi. 5	45[b]	2.76[cd]
'Lapins' / Gi. 5	55[c]	2.84[de]
'Lapins' / Gi. 6	64[d]	2.90[e]
'Kordia' / Gi. 5	41[a]	2.70[bc]
'Kordia' / Gi. 3	39[a]	2.64[b]

*Duncan's multiple ranges test. Mean values followed by the same letter within a column are not significantly different (P≤0.05)

Results regarding generative processes showed adequate tendencies of flowering, so the blooming started in 2021, one year after planting, but with few flowers, trees began flowering with abundance in 2022 (Table 14) and culminating in 2023, with a very good flower set, which continued in 2024 also.

Table 14. The generative buds evaluation and flowering abundance in the cherry plot (2022)

Cultivar/ rootstock	2022	
	Generative buds evaluation (%)	Flowering abundance (0-5 score scale[1])
'Tamara' / Gi. 3	48[a]	3.2[a]
'Tamara' / Gi. 5	63[b]	3.6 [a]
'Lapins' / Gi. 5	64[a]	4.2 [a]
'Lapins' / Gi. 6	69[a]	4.5 [a]
'Kordia' / Gi. 5	47[a]	3.7 [a]
'Kordia' / Gi. 3	55[a]	3.9 [a]

[1]0 - no flowers; 5 - abundant flowering

*Duncan's multiple ranges test. Mean values followed by the same letter within a column are not significantly different (P≤0.05)

Generative buds evaluation (Tables 14-16) showed that more than 61% of buds transformed to generative buds, this percent being even higher in 2023. Highest generative buds were formed in 2023 in 'Lapins'/'GiSelA 6' and 'Tamara'/'GiSelA 5' cultivars (Table 15).

Table 15. The generative buds evaluation and flowering abundance in the cherry plot (2023)

Cultivar / rootstock	2023	
	Generative buds evaluation (%)	Flowering abundance (0-5 score scale[1])
'Tamara' / Gi.3	54 [a]	3.6 [a]
'Tamara' / Gi.5	63 [a]	4.0 [a]
'Lapins' / Gi.5	61 [a]	4.3 [a]
'Lapins' / Gi.6	65 [a]	4.6 [a]
'Kordia' / Gi.5	48 [a]	3.1 [a]
'Kordia' / Gi.3	57 [a]	3.2 [a]

[1]0 - no flowers; 5 - abundant flowering
*Duncan's multiple ranges test. Mean values followed by the same letter within a column are not significantly different (P≤0.05)

Generative buds in 2024 showed the same tendency, ranged between 57-69 %.
Flowering tendency with highest scores were found in 'Lapins' cultivar on both rootstocks followed by 'Kordia' and 'Tamara' on 'GiSelA 5' rootstock (Table 16). Data showed that values of 4.7-4.9 on a scale with a maximum of 5.0 scoring the maximum points represent the highest rankings, thus adequate flowering process was registered. The other rootstock/ scion combinations behaved also appropriate, however the 'Lapins' cultivar being considered the most abundant variant regarding blooming.

Table 16. The generative buds evaluation and flowering abundance in the cherry plot (2024)

Cultivar / rootstock	2024	
	Generative buds evaluation (%)	Flowering abundance (0-5 score scale[1])
'Tamara' / Gi. 3	57 [a]	3.7 [a]
'Tamara' / Gi. 5	65 [a]	3.9 [a]
'Lapins' / Gi. 5	57 [a]	4.7 [a]
'Lapins' / Gi. 6	69 [a]	4.9 [a]
'Kordia' / Gi. 5	65 [a]	3.8 [a]
'Kordia' / Gi. 3	58 [a]	3.7 [a]

*Duncan's multiple ranges test. Mean values followed by the same letter within a column are not significantly different (P≤0.05)

Regarding blooming stages, we can conclude that in 2022 the pedicel elongation and the starting of flowering was in the 27.04-02.05 period, depending on the studied cultivars (Table 17). No differences in blooming were observed due the rootstocks.
In 2022 full flowering was observed in early May, 'Lapins' cultivar being earlier with a few days than the other two studied cultivars.

Table 17. Phenology of flowering sweet cherry cultivars on 'GiSelA' rootstocks '3', '5', '6' in 2022

Cultivar/ rootstock	2022		
	Starting of flowering	Full flowering	Flowers fading
'Tamara' /Gi. 3 'Tamara' / Gi. 5	27.04	05.05	10.05
Lapins' /Gi. 5 'Lapins' / Gi.6	02.05	03.05	15.05
'Kordia' /Gi.5 'Kordia' / Gi.6	29.04	04.05	11.05

In 2023 full flowering occurred earlier when compared with 2022 between 24.04-01.05.2023, with slight differences in blooming depending the cultivar (Table 18).

Table 18. Phenology of flowering sweet cherry cultivars on 'GiSelA' rootstocks '3', '5', '6' in 2023

Cultivar/ rootstock	2023		
	Starting of flowering	Full flowering	Flowers fading
'Tamara' /Gi. 3 'Tamara' / Gi. 5	22.04	27.04	02.05
'Lapins' /Gi. 5 'Lapins' / Gi. 6	20.04	24.04	30.04
'Kordia' /Gi. 5 'Kordia' / Gi. 6	27.04	01.05	07.05



Figure 1. Flowering phase in the research plot (2024)

In 2024 the start of flowering was observed at the end of April between 30.03-05.04, the full flowering occurred between 02-11 April (Table 18).

Table 19. Phenology of flowering sweet cherry cultivars on 'GiSelA' rootstocks '3', '5', '6' in 2024

Cultivar/ rootstock	2024		
	Starting of flowering	Full flowering	Flowers fading
'Tamara' /Gi. 3/5	02.04	11.04	20.04
'Lapins' /Gi. 5/6	30.03	02.04	15.04
'Kordia' /Gi. 5/6	05.04	10.04	22.04

Regarding the yield per tree, results showed that scion/rootstock combinations behaved differently.

In the experiment conducted on both rootstocks, the 'Tamara' cultivar demonstrated a similar yield (Table 20), ranging from 0.88-1.20 kg/tree in 2022 and 1.61-1.63 kg/tree in 2023.

Table 20. Yield (kg/tree) in the research plot

Cultivar/roots.	2022	2023	2024	Cumul.
'Tamara' /Gi. 3	0.9 [a]	1.7 [a]	8.8 [a]	11.3
'Tamara' / Gi. 5	1.2 [a]	1.6 [a]	7.3 [a]	10.1
'Lapins' / Gi. 5	0.9 [a]	3.7 [b]	9.3 [a]	13.4
'Lapins' / Gi. 6	1.4 [a]	4.9 [c]	10.2 [a]	16.5
'Kordia' / Gi. 5	0.4 [a]	0.7 [a]	7.6 [a]	13.4
'Kordia' / Gi. 3	1.2 [a]	0.9 [a]	8.0 [a]	10.4

In 2023, 'Kordia' and 'Tamara' cultivars provided quite similar values in terms of yield, being in the same statistical class, higher values were registered and 'Lapins'/'GiSelA 5' cultivar (3.7 kg/tree) and even higher values at 'Lapins'/'GiSelA 6' cultivar combination (4.9 kg/tree). In 2024 again the highest yield was recorded at 'Lapins'/'GiSelA 6' rootstock followed by 'Lapins'/'GiSelA 5' rootstock (9.3-10.2 kg/tree). There were no statistical differences between the means. Our findings indicate that the 'Lapins'/'GiSelA 6' rootstock yielded the highest cumulative yield followed by the cultivar 'Kordia' and 'Lapins' cultivars on the same 'GiSelA 5' rootstock.

When compared with 2024 a significant growth of yield was observed, thus was a 7.3-8.8 kg/tree production recorded.

In researches effectuated by Suran et al. (2022), differences in yield and yield efficiency were significant, the experimental plot being in the 4th year. In 'GiSelA 6', the yield was very low with 0.88 kg tree^{-1}. The highest yield was in 'GiSelA 3' and 'PiKu 1' with 5.17 and 4.05 kg tree^{-1}.

Being in the first years of development of trees, the mean fruit weight (Table 21) of 'Tamara' cultivar was found to have close values (14.57 g in 2022, 14.82 g in 2023, 13.70 g in 2024) between the two rootstocks, but on 'GiSelA 5' the fruits seemed to be larger, however the differences were small. Fruit size may vary depending on many factors, such as climate, high temperatures, rainfall or adverse negative effects (dry periods in summer).

Similar results of fruit parameters were obtained by researches in Czech Republic (Vavra et al., 2020), reporting fruit sizes of 29.5 mm, weight 12.4 g, SSC of 18.3°Brix, but in a slight colder climate with 8.1°C and an annual average rainfall of 655 mm. In our research the results obtained indicate higher values of average fruit weight and SSC values due to probable higher average annual temperatures and drip irrigated conditions.

The results presented in Table 21 shows that the soluble solids content of sweet cherries grown in 2022 ranged from 22.80-23.92°Brix.

Table 21. Average fruit weight and soluble solids content of fruit during 2022 in the cherry plot

Cultivar/ rootstock	2022	
	Average fruit weight (g)	Soluble solids (°Brix)
'Tamara'/ Gi. 3	14.47 [a]	22.80 [a]
'Tamara'/ Gi. 5	14.68 [a]	23.92 [a]
'Lapins' / Gi. 5	10.6 [a]	19.8 [a]
'Lapins' / Gi. 6	10.3 [a]	19.7 [a]
'Kordia' / Gi. 5	9.8 [a]	19.8 [a]
'Kordia' / Gi. 3	10.6 [a]	19.9[a]

*Duncan's multiple ranges test. Mean values followed by the same letter within a column are not significantly different (P≤0.05)

The soluble solids content in 2023 ranged from 23.75-24.30º Brix (Table 22).

Table 22. Average fruit weight and soluble solids content of fruit during 2023 in the cherry plot

Cultivar/ rootstock	2023	
	Average fruit weight (g)	Soluble solids (°Brix)
'Tamara'/ Gi. 3	14.78 [a]	23.75 [a]
'Tamara'/ Gi. 5	14.87 [a]	24.30 [a]
'Lapins' / Gi. 5	9.85 [a]	19.9 [a]
'Lapins' / Gi. 6	10.35 [a]	20.1 [a]
'Kordia' / Gi. 5	10.53 [a]	19.5 [a]
'Kordia' / Gi. 3	10.12 [a]	20.1 [a]

*Duncan's multiple ranges test. Mean values followed by the same letter within a column are not significantly different (P≤0.05)

In 2024 soluble solids presented a similar tendency but the registered values had a lower level. The 'GiSelA 3', 'GiSelA 5' and 'GiSelA 6' varieties showed little influence in their soluble solids content in the analysed period given the absence of significant differences between the values from each cultivar grafted on different rootstock (Table 23).

Table 23. Average fruit weight and soluble solids content during 2024 in the cherry plot

Cultivar/ rootstock	2024	
	Average fruit weight (g)	SSC content of fruits (°Brix)
'Tamara'/ Gi. 3	13.70 [a]	19.5 [a]
'Tamara'/ Gi. 5	13.34 [a]	20.5 [a]
'Lapins' / Gi. 5	10.53 [a]	20.16 [a]
'Lapins' / Gi. 6	9.63 [a]	19.9 [a]
'Kordia' / Gi. 5	10.53 [a]	22.1 [a]
'Kordia' / Gi. 3	10.21 [a]	21.6 [a]

*Duncan's multiple ranges test. Mean values followed by the same letter within a column are not significantly different (P≤0.05)

Observations showed that there were also higher values of individual fruits in Tamara measured above 13 g in our researches, showing the fact that there is an inner genetical capacity of the cultivar to bear higher weight fruits in optimal environmental and nutrition conditions.

According polish researches (Spadzik et al., 2022) in the climate conditions of Central Poland 'Tamara' fruits weighed 12 g and were around 30 mm in diameter.

It is important to note that the tasting characteristics of cherry fruits depend also on their soluble solids content but other characteristics too. According to research conducted in Nordic countries (Lanauskas, 2023) such as Lithuania and Norway, the acceptable threshold for SSC values ranges between 14.2 and 16°Brix. Our results corroborated with the results of other foreign and Romanian researchers (Balan et al., 2024) confirmed that the SSC values of the sweet cherries during our experiments were in the optimum range, indicating that they meet consumer demands

CONCLUSIONS

The growth vigor and generative characteristics of 'Lapins', 'Tamara' and 'Kordia' sweet cherry cultivar was evaluated in the present research, rafted on rootstocks 'GiSelA 3' and 'GiSelA 5' and 'GiSelA 6', general conclusions based on several biometrical characteristics showed different growth and bearing characteristics of scion rootstock-combinations. In terms of trunk cross-sectional area 'Lapins' cultivar grafted on both 'GiSelA 5' and 'GiSelA 6' cultivars grew the most intensively followed by 'Kordia' cultivar on 'GiSelA 5' and 'Tamara' cultivar on both 'GiSelA 3' and 'GiSelA 5' cultivars. Regarding the shoot length parameter generally conclusion is that 'Lapins' and 'Kordia' cultivars are in the same statistical class on the three rootstocks ('GiSelA 3', '5', '6'), 'Tamara' cultivar presented lower values on 'GiSelA 3' and '5'. The height of the trees indicator was similar for both rootstock systems ('GiSelA 5', '6') achieving good and optimum growing heights, specific for tall spindle axe training systems. A small tree die-back phenomenon was still observed in 2023-2024, ranging from 7-14% of trees, with no specific explanation, however due to natural causes. The canopy volume of trees showed significant differences between scion-rootstock combinations, the highest values being observed on 'GiSelA 6' and '5' rootstock systems. 'GiSelA 3' grafted trees ('Tamara' and 'Kordia') showed smaller values. The number of shoots indicator showed the same pattern, the highest number of shoots was recorded on 'Lapins' cultivar on both rootstocks, and in opposite way the lowest number was recorded on 'Tamara' and 'Kordia' on the 'GiSelA 3' rootstock.

Trees began flowering with abundance in 2022 and this tendency appeared to remain in all the other years with a very good flower set. In the experimental years no significant differences induced by the rootstock were observed until

now. Regarding the cumulative yield per tree indicator results showed that the highest values were obtained at 'Lapins' cultivar grafted on 'GiSelA 6' and 'GiSelA 5' with close cumulative values, followed by 'Kordia' cultivar grafted on 'GiSelA 5' cultivar. The average fruit weight was the highest in 'Tamara' cultivar with a good soluble solids content, with very good aroma and tasting qualities, probably due also to the complex genetics of the cultivar and also to soil-climate phenomena. Until now, a very good soil and climate adaptability was observed in the studied rootstock-scion combinations and no compatibility problems or other phyisiological problems were detected.

ACKNOWLEDGEMENTS

This research was funded with the support of the Ministry of Agriculture and Rural Development through Sectorial Project ADER 633/2023. We are grateful to Fruit Research & Development Station Bistrita, Romania, for the generous help and assistance.

REFERENCES

Aglar, E., & Yildiz, K. (2014). Influence of rootstocks ('GiSelA 5', 'GiSelA 6', MaxMa, SL 64 on performance of'0900 Ziraat'sweet cherry. *Journal of Basic & Applied Sciences*, *10*, 60.

Balan, V., S. Vămăsescu, Cornealiu Buză, Dumitru Talpalariu, Stanislav Russu (2024). The impact of the cultivation system on the yield and quality of sweet cherries. *Scientific papers. Series B. Horticulture*. *Vol. LXVIII*, No. 2, 24-34 2024.

Bhatt, M., Chanda, S.V. (2003). Prediction of leaf area in *Phaseolus vulgaris* by non-destructive method. *Bulgarian Journal of Plant Physiology*, 29, 96-100

Boskov, D., Milatovic, D.; Rakonjac, V.; Zec, G.; Hudina, M.; Veberic, R.; Mikulic-Petkovsek, M. (2023). The Phenolic Profile of Sweet Cherry Fruits Influenced by Cultivar/ Rootstock Combination. *Plants*, 12, 103. https://doi.org/10.3390/ plants12010103

Bujdosó, G. Hrotkó, K. (2012). Preliminary results on growth, yield and fruit size of some new precocious sweet cherry cultivars on Hungarian bred mahaleb rootstocks. *Acta Horticulturae*, 1058: 559-564.

Călinescu,M., Mazilu Ivona, Chițu E., Plăiașu F., Paraschiv, M. (2024). Biostimulators effect on growth effect on fruiting processes of two sweet cherry cultivars grafted on 'GiSelA 3' rootstock. *Fruit Growing Research*, vol. XXXX, 2024

Dziadek, K., Kope´c, A., Tabaszewska, M. (2019). Potential of sweet cherry (*Prunus avium* L.) by-products: Bioactive compounds and antioxidant activity of leaves and petioles. *Eur. Food Res. Technol*., 245, 763–772 (20).

FAOSTAT. Available online: https://www.fao.org/faostat/ en/#data/QCL (accessed on 13 March 2024).

Franken-Bembenek, S. (2010). GiSelA's, PIKUs und neue Giessener Klone: Ergebnisse aus europäischen und nordamerikanischen Kirschenunterlagenversuchen. Erwerbs-Obstbau, 52(1), 17–25.

Gjamovski, Viktor; Kiptijanovski, Marjan; and Arsov, Tosho (2016). Evaluation of some cherry varieties grafted on 'GiSelA 5' rootstock, *Turkish Journal of Agriculture and Forestry*: Vol. 40: No. 5, Article 9. https://doi.org/10.3906/tar-1601-80

Gyeviki,M., Bujdoso,G., Hrotko, K. (2008). Results of cherry rootstock evaluations in Hungary. *International Journal of Horticultural Science*, 14(4):11–14

Gutierrez, T.; Lavin, A. (2000). Linear measurements for non destructive estimation of leaf area in 'Chardonnay' vines. *Agricultura Técnica*, 60(1), 69–73.

Hrotkó, K., Magyar L., Hoffmann, S. & Gyeviki, M. (2009). Rootstock evaluation in intensive sweet cherry (*Prunus avium* L.) orchard. *International Journal of Horticultural Science*, 15(3): 7–12.

Jakab-Ilyefalvi Z., Chiorean A.M., Vlaşin L.D., Moldovan C., Guzu G.M., Roşu-Mareş S.D. (2024a). Research on Early Identification of Nutritional Status in High Density Fertigated Cherry Orchards through Ion Selective Electrode Determination Validated by Foliar Diagnosis. *Fruit Growing Research*, Vol. XXXX, 129–137

Jakab-Ilyefalvi, Z., Chiorean, A. (2022a). Preliminary results on early crop load and growth responses of 'Lapins' sweet cherry cultivar (*Prunus avium* L.) grafted on 'GiSelA 5'and 'GiSelA 6' rootstock in drip irrigated field trial. *Scientific Papers. Series B, Horticulture*, Vol. LXVI, Issue 1, USAMV of Bucharest, Print ISSN 2285-5653, 121-126

Lanauskas, J., Kviklys, D., Uselis, N., Stanys, V. (2023). Performance of Sweet Cherry Cultivars and Advanced Selections on 'GiSelA 5' Rootstock in Young Orchards. *Plants*, 12, 614. https://doi.org/ 10.3390/plants12030614

Martins, V., Silva, V., Pereira, S., Afonso, S., Oliveira, I., Santos, M., Ribeiro, C., Vilela, A., Bacelar, E., Silva, A. P., & Gonçalves, B. (2021). Rootstock Affects the Fruit Quality of 'Early Bigi' Sweet Cherries. *Foods*, *10*(10), 2317. https://doi.org/10.3390/foods10102317

Martini, S.; Conte, A.; Tagliazucchi D. (2017). Phenolic compounds profile and antioxidant properties of six sweet cherry (*Prunus avium*) cultivars. *Food Res. Int.*, 97, 15–26 (26).

Milic, B., Kalajdzic, J., Keserovic, Z., Magazin, N., Ognjanov, V., Miodragovic, M., & Popara. (2019). Early performance of four sweet cherry cultivars grafted on 'GiSelA 5' and Colt rootstocks in a high density growing system. Acta Scientiarum Polonorum. *Hortorum Cultus*, 18(1).

Nawirska-Olszanska, A.; Kolniak-Ostek, J.; Oziembłowski, M.; Ticha, A.; Hyšpler, R.; Zadak, Z.; Židová, P.; Paprstein, F. (2017). Comparison of old cherry cultivars grown in Czech Republic by chemical composition and bioactive compounds. *Food Chem*., 228, 136–142 (4).

Roversi, A., Ughini V. (1996). Influence of weather conditions of the flowering period on sweet cherry fruit set. *Acta Hortic*, 410: 427–433.

Radek Vavra, (2020). Sweet cherry fruit characteristic in covered orchards, Slovaka Universitas Agriculturaw Nitrae, pp. 5–7, doi:10.2478.

Suran P., K. Scháňková and L. Zeleny (2022). Sweet cherry rootstocks in high density orchards without irrigation in Central Europe, *Acta Hortic*. 1346. ISHS 2022. DOI 10.17660/ActaHortic.2022.1346.86 Proc. XII IS on Integ. Canopy, Rootstock and Environ. Physiol. in Orchard Sys.

Sitarek, M., and Bartosiewicz, B. (2012). Influence of five clonal rootstocks on the growth, productivity and fruit quality of 'Sylvia' and 'Karina' sweet cherry trees. J. Fruit Ornam. Plant Res., *20*(*2*), 5–10 https://doi.org/10.2478/v10290-012-0010-z.

Sjaak Walraven (2024). Weigi®, dwarfing and semi-dwarfing rootstocks for cherry trees.

https://cherrytimes.it/en/news/Weigi-dwarfing-and-semi-dwarfing-rootstocks-for-cherry-trees

Szpadzik E, Krupa T, Molska-Kawulok K, Przybyłko S. (2022). Fruit Quality and Contents of Some Bioactive Compounds in Selected Czech Sweet Cherry (*Prunus avium* L.) Cultivars under Conditions of Central Poland. *Agriculture*. 2022; 12(11): 1859. https://doi.org/10.3390/agriculture12111859

Whiting, M.D., Lang, G., Ophardt, D. (2005). Rootstock and training system affect sweet cherry growth, yield and fruit quality. *Hort Science*, 40, 582–586 (30).

Zhao, D., Yuan, J., Xu., K., Cheng, C., Li, H. (2016). Selection of morphological, physiological and biochemical indices: evaluating dwarfing apple interstocks in cold climate zones. *New Zealand Journal of Crop and Horticultural Science*, 44(4): 291–311.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

POMOLOGICAL AND CHEMICAL CHARACTERIZATION OF SOME SWEET CHERRY VARIETIES GROWN IN URBAN ORCHARD

Lehel LUKACS[1], Gabriela Maria ROMAN[2], Ioana MOLDOVAN[2], Sándor RÓZSA[1], Tincuța-Marta GOCAN[1], Orsolya BORSAI[1]

[1]University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, 3-5 Calea Mănăştur Street, Cluj-Napoca, Romania
[2]Horticultural Research Station Cluj-Napoca, 5 Horticultorilor Street, Cluj-Napoca, Romania

Corresponding author email: orsolya.borsai@usamvcluj.ro

Abstract

The increasing interest in urban agriculture highlights the need for a comprehensive understanding of fruit quality parameters in urban orchards. This study aimed to investigate the pomological and chemical characteristics of four sweet cherry (Prunus avium L.) varieties, namely, 'Napoleon', 'Van', 'Boambe de Cotnari' and 'Stella' cultivated in an urban orchard. The investigated varieties were evaluated for key pomological traits, including fruit weight, size, firmness, and skin colour, as well as chemical attributes such as soluble solids content (°Brix), titratable acidity, pH, vitamin C, total phenolic compounds and total flavonoids. The results revealed significant varietal differences in both physical and chemical properties, with some varieties demonstrating exceptional balance between sweetness and acidity, making them highly suitable for fresh consumption. Furthermore, the urban growing environment was found to influence certain quality parameters, potentially due to microclimatic conditions. This research underscores the potential of urban orchards to produce high-quality sweet cherries while contributing to local food systems. Insights from this study may aid in the selection of cherry varieties best suited for urban cultivation, enhancing productivity and fruit quality.

Key words*: yield, fruit quality, Prunus avium, TSS, TA, phenolic content, vitamin C.*

INTRODUCTION

Sweet cherry (*Prunus avium* L.) is a highly valued fruit species cultivated for its exceptional taste, attractive appearance, and notable nutritional properties (Correia et al., 2017). As consumer demand increasingly shifts toward fresh, health-promoting products, the interest in sweet cherry has expanded beyond commercial orchards to include urban and peri-urban agricultural systems. Urban orchards, though limited in scale, offer unique microclimatic conditions and opportunities for sustainable fruit production close to consumer markets, contributing to food security, biodiversity, and environmental quality in urban landscapes (Lin et al., 2015; Ghahremani et al., 2024). In urban environments, the cultivation of fruit trees like sweet cherry presents both challenges and benefits. While urban heat islands, air pollution, and soil variability can influence crop performance, urban orchards often benefit from favorable microclimates, reduced pest pressure, and proximity to markets (Eeraerts et al., 2017). For successful cultivation, careful selection of cultivars is essential, especially those that exhibit both agronomic adaptability and desirable fruit quality under urban conditions. Evaluating the pomological traits, such as fruit size, firmness, total soluble solids, and acidity is essential for understanding fruit quality, market value, and consumer preference (Kappel et al., 1996; Boghean et al., 2025). Beyond appearance and taste, consumers and producers are increasingly interested in the functional quality of fruits, particularly their content of bioactive compounds like vitamin C, total phenolics, and flavonoids, which contribute to antioxidant activity and confer various health benefits (Sun-Waterhouse, 2011). These compounds play a vital role in neutralizing free radicals and are linked to reduced risks of chronic diseases such as cardiovascular conditions and certain cancers (Heim et al., 2002; Wu et al., 2004; Fatih et al., 2014). In sweet cherries, phenolic compounds are especially important contributors to antioxidant capacity, with flavonoids and anthocyanins providing not only nutritional benefits but also contributing to fruit colour and shelf-life (Moutinho-Pereira et al., 2007; Muskovics et al., 2006). These biochemical

markers are strongly associated with health-promoting effects and are increasingly used to guide breeding programs and orchard management strategies (Gonçalves et al., 2024). Despite the growing interest in urban horticulture, data on the performance of sweet cherry cultivars under urban orchard conditions remain limited. Cultivar-specific responses to urban microenvironments, such as heat islands, soil heterogeneity, and localized air pollution which may lead to variability in fruit development, chemical composition, and overall fruit quality (Asănică et al., 2013; Orsini et al., 2013). Understanding these differences is essential for optimizing cultivar selection and orchard management practices adapted to urban environments. In this context, it has never been more important to provide a comprehensive evaluation of both pomological and chemical attributes to identify cultivars best suited for urban fruit production. Therefore, the current research aims to characterize and compare the pomological and chemical traits of four sweet cherry varieties, namely 'Napoleon', 'Van', 'Boambe de Cotnari', and 'Stella'grown in an urban orchard. Through detailed analysis of fruit morphology, taste-related parameters, and antioxidant profiles, this research seeks to provide a foundation for variety selection and quality optimization in urban horticulture systems.

MATERIALS AND METHODS

Site location and plant material

The current research has been carried out during 2023-2024 in a private high-density urban orchard located in Cluj-Napoca (46°48'05.1"N; 23°37'32.4"E) at 430 m above sea level on the hill of Sfântu Gheorghe. Cluj-Napoca is known for its continental climate (Kottek et al., 2006) characterised by cold winter and hot summers with an annual average rainfall ranging from 600 to 700 mm and an annual average temperature of 8.9°C reflecting the city's humid continental climate (Climate-data, 2025). The sweet cherry trees were planted in 1997 at a distance of 5 m between the rows and 4 m between the trees within a row. The trees were grafted onto the same rootstock (Mahaleb) and the canopies were trained to the standard open vase grown under standard conditions of irrigation, fertilization, and pest and disease management practices (Table 1).

Table 1. Fertilization, pest and disease management practices applied in the orchard

No	Agricultural practice	Moment of application
1	Spraying with Funguran	dormant season
2	Spraying with Chorus and Optil	pink bud
3	Luna Care, Karate Zeon and urea	petal fall
4	Folicur Solo, Krima and $Ca(NO_3)_2$	beginning of ripening
5	Folicur Solo, Fastac and Optical	2 weeks before harvest

Fruits from four sweet cherry (*Prunus avium*) cultivars ('Napoleon', 'Van', 'Boambe de Cotnari' and 'Stella') were harvested at the commercial ripening stage (Figure 1).

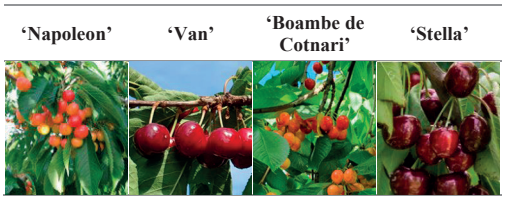


Figure 1. Sweet cherry varieties before harvest

Ten trees were randomly selected from each cultivar and 60 fruits/plant from different parts of the canopy were harvested and transferred immediately to the laboratory to determine the physical (fruit colour, width, thickness, length, weight and firmness) and chemical fruit quality parameters such as total soluble solid content (SSC), titratable acidity (TA), SSC/TA, pH, Vitamin C, total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity (DPPH). In addition, the physical traits of the stone and fruit peduncle were also determined followed by total yield/tree which was monitored at every harvest and summarized at the end of the harvesting period.

Determination of fruit colour

The chromatic characteristics of the harvested cherry fruits were analysed by the Colour Picker Software (Version 1.5.0) by using the CIE colour space as described by Acero et al., 2019. The CIE colour space is defined by L*, a* and b* coordinates which indicate lightness (L*)-black/white coordinate; a*-red/green coordinate, b*- yellow/blue coordinate (Gonçalves et al., 2007). Hue angle, which represents the dominant colour as perceived by

an observer, was calculated as arctan (b*/a*). Chroma was obtained following the formulae: $\sqrt{a^{*2} + b^{*2}}$ (Jha, 2010). Each measurement is the average of two measurements performed on opposite points along the equatorial diameter of each cherry.

Physical traits of fruits, stone and peduncle

Fruit weight, length, width and thickness were determined with a digital balance and a digital caliper. Fruit firmness was determined by a hand-held penetrometer (Sekse & Wermund, 2008). Stone weight was determined after the mechanical extraction and cleaning of the stone. During data processing the stone/flesh ration was calculated and expressed as percentage. Peduncle length and weight were measured individually after detaching from the fruit using a digital balance and a ruler.

Chemical characteristics of sweet cherries

The soluble solid content was individually determined from each fruit. The fruits were squeezed and the fresh juice was used to read the SSC content on a digital Atago PAL-BX ACID refractometer (Tokyo, Japan) and expressed as °Brix (Esmaeili et al., 2019).

Titratable acidity was determined by titrating 10 mL of cherry juice diluted with 40 mL of distilled water against 0.1 N NaOH using phenolphthalein as an indicator. Results were expressed as a percentage of malic acid equivalents as described by Usenik et al., (2008). After data collection the SCC/TA ratio was calculated to provide a comprehensive measure of fruit quality (Wu et al., 2020).

Active acidity of the fruits was expressed as pH of each sample and measured using a WTW 3310 pH meter as described by Henríquez et al., (2011).

The Vitamin C content of the fruits was ascertained using the 2,6-dichlorophenolindophenol (DCPIP) titrimetric method (AOAC, 2000). A known volume of juice was titrated against a standardized DCPIP solution until a persistent pink endpoint was observed. Results were calculated based on a calibration curve using ascorbic acid standards and expressed as mg ascorbic acid per 100 g fresh weight (FW).

The total phenolic content of the fruits was determined according to the Folin- Ciocalteu procedure as described by Singleton & Rossi (1965). An aliquot of fruit extract (0.5 mL) was mixed with 2.5 mL of 10% Folin-Ciocalteu reagent and 2 mL of 7.5% sodium carbonate. The mixture was incubated in the dark at room temperature for 30 minutes, and absorbance was measured at 765 nm using a UV–VIS spectrophotometer. Results were expressed as mg gallic acid equivalents (GAE) per 100 g FW.

The total flavonoid content was quantified by aluminium trichloride method using catechin as reference compound as previously described by Zhishen et al. (1999). The methanolic extracts were diluted with distilled water up to 5 ml, adding 300 µl of 5% $NaNO_2$ solution. After 5 minutes, the mixture was treated with 300 µl 10% $AlCl_3$ solution and kept in the dark for another 5 minutes, and then 2 ml of 1N NaOH solution and 6.4 ml of distilled water were added. After homogenization and 5 minutes of incubation the absorbance was read at 510 nm using a UV-VIS 1700 Shimadzu spectrophotometer, the total flavonoid content was calculated as quercetin equivalent from the calibration curve of quercetin standard solutions and expressed as milligrams of quercetin per 100 g of fresh weight.

The potential antioxidant activity of the sweet cherry varieties was determined based on the scavenging activity of the 70% aqueous methanol sweet cherry extracts of the stable 1,1`-diphenyl-2-picrylhydrazyl (DPPH) free radicals (Gonçalves, 2016). Over 90 µL of methanol extract 10 µl of distilled water and 3.9 ml of DPPH solutions were added. After 30 minutes of incubation in the dark, the absorbance was read at 515 nm using a UV-VIS spectrophotometer. DPPH-radical scavenging activity was expressed as the percentage inhibition activity and was calculated from [(A0–A1)/A0] x 100, where A0 is the absorbance of the control, and A1 is the absorbance of the extract assuming that the sample with the higher percentage has higher scavenging capacity (Kumaran & Karunakaran, 2007). All measurements were done in triplicate. The results were expressed as means of the triplicates.

Statistical analyses

Statistical significance was assessed by the analysis of variance followed by the comparison of means by Tukey's multiple range test a at $p<0.05$ using the statistical package SPSS

Version 20 (SPSS Inc., Chicago, IL, USA). Furthermore, Pearson's correlation was performed to reveal the relationships between chemical compounds and DPPH radical scavenging activity in the four sweet cherry cultivars.

RESULTS AND DISCUSSIONS

The results of this research highlight the varietal differences regarding the analysed physical and chemical traits of the sweet cherry varieties analysed.

Fruit colour

The chromatic parameters of sweet cherry fruits are presented in Table 2. The results indicate 'Napoleon' and 'Boambe de Cotnari' are significantly lighter and more colorful, while 'Van' and 'Stella' are darker and less saturated. These visual differences are also confirmed by significant differences found in all parameters between some cherry varieties. It is noticeable that in terms of lightness (L*), yellow/blue coordinate (b*), hue angle and chroma 'Napoleon' and 'Boambe de Cotnari' varieties are not significantly different (Table 2 and Figure 2). 'Napoleon' is a light-colored fruit (L* = 65.1) with high chroma (50.3) exhibiting a vibrant and bright skin colour. Moderate redness (a*) and yellowness (b*) indicate a strong red-yellow tone, while the low hue angle (0.7°) shows that the fruit is skewed strongly toward red. Significantly brighter and more colorful than 'Van' and 'Stella'. 'Boambe de Cotnari' has a very similar lightness (L* = 64.9) to 'Napoleon', but with the highest chroma (54.9) making it the most intense in color. High a* and b* values suggest a bright red-yellow tone. Low hue angle (0.8°) confirms strong red direction.

Table 2. Chromatic characteristics of the analysed sweet cherry varieties

	Colour coordinates				
	L*	a*	b*	Hue angle (h°)	Chroma (C*)
'Napoleon'	65.1 ± 5.2 b	31.8 ± 5.9 a	39.0 ± 4.3 c	0.7 ± 0.1 a	50.3 ± 3.2 b
'Van'	43.4 ± 2.6 a	31.7 ± 3.2 a	4.4.0 ± 3.8 b	1.4 ± 0.1 b	32.0 ± 4.1 a
'Boambe de Cotnari'	64.9 ± 5.2 b	39 ± 6.7 b	38.6.0 ± 2.1 c	0.8 ± 0.1 a	54.9 ± 3.4 b
'Stella'	41.1 ± 4.0 a	30.5 ± 2.5 a	-7.1.0 ± 2.7 a	1.8 ± 0.1 c	31.3 ± 2.2 a

The results are expressed as meand±standard error (n=40). Different lowercase letters indicate statistically significant differences between varieties for each variable according to Tukey's HSD test, $p<0.05$.

The darkest fruit (L* = 41.1) with the lowest b* (–7.1) appeared to be 'Stella'. The highest hue angle (1.8°) reflects this cooler tone and is confirmed by the low value chroma (31.3) which denotes a duller and cooler fruit colour as compared to other varieties. Visually, 'Stella' appears dark red with a hint of purple/blue. However, data are limited on chromatic characteristics of sweet cherries, Acero et al., (2019) in a previous study reported similar chromatic coordinates for 'Van' variety.

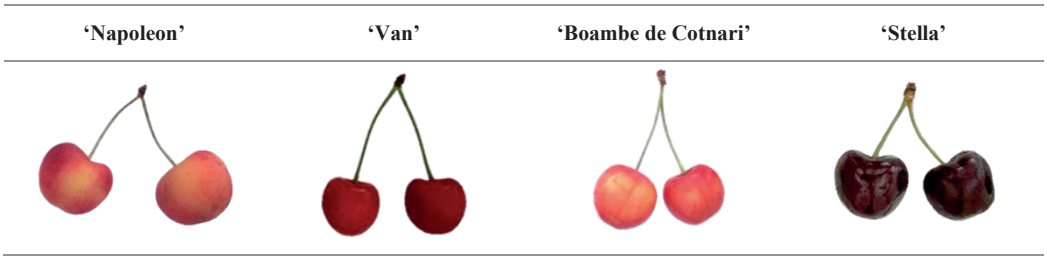


Figure 2. Fruit skin colour of the investigated sweet cherry varieties

Physical traits of fruits, stone and peduncle

The physical characteristics of four sweet cherry (*Prunus avium* L.) varieties, namely 'Napoleon', 'Van', 'Boambe de Cotnari', and 'Stella' are summarized in Table 3. Significant varietal differences at $p < 0.05$ were observed in fruit

size, weight, and yield per tree. The results show that 'Stella' had the largest fruit dimensions, with a mean width of 26.68 ± 0.21 mm, thickness of 23.00 ± 0.19 mm, and length of 24.96 ± 0.24 mm. These traits corresponded to the highest average fruit weight (10.34 ± 0.22 g), indicating this variety's potential for fresh market appeal due to its superior fruit size, a key consumer preference criterion (Habib et al., 2017; Romano et al., 2006). In comparison, Kappel et al. (2011) reported average fruit weights of 8.4 g for 'Bing' and 9.3 g for 'Lapins', placing 'Stella' above these commonly cultivated varieties. Other varieties such as 'Napoleon', 'Van', and 'Boambe de Cotnari' displayed similar values for fruit width and thickness, with no statistically significant differences. However, 'Boambe de Cotnari' and 'Van' showed slightly greater fruit length compared to 'Napoleon'. Fruit weight among these three varieties ranged from 7.76 g ('Van') to 8.22 g ('Napoleon'), all significantly lower than that of 'Stella'. Slightly higher values (8.25-8.60 g) for fruit weight for 'Van' variety has been reported by Sirbu et al. (2018) claiming that the position of fruits within trees' architecture may influence differently the fruit size and biochemical content of the fruits. In this regard, it was observed that fruit size was greater in the lower part of the tree crown. 'Napoleon', 'Van', and 'Boambe de Cotnari' exhibited comparable fruit widths (24.31-24.75 mm) and thicknesses (21.46-22.07 mm), with no statistically significant differences. However, 'Boambe de Cotnari' and 'Van' had slightly greater fruit lengths than 'Napoleon'. Fruit weight among these three cultivars ranged from 7.76 g ('Van') to 8.22 g ('Napoleon'), aligning closely with values reported by Giménez et al. (2014) and Girard et al. (1988) for similar mid-season cultivars in Mediterranean regions.

Table 3. Physical fruit traits and productivity of the selected sweet cherry varieties

Variety name	Fruit width (mm)	Fruit thickness (mm)	Fruit length (mm)	Fruit weight (g)	Fruit firmness (kgf/cm^2)	Yield/tree (kg)
'Napoleon'	24.75±0.48 *a*	21.46±0.35 *a*	20.23±0.32 *a*	8.22±0.36 *a*	0.30±0.03 *c*	20.38±0.52 *a*
'Van'	24.31±0.28 *a*	21.76±0.21 *a*	21.03±0.28 *ab*	7.76±0.18 *a*	0.24±0.02 *b*	24.25±0.56 *b*
'Boambe de Cotnari'	24.38±0.28 *a*	22.07±0.21 *ab*	21.19±0.22 *b*	7.92±0.23 *a*	0.24±0.01 *b*	28.16±0.28 *c*
'Stella'	26.68±0.21 *b*	23.00±0.19 *b*	24.96±0.24 *c*	10.34±0.22 *b*	0.23±0.03 *a*	21.26±0.20 *a*

The results are expressed as meand±standard error (n=40). Different lowercase letters indicate statistically significant differences between varieties for each variable according to Tukey's HSD test, $p<0.05$.

Fruit firmness varied slightly among the four sweet cherry cultivars analyzed. 'Napoleon' exhibited the highest firmness value at 0.30 N, followed by 'Van' and 'Boambe de Cotnari' at 0.24 N, while 'Stella' showed the lowest firmness at 0.23 N. These differences, although modest, may have implications for postharvest handling and consumer preference. Firmer fruits like 'Napoleon' are generally more resistant to mechanical damage during harvesting and transportation, which can extend shelf life and reduce losses (Einhorn et al., 2013; Hampson et al., 2014). Conversely, the slightly softer texture of 'Stella' might be attractive to consumers who prefer a more tender bite but could be more susceptible to bruising and faster deterioration (Crisosto et al., 1995). Fruit firmness is influenced by several factors, including cell wall composition, water content, and cultivar genetics. The higher firmness observed in 'Napoleon' may be associated with greater cell wall integrity or lower enzymatic activity related to softening, as suggested by previous research on sweet cherries (Muskovics et al., 2006).

Considering the productivity of the trees, the results showed that out of the four investigated sweet cherry varieties, 'Boambe de Cotnari' achieved the highest yield per tree (28.16 ± 0.28 kg), significantly overperforming the other cultivars. This trait makes it a compelling candidate for high-output commercial orchards. In comparison, 'Van' yielded 24.25 ± 0.56 kg, while 'Napoleon' and 'Stella' had lower productivity at 20.38 ± 0.52 kg and 21.26 ± 0.20 kg, respectively. Usenik et al. (2011) similarly reported that high-yielding cultivars like

'Kordia' and 'Regina' often exhibit moderate fruit size but excellent productivity, being consistent with the results obtained in 'Boambe de Cotnari'. These results highlight a typical negative correlation between fruit size and yield-where cultivars with larger fruits, like 'Stella', may have reduced overall productivity (Gonçalves et al., 2006).

Stone and peduncle characteristics are critical traits in the evaluation of sweet cherry varieties, as they directly influence both consumer acceptance and postharvest handling performance. Stone weight and the stone-to-flesh ratio affect the edible portion of the fruit, which is a key quality attribute for fresh consumption and processing industries (Kappel & MacDonald, 2007). Lower stone-to-flesh ratios are generally preferred, as they offer a greater proportion of consumable pulp relative to inedible seed. Peduncle attributes, especially length and weight, play an important role in fruit detachment resistance, shelf life, and visual appeal. Longer and firmer peduncles may enhance the resistance of cherries to mechanical injury and dehydration during storage and transport, contributing to better fruit quality and marketability (Demir & Kalyoncu, 2003). In this regard, the results revealed significant differences among the four cultivars at $p < 0.05$ (Table 4).

Table 4. Physical traits of stone and peduncle of the selected sweet cherry varieties

Variety name	Stone weight (g)	Stone-flesh rate (%)	Peduncle length (mm)	Peduncle weight (g)
'Napoleon'	0.48±0.01 *b*	6.05±0.19 *a*	3.38±0.12 *b*	0.04±0.00 *a*
'Van'	0.45±0.01 *a*	5.79±0.16 *a*	2.88±0.06 *a*	0.07±0.00 *b*
'Boambe de Cotnari'	0.52±0.01 *c*	6.69±0.15 *b*	3.88±0.09 *c*	0.07±0.00 *b*
'Stella'	0.57±0.01 *d*	5.52±0.09 *a*	4.28±0.07 *d*	0.10±0.00 *c*

The results are expressed as meand±standard error (n=40). Different lowercase letters indicate statistically significant differences between varieties for each variable according to Tukey's HSD test, $p<0.05$.

'Stella' exhibited the highest stone weight (0.57 ± 0.01 g), followed by 'Boambe de Cotnari' (0.52 ± 0.01 g), while 'Van' had the lowest (0.45 ± 0.01 g). However, the stone-flesh ratio, an important parameter for consumer appeal and fruit processing, was highest in 'Boambe de Cotnari' ($6.69 \pm 0.15\%$), suggesting a relatively larger proportion of stone to edible flesh. In contrast, 'Stella' had the lowest stone-flesh ratio ($5.52 \pm 0.09\%$), making it potentially more desirable for fresh consumption, as consumers typically prefer fruits with more flesh and smaller stones (Kappel & MacDonald, 2007).

Peduncle characteristics are important indicators of harvestability and market preference. 'Stella' displayed the longest peduncle (4.28 ± 0.07 mm) and the heaviest (0.10 ± 0.00 g), which may benefit mechanical harvesting and reduce fruit detachment during handling and transport. Conversely, 'Van' had the shortest and lightest peduncles (2.88 ± 0.06 mm; 0.07 ± 0.00 g), which could increase the likelihood of fruit drop and mechanical damage.

Longer peduncles are also associated with improved postharvest shelf life due to reduced risk of stem detachment and dehydration (Demir & Kalyoncu, 2003). These finding are in accordance with previous studies that emphasize the role of varietal differences in determining both consumer preference and postharvest behavior (Crisosto et al., 1999).

While 'Stella' offers favorable traits in terms of low stone-flesh ratio and longer peduncles, 'Boambe de Cotnari' combines a larger stone with moderate peduncle traits, potentially making it more suited to processing than fresh markets.

Chemical characteristics of sweet cherries

The results of the biochemical analyses showed significant variations in soluble solids content (SSC), titratable acidity (TA), SSC/TA ratio, and pH (Table 5). The SSC values ranged from 15.74 ± 0.29 °Brix in 'Boambe de Cotnari' to 18.73 ± 0.40 °Brix in 'Stella', indicating significant differences among cultivars ($p < 0.05$). 'Stella' exhibited the highest sugar concentration, consistent with findings in previous studies where higher SSC is often associated with enhanced consumer preference due to perceived sweetness (Muskovics et al., 2006; Serrano et al., 2005).

Table 5. Chemical characteristics of the sweet cherry varieties under study

Variety name	SSC (°Brix)	Titratable acidity (mg malic acid 100^{-1} mL)	TSS/TA	pH
'Napoleon'	18.12 ± 0.51 *b*	1.12 ± 0.01 *a*	16.11 ± 0.31 *c*	4.02 ± 0.01 *b*
'Van'	17.85 ± 0.39 *b*	1.35 ± 0.01 *b*	13.20 ± 0.11 *b*	4.11 ± 0.01 *c*
'Boambe de Cotnari'	15.74 ± 0.29 *a*	1.50 ± 0.01 *c*	10.47 ± 0.15 *a*	4.16 ± 0.02 *c*
'Stella'	18.73 ± 0.40 *c*	1.07 ± 0.01 *a*	17.49 ± 0.21 *d*	3.70 ± 0.01 *a*

The results are expressed as meand±standard error (n=40). Different lowercase letters indicate statistically significant differences between varieties for each variable according to Tukey's HSD test, p<0.05.

TA values were lowest in 'Stella' (1.07 ± 0.01 mg malic acid/100 mL) and 'Napoleon' (1.12 ± 0.01 mg), while 'Boambe de Cotnari' had the highest acidity (1.50 ± 0.01 mg). Similar values have been reported by Skrzyński et al., (2016) for 'Napoleon' when analysing the chemical attributes of various sweet cherry cultivars. The differences in acidity may influence taste perception and shelf life, with higher TA potentially conferring greater microbial stability but less palatability (Crisosto et al., 2003).

SSC/TA ratio, a key indicator of flavor balance, varied significantly among the cultivars. 'Stella' recorded the highest ratio (17.49 ± 0.21), indicating a sweeter and more balanced taste, while 'Boambe de Cotnari' showed the lowest (10.47 ± 0.15), suggesting a more acidic flavor profile. The SSC/TA ratio is frequently used to assess fruit maturity and acceptability, with higher values generally favored by consumers (Kader, 2008). The pH ranged from 3.70 in 'Stella' to 4.16 in 'Boambe de Cotnari'. Interestingly, despite having the highest acidity, 'Boambe de Cotnari' also had the highest pH, potentially due to buffering compounds present in the juice matrix. This suggests that pH alone may not be a sufficient predictor of perceived acidity, aligning with prior findings (Mattheis & Fellman, 1999).

Assessing vitamin C levels among the selected sweet cherry varieties provide valuable insights into their potential health benefits and commercial appeal. The results indicate that 'Van' had the highest vitamin C content (9.52 ± 0.02 mg/100 g FW), while 'Stella' had the lowest (8.10 ± 0.03 mg/100 g FW) as shown in Table 6).

Table 6. Bio-chemical compounds and antioxidant activity of the investigated sweet cherry varieties

Variety name	Vitamin C (mg/100 g FW)	TPC (mg GAE/100 g FW)	TFC (mg QE/100 g FW)	DPPH (%)
'Napoleon'	8.51 ± 0.01 *b*	103.21 ±2.51 a	22.79 ± 0.99 b	25.1 ± 0.10 a
'Van'	9.52 ± 0.02 *d*	356.26 ±4.39 b	53.11 ± 2.36 d	45.8 ± 0.73 b
'Boambe de Cotnari'	8.93 ± 0.01 *c*	98.33 ± 2.29 a	9.92 ± 0.78 a	23.9 ± 0.12 a
'Stella'	8.10 ± 0.03 *a*	398.66 ± 3.19 b	40.22 ± 1.88 c	53.3 ± 0.16 c

The results are expressed as meand±standard error (n=40). Different lowercase letters indicate statistically significant differences between varieties for each variable according to Tukey's HSD test, p<0.05.

These values are within the typical range reported for sweet cherries (Muskovics et al., 2006). Vitamin C, an essential water-soluble antioxidant, contributes to the nutritional value of cherries and enhances their oxidative stress resistance (Wu et al., 2004). The differences observed may be attributed to genetic variability among cultivars as well as environmental and agronomic factors (Fenech et al., 2019).

Phenolic compounds are crucial contributors to the antioxidant properties of fruits and have been linked to various health benefits, including anti-inflammatory and anticancer effects (Fonseca et al., 2021; Paskucza et al., 2021; Cuevas-Cianca et al., 2023).

The highest TPC was recorded in 'Stella' (398.66 ± 3.19 mg GAE/100 g FW) and 'Van' (356.26 ± 4.39 mg), while both 'Napoleon' and

'Boambe de Cotnari' exhibited significantly lower levels (103.21 ± 2.51 and 98.33 ± 2.29 mg, respectively) as presented in Table 6. These results are compatible with those obtained by Corneanu et al. (2021) who found very similar TPC in 'Van' (323.56 mg GAE/100 mL), 'Boambe de Cotnari' (92.25 mg GAE/100 mL) and 'Stella' (363.50 mg GAE/100 mL). The results align also with other previous findings that TPC is cultivar-dependent and often correlates with antioxidant capacity (Moutinho-Pereira et al., 2007).

Flavonoids are a subclass of phenolics with strong antioxidant activity, and their abundance enhances the nutraceutical quality of fruits (Heim et al., 2002; Nikolova et al., 2013).

In terms of TFC, 'Van' exhibited the highest TFC (53.11 ± 2.36 mg QE/100 g FW), followed by 'Stella' (40.22 ± 1.88 mg).

'Boambe de Cotnari' had the lowest TFC (9.92 ± 0.78 mg), indicating a much lower concentration of these bioactive compounds (Table 6).

The TFC pattern resulted was very similar to that of TPC, suggesting a strong interrelationship between these two parameters.

Antioxidant capacity, as measured by DPPH free radical scavenging, ranged from 23.9% in 'Boambe de Cotnari' to 53.3% in 'Stella'. 'Stella' and 'Van' (45.8%) showed considerably higher antioxidant activities, consistent with their elevated levels of phenolics and flavonoids. These findings reinforce the well-established correlation between polyphenolic content and antioxidant potential in sweet cherries (Serra et al., 2012). Meanwhile, the relatively low antioxidant capacity in 'Napoleon' and 'Boambe de Cotnari' reflects their modest bioactive compound profiles.

Pearson correlation analysis revealed strong associations among several biochemical traits and their relationship with antioxidant activity (DPPH). The most significant finding was the very strong positive correlation between total phenolic content (TPC) and DPPH activity ($r = 0.99$, $p < 0.05$), indicating that phenolics are the primary contributors to the antioxidant potential in sweet cherry fruits (Table 7). This is consistent with numerous studies highlighting phenolic compounds as major antioxidants in cherries and other fruits (Serra et al., 2012; Moutinho-Pereira et al., 2007).

Table 7. Pearson's correlation coefficients between chemical features of the sweet cherry varieties

Variables	SSC	TA	pH	Vit C	TPC	TFC	DPPH
TSS	1						
TA	-0.91	1					
pH	-0.74	0.83	1				
Vit C	-0.37	0.70	0.78	1			
TPC	0.64	-0.36	-0.60	0.01	1		
TFC	0.69	-0.32	-0.33	0.31	0.89	1	
DPPH	0.67	-0.43	-0.68	-0.09	0.99*	0.85	1

*Significant correlation at p<0.05. TSS-total soluble solid content; TA-titratable acidity; TPC-total phenolic content; TFC-total flavonoid content; DPPH-2,2′-diphenyl-1-Picrylhydrazyl.

A similarly strong positive correlation was observed between total flavonoid content (TFC) and DPPH ($r = 0.85$), supporting the role of flavonoids as key antioxidant constituents (Heim et al., 2002). The high intercorrelation between TPC and TFC ($r = 0.89$) suggests a substantial overlap in these two groups of compounds, both structurally and functionally, which collectively enhance the radical scavenging capacity of the fruit.

In contrast, vitamin C showed no meaningful correlation with DPPH activity ($r = –0.09$), indicating a limited contribution of ascorbic acid to overall antioxidant potential in these cultivars. This finding confirms previous reports suggesting that in cherries, polyphenols are more influential contributors than vitamin C when determining antioxidant capacity (Wu et al., 2004; Muskovics et al., 2006).

Interestingly, soluble solids content (TSS) was moderately positively correlated with both TPC ($r = 0.64$) and DPPH ($r = 0.67$), suggesting that cultivars with higher sugar content may also have increased polyphenol accumulation,

possibly due to shared metabolic pathways (Scalbert et al., 2005). On the other hand, titratable acidity (TA) showed a strong negative correlation with TSS (r = –0.91) and moderate negative correlation with DPPH (r = –0.43), while pH was negatively associated with DPPH (r = –0.68), reinforcing the idea that sweeter, less acidic fruits tend to exhibit greater antioxidant activity.

CONCLUSIONS

The pomological and chemical evaluation of the sweet cherry varieties grown in the urban orchard revealed significant variability in both morphological traits and biochemical composition among the studied cultivars. Differences in fruit size, firmness, soluble solids content, titratable acidity, and vitamin C levels highlights the influence of genetic factors and urban environmental conditions on fruit quality. These findings also shed light to the potential of specific varieties for urban horticulture based on their superior agronomic and nutritional characteristics. These insights are valuable to understand better the varietal performance in non-traditional growing environments and support informed selection for urban orchard planning, local consumption, and potential health benefits.

ACKNOWLEDGEMENTS

The authors express their gratitude to the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca and Horticultural Research Station for supplying all facilities and cooperation to carry out this research.

REFERENCES

Acero, N., Gradillas, A., Beltran, M., García, A., & Mingarro, D. M. (2019). Comparison of phenolic compounds profile and antioxidant properties of different sweet cherry (*Prunus avium* L.) varieties. *Food chemistry*, 279, 260-271.

AOAC. (2000). Official Method 967.21 – Ascorbic Acid in Vitamin Preparations and Juices. In Official Methods of Analysis (17th ed.). Association of Official Analytical Chemists.

Asănică, A., Tudor, V., & Teodorescu, R. (2013). Distinctive behaviour of some sweet cherry cultivars related to rootstock type. *AgroLife Scientific Journal*, *2*(1).

Boghean, S. O., Militaru, M., Gherghina, E., Sestras, R. E., Borsai, O., Andrecan, A. F., ... & Butiuc-Keul, A. L. (2025). The Effect of the Ripening Period on the Quality Attributes of Pear Fruit. *Horticulturae*, *11*(5), 468.

Climate Data, 2025. https://en.climate-data.org/europe/romania/cluj-511/

Corneanu, M., Iurea, E., & Sirbu, S. (2021). Comparison of five new sweet cherry cultivars bred in Romania, with their parental forms. *Journal of Horticultural Research*, *29*(1), 1-8.

Correia, S., Schouten, R., Silva, A. P., & Gonçalves, B. (2017). Factors affecting quality and health promoting compounds during growth and postharvest life of sweet cherry (*Prunus avium* L.). *Frontiers in plant science*, *8*, 2166.

Crisosto, C. H. (1999). Optimum procedures for ripening stone fruit. *Management of Fruit Ripening. University of California, Davis, Postharvest Horticulture Series*, *9*, 28-30.

Crisosto, C. H., Crisosto, G. M., & Metheney, P. (2003). Consumer acceptance of 'Brooks' and 'Bing'cherries is mainly dependent on fruit SSC and visual skin color. *Postharvest Biology and Technology*, *28*(1), 159-167.

Crisosto, C. H., Mitchell, F. G., & Johnson, S. (1995). Factors in fresh market stone fruit quality. *Postharvest news and information*, *6*(2), 17-21.

Cuevas-Cianca, S. I., Romero-Castillo, C., Gálvez-Romero, J. L., Juárez, Z. N., & Hernández, L. R. (2023). Antioxidant and anti-inflammatory compounds from edible plants with anti-cancer activity and their potential use as drugs. *Molecules*, *28*(3), 1488.

Demir, F., & Kalyoncu, I. H. (2003). Some nutritional, pomological and physical properties of cornelian cherry (*Cornus mas* L.). *Journal of Food Engineering*, *60*(3), 335-341.

Eeraerts, M., Meeus, I., Van Den Berge, S., & Smagghe, G. (2017). Landscapes with high intensive fruit cultivation reduce wild pollinator services to sweet cherry. *Agriculture, Ecosystems & Environment*, *239*, 342-348.

Einhorn, T. C., Wang, Y., & Turner, J. (2013). Sweet cherry fruit firmness and postharvest quality of late-maturing cultivars are improved with low-rate, single applications of gibberellic acid. *HortScience*, *48*(8), 1010-1017.

Esmaeili, N., Naghshband, R., & Zare Nahandi, F. (2019). Evaluation of the effect of harvest time and fruit cold storage period on some of qualitative characteristics of Cornelian cherry fruit. *Food Research Journal*, *29*(3), 69-84.

Fatih, S. E. N., Oksar, R. E., Golkarian, M., & Yaldiz, S. (2014). Quality changes of different sweet cherry cultivars at various stages of the supply chain. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, *42*(2), 501-506.

Fenech, M., Amaya, I., Valpuesta, V., & Botella, M. A. (2019). Vitamin C content in fruits: Biosynthesis and regulation. Frontiers in plant science, 9, 2006.

Fonseca, L. R., Silva, G. R., Luís, Â., Cardoso, H. J., Correia, S., Vaz, C. V., ... & Socorro, S. (2021). Sweet cherries as anti-cancer agents: From bioactive compounds to function. *Molecules*, *26*(10), 2941.

Ghahremani, V., Noori, O., Deihimfard, R., & Veisi, H. (2024). Understanding resilience: Contributions of urban agriculture to the resilience of urban landscapes. *Urban Agriculture & Regional Food Systems*, *9*(1), e70003.

Giménez, M. J., Valverde, J. M., Valero, D., Guillén, F., Martínez-Romero, D., Serrano, M., & Castillo, S. (2014). Quality and antioxidant properties on sweet cherries as affected by preharvest salicylic and acetylsalicylic acids treatments. *Food chemistry*, *160*, 226-232.

Girard, B., & Kopp, T. G. (1998). Physicochemical characteristics of selected sweet cherry cultivars. *Journal of Agricultural and Food Chemistry*, *46*(2), 471-476.

Gonçalves, A. C. A. (2016). Sweet cherries from Fundão as health promotors: chemical characterization and biological potential. Master's thesis, Universidade da Beira Interior (Portugal).

Gonçalves, B., Aires, A., Oliveira, I., Baltazar, M., Cosme, F., Afonso, S., ... & Silva, A. P. (2024). From Orchard to Wellness: Unveiling the Health Effects of Sweet Cherry Nutrients. *Nutrients*, *16*(21), 3660.

Gonçalves, B., Moutinho-Pereira, J., Santos, A., Silva, A. P., Bacelar, E., Correia, C., & Rosa, E. (2006). Scion–rootstock interaction affects the physiology and fruit quality of sweet cherry. *Tree Physiology*, *26*(1), 93-104.

Gonçalves, B., Silva, A. P., Moutinho-Pereira, J., Bacelar, E., Rosa, E., & Meyer, A. S. (2007). Effect of ripeness and postharvest storage on the evolution of colour and anthocyanins in. *Food Chemistry*, *103*(3), 976-984.

Habib, M., Bhat, M., Dar, B. N., & Wani, A. A. (2017). Sweet cherries from farm to table: A review. *Critical reviews in food science and nutrition*, *57*(8), 1638-1649.

Hampson, C. R., Stanich, K., McKenzie, D. L., Herbert, L., Lu, R., Li, J., & Cliff, M. A. (2014). Determining the optimum firmness for sweet cherries using Just-About-Right sensory methodology. *Postharvest Biology and Technology*, *91*, 104-111.

Heim, K. E., Tagliaferro, A. R., & Bobilya, D. J. (2002). Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. *The Journal of nutritional biochemistry*, *13*(10), 572-584.

Henríquez, C., Almonacid, S., Chiffelle, I., Valenzuela, T., Araya, M., Cabezas, L., ... & Speisky, H. (2011). Determination of antioxidant capacity, total phenolic content and mineral composition of different fruit tissue of five apple cultivars grown in Chile.

Jha, S. N. (2010). Colour measurements and modeling. In *Nondestructive evaluation of food quality: Theory and practice* (pp. 17-40). Berlin, Heidelberg: Springer Berlin Heidelberg.

Kader, A. A. (2008). Flavor quality of fruits and vegetables. *Journal of the Science of Food and Agriculture*, *88*(11), 1863-1868

Kappel, F., & MacDonald, R. (2007). Early gibberellic acid sprays increase firmness and fruit size of'Sweetheart'sweet cherry. *Journal of the American Pomological Society*, *61*(1), 38.

Kappel, F., Fisher-Fleming, B., & Hogue, E. (1996). Fruit characteristics and sensory attributes of an ideal sweet cherry.

Kappel, F., MacDonald, R., Brownlee, R., & McKenzie, D. L. (2011). SPC103 (Sentennial™) sweet cherry. *Canadian Journal of Plant Science*, *91*(1), 179-181.

Kottek, M., Grieser, J., Beck, C., Rudolf, B., & Rubel, F. (2006). World map of the Köppen-Geiger climate classification updated.

Kumaran, A., & Karunakaran, R. J. (2007). In vitro antioxidant activities of methanol extracts of five Phyllanthus species from India. *LWT-Food Science and Technology*, *40*(2), 344-352.

Lin, B. B., Philpott, S. M., & Jha, S. (2015). The future of urban agriculture and biodiversity-ecosystem services: Challenges and next steps. *Basic and applied ecology*, *16*(3), 189-201.

Mattheis, J. P., & Fellman, J. K. (1999). Preharvest factors influencing flavor of fresh fruit and vegetables. *Postharvest biology and technology*, *15*(3), 227-232.

Moutinho-Pereira, J., Meyer, A. S., Gonçalves, B., Silva, A. P., Rosa, E., & Bacelar, E. (2007). Effect of ripeness and postharvest storage on the evolution of colour and anthocyanins in cherries (*Prunus avium* L.).

Muskovics, G., Felföldi, J., Kovács, E., Perlaki, R., & Kállay, T. (2006). Changes in physical properties during fruit ripening of Hungarian sweet cherry (*Prunus avium* L.) cultivars. *Postharvest biology and technology*, *40*(1), 56-63.

Nikolova, M., Vitkova, A., Zayova, E., & Petrova, M. (2013). Flavonoid Profiles, Polyphenolic Content And Antiradical Properties of Cultivated Plants of Arnica Montana. *Agrolife Scientific Journal*, *2*(2).

Orsini, F., Kahane, R., Nono-Womdim, R., & Gianquinto, G. (2013). Urban agriculture in the developing world: a review. *Agronomy for sustainable development*, *33*, 695-720.

Paskucza, S., Carpa, R., Culda, C. A., Butiuc-Keul, A. L., Dobrota, C., Berchez, O., & Rusu, T. (2021). Biochemical composition of blackcurrant fruits in a plantation from Jibou area. *AgroLife Scientific Journal*, *10*(1).

Romano, G. S., Cittadini, E. D., Pugh, B., & Schouten, R. (2006). Sweet cherry quality in the horticultural production chain. *Stewart Postharvest Rev*, *6*(2), 1-9.

Scalbert, A., Johnson, I. T., & Saltmarsh, M. (2005). Polyphenols: antioxidants and beyond. *The American journal of clinical nutrition*, *81*(1), 215S-217S.

Sekse, L., & Wermund, U. (2008). Fruit flesh firmness in two plum cultivars: comparison of two penetrometers. In *IX International Symposium on Plum and Prune Genetics, Breeding and Pomology 874* (pp. 119-124).

Serra, A., Macià, A., Romero, M. P., Reguant, J., Ortega, N., & Motilva, M. J. (2012). Metabolic pathways of the colonic metabolism of flavonoids (flavonols, flavones and flavanones) and phenolic acids. *Food chemistry*, *130*(2), 383-393.

Serrano, M., Díaz-Mula, H. M., Zapata, P. J., Castillo, S., Guillén, F., Martínez-Romero, D., ... & Valero, D.

(2009). Maturity stage at harvest determines the fruit quality and antioxidant potential after storage of sweet cherry cultivars. *Journal of Agricultural and Food Chemistry*, *57*(8), 3240-3246.

Serrano, M., Guillén, F., Martínez-Romero, D., Castillo, S., & Valero, D. (2005). Chemical constituents and antioxidant activity of sweet cherry at different ripening stages. *Journal of Agricultural and Food Chemistry*, *53*(7), 2741-2745.

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American journal of Enology and Viticulture, 16(3), 144-158.

Sirbu, S., Oprica, L., Poroch, V., Iurea, E., Corneanu, M., & Grigore, M. N. (2018). Physical parameters, total phenolics, flavonoids and vitamin C content of nine sweet cherry cultivars. *Revista de Chimie*, *69*(1), 125-129.

Skrzyński, J., Leja, M., Gonkiewicz, A., & Banach, P. (2016). Cultivar effect on the sweet cherry antioxidant and some chemical attributes. *Folia Horticulturae*, *28*(1), 95-102.

Sun-Waterhouse, D. (2011). The development of fruit-based functional foods targeting the health and wellness market: A review. *International Journal of Food Science and Technology*, *46*(5), 899-920.

Usenik, V., & Stampar, F. (2011). The effect of environmental temperature on sweet cherry phenology. *European Journal of Horticultural Science*, *76*(1), 1.

Usenik, V., Fabčič, J., & Štampar, F. (2008). Sugars, organic acids, phenolic composition and antioxidant activity of sweet cherry (Prunus avium L.). *Food Chemistry*, *107*(1), 185-192.

Wu, X., Beecher, G. R., Holden, J. M., Haytowitz, D. B., Gebhardt, S. E., & Prior, R. L. (2004). Lipophilic and hydrophilic antioxidant capacities of common foods in the United States. *Journal of agricultural and food chemistry*, *52*(12), 4026-4037.

Wu, Y., Li, L., Li, M., Zhang, M., Sun, H., & Sigrimis, N. (2020). Optimal fertigation for high yield and fruit quality of greenhouse strawberry. *PLoS One*, *15*(4), e0224588.

Zhishen, J., Mengcheng, T., & Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food chemistry*, *64*(4), 555-559.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

THE IMPACT OF CLIMATE CHANGES ON THE PHENOLOGICAL DYNAMICS OF SOME PEAR VARIETIES IN THE MĂRĂCINENI AREA, ROMANIA

Eugenia GHERGHINA (MAREŞI)[1], Mădălina MILITARU[2], Mădălina BUTAC[2], Dorel HOZA[1]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăşti Blvd, District 1, Bucharest, Romania
[2]Research Institute for Fruit Growing Piteşti - Mărăcineni, 402 Mărului Street, Mărăcineni, Piteşti, Romania

Corresponding author email: genimaresi@gmail.com

Abstract

Climate change has impact on the phenology of fruit trees brings changes on the quality and productivity of the fruits, on the resistance/tolerance to frost, diseases and pests and implications on the zoning of the fruit tree species. The study was carried out in pear collection from ICDP Pitesti - Maracineni on seven Romanian pear varieties ('Argessis', 'Carpica', 'Monica', 'Ervina', 'Paramis', 'Paradox', 'Isadora') compared with 'Packham's Triumph' cv., between 2014-2024 periods. It has been studied for 11 years the phenophases of flowering in relation with climatic factors (temperature, and precipitation). Thus, from the multiannual observations it was found that all the flowering phenophases of pear were realized earlier, with about 9 days (for beginning of flowering) and 10 days (for end of flowering). Climate accidents were also recorded in 2017 (late spring frosts, -4.2°C on April, 21-22th), 2020 (late spring frost, -6.2°C ...-7.1°C on March, 16-17th and on April, 1-7th), 2022 (late spring frosts -1.8...5.5°C on April, 18-21th), in 2023 (low temperatures -4°C... -6.4°C on March, 29-30th), and 2024 (high temperature, +27°C, April, 1st). All these climatic accidents had visible negative effects on fruit production (e.g.: low temperatures from April, 2017 led to the loss of yield in a proportion of 95 % on 'Monica' cv.).

Key words*: changes climatic, genetic resources, pear, phenology, late frost.*

INTRODUCTION

Climatic changes cause significant agricultural obstacles, negatively influencing productivity, water and soil resources. Extreme weather events negatively affect global agriculture and food security, disrupt fruit production leading to increased food prices (Jovović et al., 2024; Razzaghi, 2024).

The quantitative and qualitative basis of production is the result of the interaction between genetic, technological and environmental factors.

Fruit species, as perennial organisms composed of cultivar and rootstocks that interact, are strongly influenced by the environment throughout the life period, during which the annual cycles follow each other, each involving the passage through growth, fructification and physiological processes (Chitu et al., 2023).

In fruit growing, the adaptation of varieties and hybrids to the different climatic conditions from year to year constitutes a breeding objective that remains current.

The adaptability of the genotypes is closely correlated with different factors: rootstocks, precocity and resistance to low temperatures, late spring frosts, high temperatures (drought) or excess humidity.

The positive response to climatic changes can be obtained by appropriate technologies or cultural means; by cultivating in favourable areas (appropriate zoning) or by breeding programs that follow the creation of varieties that incorporate genes for resistance to these extreme factors. Some of the physiological processes of trees, such as vegetative growth, flowering, fruiting and quality of fruits are strongly influenced by climate changes. And in the pear species, phenology is influenced by climatic changes (especially temperature, drought and precipitation), with an interaction between genotype and environmental factors.

In Europe, previous research shows that, due to

the warming of the climate, flowering phenology of fruit trees is advancing, with studies indicating earlier flowering in apples, sweet cherries, pears and plums (Sparks et al., 2005; Craufurd and Wheeler, 2009; Unterberger et al., 2018; Reeves et al., 2022). This research area is a concern for the agricultural sector, given the possible influence on pollination, yield capacity and ultimately food security (Craufurd and Wheeler, 2009).

In Romania, according to Cosmulescu et al. (2010) and Chițu and Paltineanu (2020), there was an advance between 2 - 10 days in the pear phenology, this being smaller than at the apple, at which the advance is 7-15 days.

The increases of temperatures between January and March and the advance of the flowering phenophases have increased the risk of frost damages. Also, late spring frost from the beginning or ending of flowering can cause damage in flower buds or destroy flower elements with negative implications on fruit production.

The aim of this paper is to study the major effects of the climate changes on the pear flowering phenology and the damages caused by the late frosts in the Southern part of Romania.

MATERIALS AND METHODS

The studies were carried out in pear collection of Genetic and Breeding Department from Research Institute for Fruit Growing Pitești (RIFG), Romania in 2014-2024 periods using a climate database from 1969 to 2024. Phenological data on pear flowering were collected according to BBCH Meier (2001), ECPGR Characterization and Evaluation Descriptors for Pear Genetic Resource (2022), respectively: beginning of flowering - code 61 and end of flowering - code 69 (Table 1).

Table 1. The stages of phenological development at pear (after Meier, 2001)

Growth stage	Code	Description
Flowering (main shoot)	61	Beginning of flowering: about 10% flowers open
	69	End of flowering: all petals fallen

The appreciation of the flowering phenophase was performed on seven pear Romanian cultivars ('Argessis', 'Carpica', 'Monica', 'Ervina', 'Paramis', 'Paradox', 'Isadora') compared with 'Packham's Triumph' cv., very widespread in the plantations from Europe.

The data were included in an Excel and interpreted statistical using average, standard deviation and coefficient of variability.

RESULTS AND DISCUSSIONS

In order to determine the effect of climatic factor on the phenological stages of pear cultivars studied the evolution of meteorological parameters was analyzed. In the South part of Romania, the climate it is temperate-continental, characterized by an average annual temperature of 10.1ºC and an amount of precipitation of 673.2 mm for the period 1969-2023 (Figure 1).

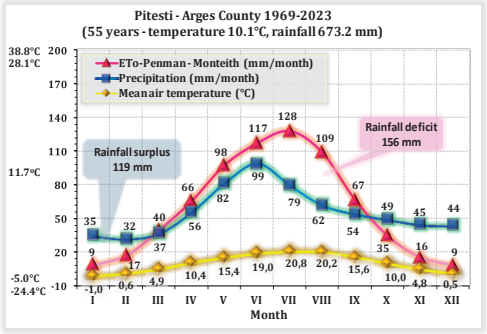


Figure 1. The values of the main meteorological parameters (1969-2023)

During the study period (2014-2024) the average annual temperature was 11.5ºC being with 1.4ºC higher than the multiannual average; the average maximum temperature was with 1.6ºC higher than the multiannual average; the average minimum temperature was with 0.8ºC higher than the multiannual average and the average amount of precipitation was 677.8 mm, closely to the multiannual average, but with decreasing trends in the last 3 years (Table 2).

In Romania, significant increases of average air temperature were also noted in January, February and March which causes an early start of trees in vegetation with negative influences on resistance to late spring frosts (Chițu et al., 2013; Florea et al., 2019; Chițu and Păltineanu, 2020). Also, in different countries from Europe there have been increases in average air temperature,

such as: 1.1-1.3ºC in France; 0.6-1.9ºC in two regions of Germany; 1.2ºC in Serbia; 1.4ºC in Slovenia (Guedon and Legave, 2008; Blanke and Kunz, 2017; Drkenda et al., 2018; Waldau and Chmielewski, 2018; Milisévič and Milosévič, 2023).

In the study period (2014-2024) there is also a tendency to increase temperatures, which causes an earlier flowering. Thus, average temperature was in January with 1ºC higher, in February with 2.3ºC higher, in March with 1.5ºC higher and in April with 0.8ºC higher than the multiannual average temperature (Table 3).

Table 2. Average values of the main meteorological parameters in the period 2014-2024 compared to the baseline for 54 years period (1969-2023)

Year	Average tempera-ture (ºC)	Average maximum temperature (ºC)	Average minimum Temperature (ºC)	Precipitation (mm)
2014	10.6	16.3	5.8	1,011.3
2015	11.3	17.8	5.8	725.2
2016	11.0	17.5	5.4	776.0
2017	11.0	17.8	5.1	632.3
2018	11.2	17.8	5.8	745.9
2019	11.9	18.9	5.9	634.7
2020	11.8	18.9	5.8	679.1
2021	10.7	17.4	5.2	636.2
2022	11.6	18.9	5.4	525.3
2023	12.3	19.2	6.5	562.8
2024	12.7	20.2	6.3	527.0
2014-2024	**11.5**	**18.2**	**5.7**	**677.8**
1969-2023	**10.1**	**16.6**	**4.9**	**673.2**

Table 3. Temperature values recorded in the first four month of years (2014-2024) compared to the baseline for 54 years period

Year	**Mean temp.** (°C)			
	Jan.	Febr.	March	April
2014	0.1	1.7	8.1	10.9
2015	0.6	1.2	5.6	9.9
2016	-2.4	5.7	6.7	13.5
2017	-4.5	1.5	8.5	9.8
2018	0.6	0.6	3.7	15.2
2019	-1.2	2.6	7.8	10.8
2020	0.3	4.2	7.7	10.9
2021	0.5	3.0	4.1	8.6
2022	0.8	3.1	3.6	10.1
2023	4.0	2.6	7.1	9.7
2024	0.2	6.5	7.8	13.4
2014-2024	**-0.1**	**3.0**	**6.4**	**11.2**
1969-2023	**-1.0**	**0.7**	**4.9**	**10.4**

All these increases of temperatures from the beginning of the year have had the effect of earlier flowering than the normal flowering time in Mărăcineni - Argeș area.

The beginning of flowering is a specific trait of each cultivar, being genetically determined and is not correlated with the ripening time of fruits.

The average date of the beginning of flowering of pear is April 17 being considered a landmark for pear flowering in Mărăcineni - Argeș area.

It is observed that in the period 2014-2024 the average date regarding the beginning of flowering was April 8, the standard deviation between cultivars being 0.70 days, indicating a flowering grouped of the studied cultivars. It can also be noted that the beginning of flowering was with 9 days earlier than the average flowering date in the Mărăcineni -Argeș area (Figure 2).

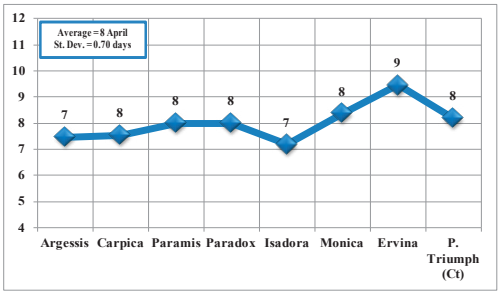


Figure 2. The oscillation of average values (2014-2024) regarding the date of beginning of flowering at the pear cultivars studied

Analyzing the phenophase beginning of flowering by the years it can be seen that the earliest blooming was achieved in the years: 2014 and 2019 - April 3, 2016 and 2024 - April 2, 2023 - April 6, 2020 - April 10.

In the years 2015, 2018, 2021 and 2022 the beginning of flowering was carried out within normal limits (April, 13-19) (Figure 3).

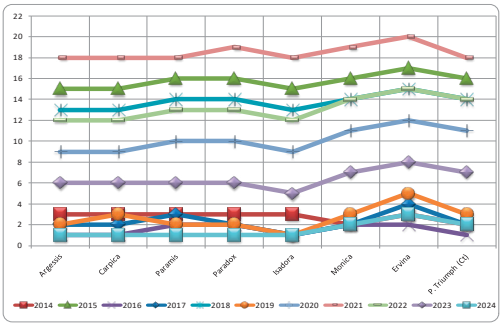


Figure 3. The oscillation of the date regarding the beginning of flowering on the pear cultivars between 2014 and 2024

On cultivars the situation is presented as follows: 'Argessis' and 'Isadora' cvs.

blossomed earlier than 'Packham's Triumph' cv., and 'Ervina' cv. blossomed a little later than the 'Packham's Triumph' cv. (Figure 2).
Regarding the end of flowering, it can be said that the average date of this phenophase is April 27 considered a landmark for the Mărăcineni-Argeș area.
In the last 11 years, the average date of the end of flowering was April 17, being with 10 days earlier than the normal average date of this phenophase (Figure 4).

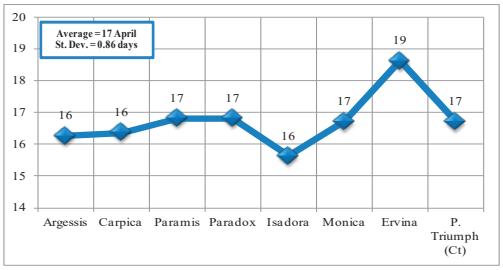


Figure 4. The oscillation of average values (2014-2024) regarding the date of ending of flowering at the pear cultivars studied

The oscillation of the average values regarding the end of flowering in the study period was similar to that of the beginning of flowering (Figure 5).
Thus, the end of flowering was earlier in the years 2014, 2016, 2017, 2019, 2023, 2024 and within normal limits in the years 2015, 2018, 2020, 2022 (Figure 5).
It can be seen that in the years when the flowering was earlier, the average air temperature of February and March were much higher than the normal of the Mărăcineni, Argeș area (Table 3, Figures 3 and 5).

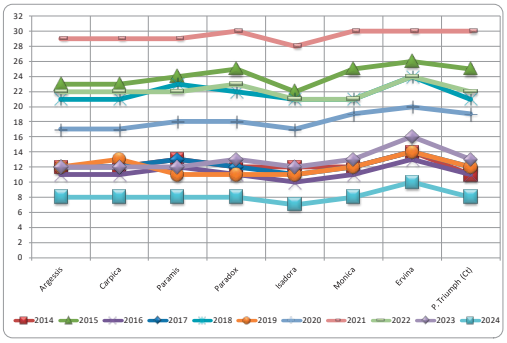


Figure 5. The oscillation of the date regarding the ending of flowering on the pear cultivars between 2014 and 2024

These advances of flowering had serious implycations on the damage caused by late frosts.
Climate change has introduced more unpredictability and extreme temperature events that can have major impacts on dormancy, cold-hardiness of wood and buds, accumulation of chilling hours and fruiting phenology of pear.
Late fall and early spring warming can shorten the number of chilling hours accumulated, particularly in warmer climates, which leads to issues with fruits production (Vyse et al., 2019; Waite et al., 2024).
The frequency of the climatic accidents occurrence between March 11 and April 10 and April 26-May 10, is low (on average every 50 years). Higher damage can be recorded between April 11 and 20, when the probability increases to 4% (so in a year of 25, the floral organs can freeze). The most critical period is April 21-25 when the probability of destroying the floral organs by frost reaches 6.3%, respectively in a year of 16 the harvest is compromised (Butac and Chițu, 2009).
In the Mărăcineni - Argeș area, in the period 2014-2024, climate accidents were recorded in 2017 (late spring frosts, -4.2°C on April, 21-22th), 2020 (late spring frost, -6.2°C …-7.1°C on March, 16-17th and on April, 1-7th), 2022 (late spring frosts -1.8...5.5°C on April, 18-21th), in 2023 (low temperatures -4°C... -6.4°C on March, 29-30th), and 2024 (high temperature, +27°C, April, 1st) (Table 4). All these climatic accidents had visible negative effects on fruit production.

Table 4. Climatic accidents in the 2014-2024 period, specific to the pear species

Year	Climatic accident	Date	Min. temp. (°C)	Phenophase	Level of damages (%)
2017	Late spring frost	April, 21-22	-4.2	End of flowering	28-95
2020	Late spring frost	March 16-17 April 1-7	-6.2..-7.1 -5	Bud burst Beginning of flowering	15-30
2022	Late spring frost	April 18-21	-1.8..-5.8	Begining of flowering	10-15
2023	Late spring frost	March, 29-30	-1.4..-6.4	Bud burst	10-67
2024	High temp.	April 1	+27	Begining of flowering	Low fruit set

In 2017, the level of damages was in average 60.83% varying between 28.00% at 'Carpica' cv. and 95.00% at 'Monica' cv. (Table 5; Figure 6).

In 2020 and 2022 the percentage of flowers destroyed was slightly lower, 20.67 in 2020 and 12.42% in 2022, the fruit production being not economically affected (Table 5).
Also in 2023, the late spring frosts from the end of March when the trees were in the phenophase bud burst had a negative effect, the average percentage of damages being 40.71% (Table 5). However, fruit production has not been much diminished.
The most sensitive cultivars were 'Argessis', 'Monica', 'Ervina' and 'Paramis' to which the percentage of flowers destroyed was higher, and implicitly the fruit production was very small or even absent at the 'Ervina' and 'Monica' cvs.

Table 5. Damage caused to pear by late frosts from March, 29-30, 2023, Romania

No.	Cultivar	Level of damages (%)			
		2017	2020	2022	2023
1	Argessis	79.33	23.67	13.33	64.67
2	Carpica	28.00	15.00	10.00	13.33
3	Monica	95.00	30.33	15.00	67.00
4	Ervina	93.00	25.00	15.00	65.33
5	Paramis	84.67	24.67	14.33	62.00
6	Paradox	32.33	15.33	10.33	20.00
7	Isadora	36.67	15.67	10.67	16.67
8	Packham's Triumph	37.67	15.67	10.67	16.67
Average		**60.83**	**20.67**	**12.42**	**40.71**
Standard deviation		**29.58**	**5.95**	**2.21**	**25.80**
Coefficient of variability		**48.62**	**28.78**	**17.79**	**63.37**



Figure 6. Pear flowers - 'Monica' cv., unaffected and affected by the late frosts from April 21-22, 2017 (Mărăcineni - Argeş)

CONCLUSIONS

Climate changes from the last years have a negative effect on the growth and fruiting of pear species.
In the Southern part of Romania, in the last time, were recorded increasing of mean, maximum and minimum temperatures.
The increase of average air temperature was also noted in January, February, March and April.
Due to the significant increase in these months, the flowering phenophases of pear were realized earlier, with about 9 days (for beginning of flowering) and 10 days (for end of flowering).
This advance had serious implications on the damage caused by late frosts.
Climate accidents were recorded in 2017 (late spring frosts, -4.2°C on April, 21-22th), 2020 (late spring frost, -6.2°C …-7.1°C on March, 16-17th and on April, 1-7th), 2022 (late spring frosts -1.8...5.5°C on April, 18-21th), in 2023 (low temperatures -4°C... -6.4°C on March, 29-30th), and 2024 (high temperature, +27°C, April, 1st).
All these climatic accidents had visible negative effects on fruit production (e.g.: low temperatures from April, 2017 led to the loss of yield in a proportion of 95 % on 'Monica' cv.).

REFERENCES

Blanke, M. and Kunz, A. (2017). Cherry phenology as bioindicator for climate change. *Acta Hortic.,* 1162: 1-8. 10.17660/ActaHortic.2017.1162.1.

Butac, M. and Chiţu, E. (2007). Impact of climatic changes on the phenological dynamics of some plum cultivars in the fruit growing Arges area. *Scientific papers of RIFG Piteşti,* vol. XXIII: 139-147.

Chiţu, E., Giosanu, D., and Mateescu, E. (2013). Seasonal and Annual Extreme Temperature Variability and Trends of the latest three decades in Romania. *Advances in Environmental Sciences - International Journal of the Bioflux Society*, Online ISSN 2065-7647. Volume 5, Issue 2: 70-88; http://www.aes.bioflux.com.ro.

Chitu, E., Paltineanu, C. (2020). Timing of phenological stages for apple and pear trees under climate change in a temperate-continental climate. *Int. J. Biometeorol*. https://doi.org/10.1007/s00484-020-01903-2, Romania.

Chiţu, E., Mazilu, I., Călinescu, M., Coman, M., Plăiaşu, F., Chivu, M., Sturzeanu M., Petrescu A., Hera O. (2023). Modelarea favorabilităţii climatice a României în vederea extinderii în cultură a unor specii pomicole de climat temperat şi subtropical, Romania (in Romanian).

Cosmulescu, S., Baciu, A., Cichi, M., Gruia, M. (2010). The effect of climate changes on phenological phases in plum tree (Prunus domestica L.) in South-Western. Rom. South West. J. *Hortic. Biol. Environ*. 1 (1), 9–20, Romania.

Craufurd, P.Q., Wheeler, T.R. (2009). Climate change and the flowering time of annual crops. *J. Exp. Bot*., 60(9),

2529–2539. https://doi.org/10.1093/jxb/erp196, England.

Drkenda, P., Musić, O., Marić, S., Jevremović, D., Radičević, S., Hudina, M., Hodžić, S., Kunz, A., Blanke, M.M. (2018). Comparison of climate change effects on pome and stone fruit phenology between Balkan Countries and Bonn. *Erwerbs-Obstbau* 60(4): 295–304. DOI: 10.1007/s10341-018-0373-y.

Florea, A., Chițu, E., and Păltineanu, C. (2019). Dynamics of phenological stages due to climate change in plum trees in southern Romania. *Acta Hortic.* 1289: 205-212. https://doi.org/10.17660/ActaHortic.2020.1289.29.

Guédon, Y., and Legave J.M. (2008). Analyzing the time-course variation of apple and pear tree dates of flowering stages in the global warming context. *Ecological Modelling*, 219 (1-2):189-199. https://doi.org/10.1016/j.ecolmodel.2008.08.010.

Jovović, Z., Velimirović, A., Yaman, N. (2024). Climate and Crop Production Crisis. In: Çetin, Ö. (eds) *Agriculture and Water Management Under Climate Change* . SpringerBriefs in Earth System

Meier, U. (2001). Federal Biological Research Centre for Agriculture and Forestry, Growth stages of mono and dicotyledonous plants, *BBCH Monograph*, Edition, 2001, Germany.

Milošević, N., and Milošević, T. (2023). Impact of climate change on plum (*Prunus domestica* L.). Chapter 11, in: *Cultivation for climate change resilience, vol. 2*. Sciences. Springer, Cham. https://doi.org/10.1007/978-3-031-74307-8_1, Turkiye.

Razzaghi, S. (2024). Empowering Agriculture in the Face of Climate Change with Smart Solutions. In: Çetin, Ö. (eds), *Agriculture and Water Management Under Climate Change* . SpringerBriefs in Earth System Sciences. Springer, Cham. https://doi.org/10.1007/978-3-031-74307-8_8, Türkiye.

Reeves, L.A., Garratt, M.P., Fountain, M.T., Senapathi, D. (2022). Climate induced phenological shifts in pears–a crop of economic importance in the UK. *Agric Ecosyst Environ*, 338:108109. https:// doi.org/ 10. 1016/j. agee. 2022. 108109

Sparks, T.H., Croxton, P.J., Collinson, N., & Taylor, P. W. (2005). Examples of phenological change, past and present, in UK farming. *Annals of applied biology*, 146(4), 531-537. https://doi.org/ 10.1111/j.1744-7348.2005.050016.x.

Unterberger, C., Brunner, L., Nabernegg, S., Steininger, K.W., Steiner, A.K., Stabentheiner, E., ... & Truhetz, H. (2018). Spring frost risk for regional apple production under a warmer climate. *PloS one*, 13(7), e0200201, https://doi.org/10.1371/journal.pone.0200201.

Vyse, K., Pagter, M., Zuther, E., & Hincha, D.K. (2019). Deacclimation after cold acclimation - a crucial, but widely neglected part of plant winter survival. *Journal of experimental botany*, 70(18), 4595-4604.

Waite, J.M., Gottschalk, C., Reinhold, L.A., Bassil, N.V., Volk, G.M., Postman, J.D., ... & Bell, R.L. (2025). Vulnerability of pear (*Pyrus*) genetic resources in the US. *Genetic Resources and Crop Evolution*, 72(1), 815-843.

Waldau, T., and Chmielewski F.M. (2018). Spatial and temporal changes of spring temperature, thermal growing season and spring phenology in Germany 1951-2015. *Meteorologische Zeitschrift*, Vol. 27, No. 4, 335–342. http://dx.doi.org/10.18452/19629.

ECPGR Characterization and Evaluation Descriptors for Pear Genetic Resource (2022).

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

MULTIVARIATE ANALYSIS OF GENOTYPE BY YEAR INTERACTION FOR SOME FRUIT TRAITS IN ROSEHIP (*ROSA CANINA* L.)

Adina Gabriela MARIŞ, Sorin CIULCA

Faculty of Engineering and Applied Technologies, University of Life Sciences "King Mihai I" from Timisoara, 119 Calea Aradului, 300645, Timisoara, Romania

Corresponding author email: c_i_sorin@yahoo.com

Abstract

Rosehip grows wildly in many different regions of Romania, revealing high environmental adaptability. Due to cross-pollination, the natural populations of rosehip present a certain degree of heterogeneity. Given the multifaceted pharmacological properties, the rosehip fruits acquire an increasingly and wide application in food, cosmetics, and pharmaceutical industries. The 24-rosehip population was collected from different Arad County locations in the West of Romania. The rosehip fruits were randomly picked from different canopy sides for three shrubs of each population. The present study was conducted to assess the variation of fruit traits for 24 populations of rosehip under the effect of different climatic conditions over three years and to select populations with good stability of desirable fruits. The results indicated significant differences among rosehip populations' fruit traits across testing years due to genotype-by-year interaction. Suitable rosehip populations with specific and broad adaptability were identified for the studied traits.

Key words*: adaptability, AMMI, fruit variation, rosehip, stability.*

INTRODUCTION

The genus Rosa includes up to 200 species distributed throughout the northern hemisphere's diverse temperate and subtropical environments. (Bruneau et al., 2007; Tomljenovic & Pejić, 2018). In the flora of Romania there are five hybrids in addition to the 29 spontaneous and sub-spontaneous species of the *Rosa* L. genus (Oarga Porumb et al., 2024).

Rosehips are pseudo-fruits that develop in a fleshy pericarp and comprise an outer hypanthium having achenes in-grained inside (Winther et al., 2016). Rosehip fruits are well known for having human health-promoting compounds like mineral nutrients, vitamins, fatty acids, and phenolic compounds (Medveckiene et al., 2023). Rosehips have antioxidant and anti-inflammatory, antibacterial, anti-mutagenic, probiotic, anti-ulcerogenic, antinociceptive, and anti-carcinogenic properties (Gruenwald et al., 2019; Gulbagca et al., 2019).

Due to the growing interest in plant agents enriched with antioxidant properties, these plants are acquiring an increasingly wider application in the food, cosmetics, and pharmaceutical industries as efficient materials to improve the quality of final products (Cosmulescu et al., 2017).

Harvesting time is very important in improving the quality and nutritional value of the rosehip-processed product. The qualitative and quantitative composition of the biologically active compounds in rosehips, such as fatty acids (Kulaitiene et al., 2020), phenolic compounds, or vitamin C (Chae et al., 2021; Elmastas et al., 2017; Medveckiene et al., 2021), varies during the stages of maturity.

Shrubs of *Rosa canina* have considerable importance in agri-environment measures due to the reduced environmental requirements, especially those from the soil. As such, rosehip can be used in different ways: the establishment on land, a consolidation role in the establishment of shelterbelts, a role in combating deflation wind, and the establishment of biological barriers against the destructive action of snow (Soare et al., 2015). Rose hip ofis important in breeding the *Rosa* genus, especially as rootstocks for ornamental roses (Shirdel et al., 2013; Soare et al., 2014).

Wild rosehip genotypes show high morpho-biochemical diversity. These genotypes possess a higher frequency of genes that trigger resistance and accumulation of different phytochemicals due to their tolerance to natural

enemies and environmental stresses (Mertoglu et al., 2024).
It is very important to select rosehip genotypes morphologically and biochemically adapted to diverse unfavourable ecological conditions due to global climate change. These possible selected genotypes can be used directly for production and as genitors in breeding programs to develop new superior genotypes (Cheikh-Affene et al., 2013; Gunes & Dolek, 2010).
The genetic variation between populations and the influence of specific environmental factors in the growing areas can explain the differences regarding rosehip fruits. Geographical location has an important contribution through specific microclimate and radiation, temperature, rainfall, and soil nutrient availability (Andronie et al., 2019; Munteanu et al., 2023).
The additive main effects and multiplicative interaction (AMMI) model has been used extensively for analyses of multi-environment trials to understand complex genotype, environment/year, and genotype-by-year interactions (Zobel et al., 1988; Gauch & Zobel, 1990).
The knowledge of the variability in the fruit traits of rosehip is valuable for fundamental and practical medicinal purposes, and for designing efficient breeding programs of these species (Butkeviciute et al., 2022). The present study was conducted to assess the variation of fruit traits for 24 populations of rosehip under the effect of different climatic conditions over three years and to select populations with good stability of desirable fruits.

MATERIALS AND METHODS

The 24-rosehip populations were collected from different Arad County locations, in the West of Romania. The ripe rosehip fruits were randomly picked from different canopy sides for three shrubs of each population. The harvested fruits were randomly sampled, and 50 fruits per shrub/replicate were selected for analysis to determine the weight and fresh pulp percentage (100* pulp weight/fruit weight). The geographical coordinates of the collection sites for rosehip populations are presented in Table 1. Amid the high temperatures during the summer associated with the very low level of rainfall, the conditions of 2024 were considered the most unfavorable.

Table 1. Geographical coordinates of the collection sites for rosehip populations from Arad County

No.	Population	Latitude	Longitude	No	Population	Latitude	Longitude
1.	'Gurba'	46°32’19.53” N	21°49’57.30” E	13	'Beliu'	46°27’49.59” N	21°59’12.12” E
2.	'Seleus'	46°23’0.31” N	21°39’59.75” E	14	'Ineu'	46°25’38.00” N	21°52’27.47” E
3.	'Zimandu Nou'	46°17’30.30” N	21°24’57.49” E	15	'Olari'	46°24’19.24” N	21°34’56.29” E
4.	'Sebis'	46°23’0.00” N	22°5’05.00” E	16	'Bocsig'	46°25’28.78” N	21°54’56.20” E
5.	'Lipova TF'	46°04’41.14” N	21°41’13.29” E	17	'Buteni'	46°19’05.47” N	22°08’02.74” E
6.	'Lipova'	46°04’51.60” N	21°40’43.15” E	18	'Siria'	46°15’08.45” N	21° 34’10.09” E
7.	'Ususau'	46°04’37.26” N	21°47’00.23” E	19	'Chesinț'	46°03’46.88” N	21°38’39.29” E
8.	'Patars'	46°04’37.26” N	21°47 00.23” E	20	'Almas'	46°17’0.73” N	22°14’55.78’’ E
9.	'Bacaul de Mijloc'	45°58’27.53” N	22°06’55.75” E	21	'Zabrani'	46°04’19,09” N	21°35’44.35” E
10.	'Capalnas'	45°58’37.29” N	22°13’41.01” E	22	'Brazi'	46°14’11.44” N	22°19’48.6” E
11.	'Cermei'	46°32’14.67” N	21°52’50.25” E	23	'Arad'	46°08’19.84” N	21°21’55.41” E
12.	'Vladimirescu'	46°08’30.74” N	21°24’48.36” E	24	'Fantanele'	46°04’52,18” N	21°30’56.73” E

The data collected for fruit weight and fruit pulp percentage were statistically processed by combined ANOVA and AMMI analysis using MATMODEL Version 3. The AMMI model which combines the standard analysis of variance with principal component analysis (Zobel et al., 1988), allows for investigating the nature of genotype x environment interaction. The means for each trait were compared using the Multiple Range Test (Ciulca, 2006). The significance of differences between means was presented based on letters, which were considered significant differences between means marked with different letters (a, b, c -for population x year comparisons; A, B, C - for years comparisons).
Populations were clustered using the UPGMA (unweighted pair group method with arithmetic

mean), with the NEIGHBOR program of the PHYLIP package, version 3.5c (Felsenstein, 1993).
The basic principle of the biplot technique was used to display each population's performance for both fruit traits during the three years in a single graph (Yan & Kang, 2002).

RESULTS AND DISCUSSIONS

Phenotypic expression and observed variation of plant growth and development are functions of genetic background, environment, and interaction, including temporal variation due to the genotype x year effect (Redpath et al., 2021).
The variation of different fruit traits or compounds can be revealed between different sites and in the same site (Stamin et al., 2024).

The combined analysis of the variance based on the AMMI 2 model for rosehip populations over the study period (Table 2) indicates that both the genotype and the yearly conditions, respectively, and their interaction had highly significant effects on fruit weight. The population x year interaction showed the highest influence (27.43%) on fruit weight variability, followed by population (27.23%), amid a lower influence (14.24%) of the yearly conditions during the study. The high contribution to this trait's variation indicates the existence of major differences between rosehip populations. Based on the first two principal components, this model fully expresses the effect of population x year interaction on fruit weight. As such, it is relevant to assess the stability of this trait in rosehip populations based on the first two principal components.

Table 2. Combined analysis of variance according to the AMMI 2 model for fruit weight of rosehip populations

Source of variation	SS	DF	MS	F	SS %[1]
Total	73.9	215			
Block	0.4	6	0.06	3.33	
Population	20.12	23	0.87	52.03**	27.23
Year	10.52	2	5.26	312.88**	14.24
Population x Year	20.27	46	0.44	26.21**	27.43 (*100*)
IPCA 1	13.17	24	0.55	32.64**	*64.97*
IPCA 2	7.10	22	0.32	19.20**	*35.03*
IPCA residuals	0	0			
Error	2.32	138	0.02		

[1]% of model sum of squares for population, year and population x year; **significant at $P \leq 0.01$.

Based on the data from Table 3, the conditions of 2023 have favoured the achievement of significantly higher values of fruit weight, while in 2024, the mean values were lower compared to other years. Under the conditions from 2022 the fruit weight recorded values between 1.27 g at 'Bacaul de Mijloc' and 2.25 g at 'Olari' population, amid a middle variability and arelatively symmetrical distribution of populations: 12.5% with values over 2 g; 16.7% with values of 1.7-2 g; 50% with values of 1.5-1.7 g; 20.8% with values below 1.5 g. This year, the populations 'Olari', 'Cermei', and 'Capalnas' were highlighted, which achieved a fruit weight of over 2 g. Given the conditions of 2023 the rosehip populations showed smaller amplitude of this trait, with the limits from 1.39 g at 'Buteni' to 2.2 g at 'Olari' population, associated with a 12.01 coefficient of variation and the following distribution: 16.7% of the populations with fruit weight over 2 g; 29.2% with values of 1.7-2 g; 41.6% with values of 1.5-1.7 g; 12.5% with values below 1.5 g. The amplitude of fruit weight in 2024 was intermediate to that recorded in previous years, with values ranging from 1.23 g in 'Brazi' to 2.15 g for the 'Gurba' population. Thus, the distribution of populations in 2024 showed a clear left asymmetry: only one population over 2 g; 25% with values of 1.7-2 g; 41.7% with values of 1.5-1.7 g; 20.8% with values below 1.5 g.

Table 3. Variation of fruit weight in rosehip populations during 2022-2024

No.	Population	Year			Population Mean
		2022	2023	2024	
1.	'Gurba'	1.48 z	1.77 y	2.15 x	1.80 bcd
2.	'Seleus'	1.62 x	1.66 x	1.31 y	1.53 fgh
3.	'Zimandu Nou'	1.48 y	1.70 x	1.35 y	1.51 gh
4.	'Sebis'	1.61 y	1.83 x	1.65 xy	1.70 cde
5.	'Lipova TF'	1.81 y	2.03 x	1.63 y	1.82 bc
6.	'Lipova'	1.65 x	1.76 x	1.68 x	1.70 cde
7.	'Ususau'	1.59 x	1.63 x	1.72 x	1.65 ef
8.	'Patars'	1.85 x	1.60 y	1.39 y	1.61 efg
9.	'Bacaul de Mijloc'	1.27 y	1.68 x	1.33 y	1.43 h
10.	'Capalnas'	2.04 x	1.50 y	1.42 y	1.65 ef
11.	'Cermei'	2.24 x	1.42 y	1.52 y	1.73 cde
12.	'Vladimirescu'	1.60 y	1.95 x	1.50 y	1.68 de
13.	'Beliu'	1.70 xy	1.59 y	1.87 x	1.72 cde
14.	'Ineu'	1.53 zy	1.69 y	1.92 x	1.71 cde
15.	'Olari'	2.25 x	2.20 x	1.89 y	2.11 a
16.	'Bocsig'	1.82 y	2.08 x	1.55 z	1.82 bc
17.	'Buteni'	1.47 x	1.39 x	1.52 x	1.46 h
18.	'Siria'	1.89 xy	1.97 x	1.74 y	1.87 b
19.	'Chesinț'	1.52 y	1.81 x	1.55 y	1.63 efg
20.	'Almas'	1.71 x	1.66 x	1.54 x	1.64 efg
21.	'Zabrani'	1.66 x	1.61 x	1.76 x	1.68 de
22.	'Brazi'	1.37 xy	1.49 x	1.23 y	1.36 i
23.	'Arad'	1.66 x	1.62 x	1.54 x	1.61 efg
24.	'Fantanele'	1.77 x	1.89 x	1.71 x	1.79 bcd
Year mean		1.69 B	1.73 A	1.60 C	

Populations $LSD_{5\%}$ = 0.13 g; Years $LSD_{5\%}$ = 0.04 g; Population x Year $LSD_{5\%}$ = 0.22 g
Different letters indicate significance at $P \leq 0.05$; x, y, z -for population x year comparisons.
A, B, C - for years comparisons; a,b,c –for populations comparisons

Regarding the annual values of fruit weight, it was found that about 29.17% of the populations did not show significant variations during the study period, while for two populations, the mean values differed significantly from one year to another. The top-ranking populations across the study period were 'Olari', 'Siria', 'Bocsig', 'Lipova TF', and 'Gurba', which achieved a mean fruit weight of over 1.8 g. In other studies, the fruit weight of rosehip genotypes was also found to vary around the mean of 2g (Bozhuyuk et al., 2021; Cheikh-Affene et al., 2013; Demir et al., 2021; Mertoglu et al., 2024).

Based on the biplot from Figure 1, the rosehip populations are more dispersed than the environments, indicating that population variability is greater than year-related variability, consistent with the results of ANOVA. Also, given the position of the year's points related to the x-axis, it can be noted that the values recorded in 2022-2023 are higher than those of 2024, indicating the favourability of the associated yearly conditions. 'Olari' population expresses an average stability of fruit weight associated with the highest mean over the study period, amid close values during 2022-2023. In the case of the 'Siria' population, the high mean weight of fruit was obtained because of a parallel response to the favourability of the climatic conditions, with the highest value in 2023 and the lowest in 2024. The variation of this trait in the 'Lipova TF' population indicates their specific adaptability to the favourable conditions. 'Fantanele' population registered a low and insignificant variation in fruit weight amid a general performance above the experience mean. The populations 'Lipova', 'Almas', and 'Zabrani' registered significantly constant values over the years, with an average fruit weight above the experimental mean. Also, low and insignificant variation of fruit weight was recorded by the 'Buteni' population, who had the third-lowest mean value.

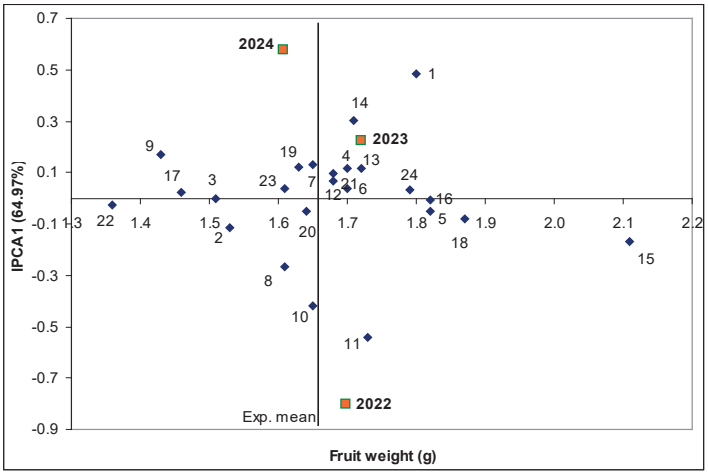


Figure 1. Biplot of mean and IPCA1 axis for fruit weight in rosehip populations during 2022-2024

Considering that the IPCA1 axis expresses approximately 64.97% of the population x year interaction from Figure 2, the conditions from 2022 showed a higher contribution to the population x year interaction, compared to those from 2023-2024. The populations close to the vector of a given year indicate a strong association with this one. Thus, the populations 'Gurba' and 'Ineu' showed a specific adaptation to the conditions of 2024 when they registered a significantly higher fruit weight than in other years. Also, the populations 'Capalnas', 'Cermei', and 'Patars' showed a strong specific adaptation to the conditions in 2022, achieving significantly higher values than those from 2023-2024. 'Bacaul de Mijloc', 'Vladimirescu', and 'Bocsig', populations recorded the highest values of fruit weight under the conditions from 2023, showing a specific adaptation to these conditions. In case of populations: 'Ususau', 'Lipova', 'Zabrani', 'Fantanele', 'Almas', and 'Arad', the close position to the origin indicates high stability of fruit weight. Considering the distance from the origin, it is observed that the populations 'Cermei', 'Gurba', and 'Capalnas' showed a low stability associated with high variation during years and different types of population x year interaction.

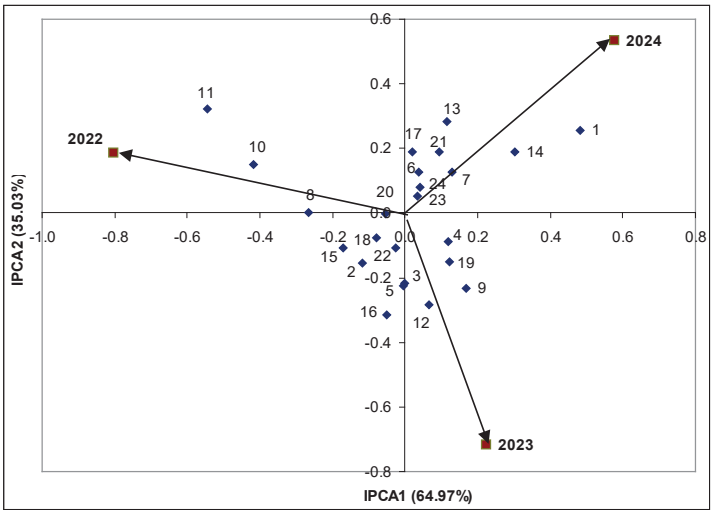


Figure 2. Biplot of interaction for principal component axis (IPCA1 and IPCA2) for fruit weight in rosehip populations during 2022-2024

The flesh ratio is one of the most important criteria regarding the quality of rosehip fruits. In developing rosehip cultivars suitable for industry, cultivars with large-sized fruits and a high flesh ratio are desired (Celik et al., 2009). The pulp percentage of rosehips increases during ripening and is highly genotype dependent (Ersoy & Salman, 2017; Medveckiene et al., 2023).

According to the analysis of the variance based on the first two components of the interaction (Table 4), it was found that all three main sources of variation had a significant influence on the fruit pulp percentage of the rosehip population during the three years. The contributions of the three sources of variation are not as balanced as in fruit weight, so the pulp percentage variability was influenced to a high extent by the population (26.18%) and the population x year interaction (23.29%). In contrast, the years had a lower influence (4.14%). Given that the first two main components fully express the effect of population x year interaction, it turns out that the AMMI2 model is suitable for assessing pulp percentage at this set of populations.

Table 4. Combined analysis of variance according to the AMMI 2 model for fruit pulp percentage of rosehip populations

Source of variation	SS	DF	MS	F	SS %[1]
Total	5295	215			
Block	73	6	12.17	1.46	
Population	1386	23	60.26	7.23**	26.18
Year	219	2	109.50	13.13**	4.14
Population x Year	1233	46	26.80	3.21**	23.29 (*100*)
IPCA 1	775	24	32.29	3.87**	*62.85*
IPCA 2	458	22	20.82	2.50**	*37.15*
IPCA residuals	0	0			
Error	1151	138	8.34		

[1]% of model sum of squares for population, year and population x year; **significant at $P \leq 0.01$.

Table 5. Variation of fruit pulp percent in rosehip populations during 2022-2024

No.	Population	Year			Population
		2022	2023	2024	Mean
1.	'Gurba'	57.46 y	67.24 x	68.16 x	64.29 cdef
2.	'Seleus'	62.34 x	60.20 x	60.24 x	60.93 ghijk
3.	'Zimandu Nou'	58.08 y	54.51 y	64.16 x	58.92 jkl
4.	'Sebis'	56.19 y	60.73 y	66.96 x	61.29 ghij
5.	'Lipova TF'	67.83 x	67.62 x	69.60 x	68.35 a
6.	'Lipova'	65.40 y	65.56 y	71.29 x	67.42 ab
7.	'Ususau'	64.42 x	62.11 x	61.71 x	62.75 defgh
8.	'Patars'	66.55 x	64.57 x	67.30 x	66.14 abc
9.	'Bacaul de Mijloc'	58.77 x	61.38 x	62.12 x	60.76 hijkl
10.	'Capalnas'	58.15 y	65.43 x	64.93 x	62.84 defgh
11.	'Cermei'	60.69 y	65.86 x	67.55x	64.70 bcde
12.	'Vladimirescu'	58.93 x	56.58 x	59.07 x	58.19 l
13.	'Beliu'	59.43 y	60.44 y	66.42 x	62.10 defghi
14.	'Ineu'	61.86 x	66.41 x	66.07 x	64.78 bcd
15.	'Olari'	64.35 xy	60.91 y	68.75 x	64.67 bcde
16.	'Bocsig'	62.78 xy	61.04 y	66.69 x	63.50 cdefg
17.	'Buteni'	62.29 x	62.58 x	61.01 x	61.96 fghi
18.	'Siria'	57.43 xy	56.31 y	61.64 x	58.46 kl
19.	'Chesinț'	66.54 x	60.61 y	61.75 y	62.97 defgh
20.	'Almas'	60.32 x	64.63 x	61.08 x	62.01 efghi
21.	'Zabrani'	67.33 x	60.35 y	61.03 y	62.90 defgh
22.	'Brazi'	60.12 y	66.38 x	60.14 y	62.21 defghi
23.	'Arad'	60.80 x	59.35 x	58.81 x	59.65 ijkl
24.	'Fantanele'	62.83 x	62.13 x	62.14 x	62.37 defgh
Year mean		61.70 B	62.20 B	64.11 A	

Populations $LSD_{5\%} = 2.69$; Years $LSD_{5\%} = 0.95$; Population x Year $LSD_{5\%} = 4.66$

Different letters indicate significance at $P \leq 0.05$; x, y, z - for population x year comparisons.

A, B, C - for years comparisons; a, b, c - for populations comparisons.

The yearly conditions during the study influenced fruit pulp percentage, causing significant variations (Table 5). Thus, the conditions from 2024 were significantly more favourable for increasing the pulp percentage of fruit compared to those of 2022-2023.

The amplitude of the plant height for the rosehip population in 2022 was 11.64, ranging from 56.19 in 'Sebis' to 67.86 in 'Lipova TF'. Thus, the distribution of genotypes showed a low asymmetry: only 33.3% of the populations had values below 60; 45.8% had values of 60-65; and 20.9% had a pulp percentage over 65.

Under the conditions from 2023 the fruit pulp percentage recorded values between 54.51 in 'Zimandu Nou' and 67.62 in 'Lipova TF' population, on the background of a right asymmetry of the population's distribution: 29.2% of the populations with values over 65; 54.2% with values of 60-65%; 16.6% with a pulp percentage of fruits below 60. The highest fruit percentage in 2022-2023 was recorded by the 'Lipova TF' population.

Given the conditions of 2024, there was an increase in fruit pulp percentage for most populations. The amplitude of this trait (12.48) was intermediate to the previous years, ranging from 58.81 in 'Arad' to 71.29 in 'Lipova' population. The distribution of genotypes in this year showed a left asymmetry, with the following structure: 41.7% of the populations had values over 65; 50% had values of 60-65; only two populations had a pulp percentage of fruit below 60. Other studies reported similar pulp percentage values between 60 and 70 (Bozhuyuk et al., 2021; Ercisli & Esitken, 2004).

Considering the annual averages of pulp percentage, approximately 50 % of the population did not register significant variations in fruit pulp percentage during the study. Most populations (54.2%) used the conditions from 2024 better, showing significantly higher pulp percentages than 2022-2023. The populations 'Lipova TF' and 'Lipova' are highlighted through significantly higher mean values compared to 75 % of the populations.

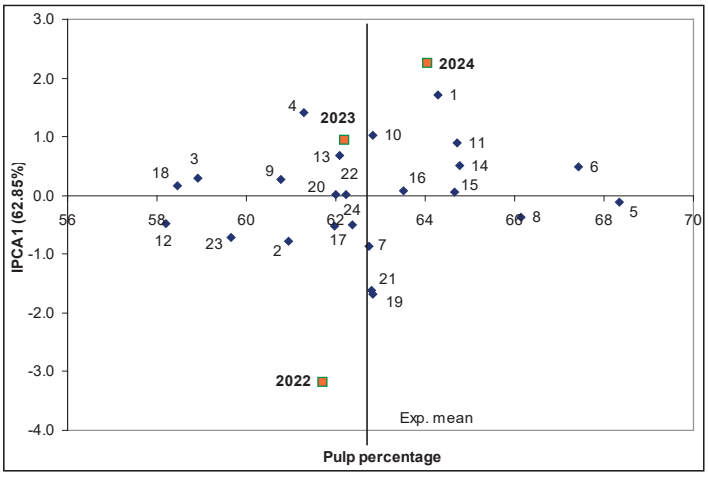


Figure 3. Biplot of mean and IPCA1 axis for pulp percent in rosehip populations during 2022-2024

Depending on the positions of the three years concerning the first component axis (Figure 3), the most favourable conditions for the growth of rosehip fruits were recorded in 2024, while the fruit weight presented the lowest values in 2022.

The high stability of the 'Lipova TF' population, revealed by its low IPCA1 value, was associated with the highest fruit pulp percentage over the three years. Also, the 'Lipova' population showed the second-ranking performance and average stability. The populations 'Olari' and 'Bocsig' achieved values above the general mean and good stability. In the case of the 'Vladimirescu' population, the high stability of this trait was associated with the lowest performance.

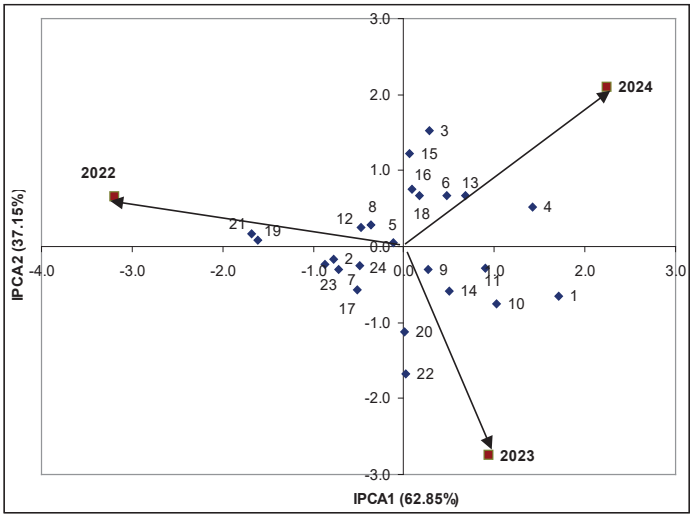


Figure 4. Biplot of interaction for principal component axis (IPCA1 and IPCA2) for pulp percent in rosehip populations during 2022-2024

The biplot from Figure 4, based on the first two components, indicates that 2022 contributed the most to the interaction between populations and climatic conditions, while 2023-2024 had considerably lower effects. According to the distance from the origin, the populations 'Lipova TF', 'Patars', 'Seleus', 'Vladimirescu', and 'Arad' presented the highest stability of fruit pulp percentage.

The length and position of the vectors for lines 'Gurba' and 'Sebis' indicate a high instability of fruit weight, associated with the highest values in 2024 and the lowest in 2022. In the 'Chesint' and 'Zabrani' populations, the low stability was associated with the highest fruit pulp percentage in 2022 and the lowest in 2023. The populations 'Almas' and 'Brazi' expressed a specific adaptation to the conditions from 2023, being positioned close to the vector of this year. 'Zimandu Nou', 'Sebiş', and 'Beliu' populations have shown a specific adaptation to the conditions from 2024, registering a significantly higher increase than in the other years.

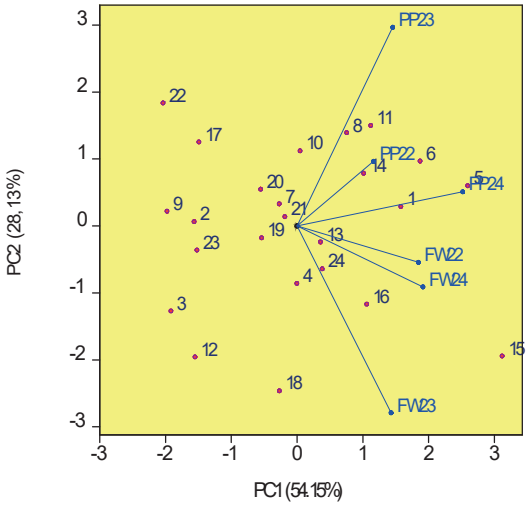


Figure 5. Biplot of the first two principal components for pulp percentage and fruit weight in rosehip populations during 2022-2024

Considering the rosehip populations' performances for the two traits during 2022-2024, the first two principal components from Figure 5 express 82.28% of the total variability. Negative PC2 values indicate populations with high fruit weight values, while positive PC2 values are characteristic of populations with a high pulp percentage. Pulp percentage is one of the most important criteria for the quality of rosehip fruit.

Depending on their position about the vectors of the various characters x years, the 'Lipova TF', 'Lipova', and 'Cermei' populations recorded the highest pulp yields over the entire period. The second-ranking group for this trait comprised the populations 'Gurba', 'Patars', and 'Ineu'. All these populations achieved fruit weight over the general mean.

The highest fruit weight of the 'Olari' population was associated with a pulp percentage above the mean, while the population 'Bocsig' presented high fruit weight with an average pulp percentage. The populations 'Siria' and 'Fantanele' have shown fruit weight over the mean with a pulp percentage below the mean. The small fruits of 'Brazi' and 'Buteni' populations were associated with an average pulp percentage. Cultivars with large-sized fruits and a high pulp percentage are desired and suitable for the industry (Celik et al., 2009).

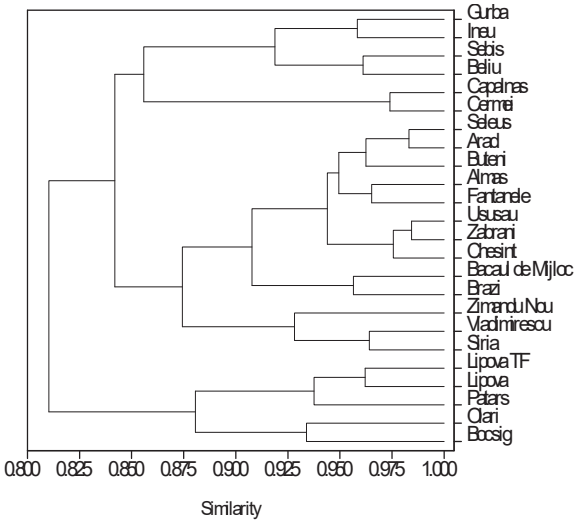


Figure 6. UPGMA clustering of rosehip populations regarding the pulp percentage and fruit weight during 2022-2024

Based on the UPGMA method, the hierarchical clustering from Figure 6 grouped the rosehip populations in four major clusters. The cluster size varied, with a larger number of populations in cluster two containing ten populations (42%), grouped into four subclusters, with an approximately 91 % intra-cluster similarity. Cluster one was the second largest, comprising six populations (25%), with a diversity of 16%. Cluster three was the smallest, with three populations sharing a 63 % similarity. Cluster four was composed of five populations, which possessed about 88% common characteristics of the two fruit traits. The diversity between the studied populations can result from the degree of heterogeneity normal in cross-pollinating plants of any natural rosehip population (Ersoy & Salman, 2017; Guler et al., 2021; Rovna et al., 2020).

CONCLUSIONS

The population has the highest contribution of 25.83-26.18% to the variability of both fruits' traits, followed by the population x year interaction with a contribution of 23.29-26.02%. The effect of the yearly conditions was lower, at 13.5% for fruit weight and 4.14 % for pulp percentage, respectively. The populations 'Lipova', 'Almas', and 'Zabrani' expressed a stable fruit weight over the years, with an average above the experience mean. The high stability

of 'Lipova TF' and 'Patars' populations was associated with fruit pulp percentage over the general mean.

The present study identified a thorn-free rosehip population ('Lipova TF'), which can be helpful both for production and breeding programs to obtain thorn-free genotypes for easy harvest. 'Olari' and 'Bocsig' populations with large fruits and high pulp percentages can be propagated vegetatively and used for production, or might be used as parents in breeding programs to develop new rosehip genotypes with large fruits.

REFERENCES

Andronie, L., Holonec, L., Ioana, P.O.P., Truta, A.M., Odagiu, A., Sălăgean, T., Sobolu, R., Coroian, A., Balta, I., & Suba, E.E. (2019). Antioxidant capacity of several Romanian forest fruits (*Rosa canina* L., *Prunus spinosa* L., *Vaccinium vitis-idaea* L., and *Cornus mas* L.). *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, *47*(4), 1178–1184.

Bozhuyuk, M.R., Ercisli, S., Karatas, N., Ekiert, H., Elansary, H.O., & Szopa, A. (2021). Morphological and biochemical diversity in fruits of unsprayed *Rosa canina* and *Rosa dumalis* ecotypes found in different agroecological conditions. *Sustainability. 13*(14), 8060.

Bruneau, A., Starr, J. R., & Joly, S. (2007). Phylogenetic relationships in the genus Rosa: new evidence from chloroplast DNA sequences and an appraisal of current knowledge. *Syst. Bot. 32*, 366–378.

Butkeviciute, A., Urbštaite, R., Liaudanskas, M., Obelevicius, K., & Janulis, V. (2022). Phenolic content and antioxidant activity in fruit of the genus Rosa L. *Antioxidants 11*, 912.

Celik, F., Kazankaya, A., & Ercisli, S. (2009). Fruit characteristics of some selected promising rose hip (Rosa spp.) genotypes from Van region of Turkey. *Afr J Agric. Res. 4* (3), 236-240.

Chae, S.H., Lee, Y.S., Kim, J.H., Han, T.H., & Ku, K.M. (2021). Metabolite and Elastase Activity Changes in Beach Rose (Rosa rugosa) Fruit and Seeds at Various Stages of Ripeness. *Plants. 10,* 1283.

Cheikh-Affene, Z.B., Haouala, F., Trabelsi, N., Boulaaba, M., Ksouri, R., & Harzallah-Skhiri, F. (2013). Pomological description and chemical composition of rosehips gathered on four *Rosa* species section caninae growing wild in Tunisia. *Int J Agric Sci Tech. 1*(3), 43–50.

Ciulca, S. (2006). *Metodologii de experimentare în agricultura si biologie (Experimental methodologies in agriculture and biology).* Agroprint, Timisoara, Romania.

Cosmulescu, S., Trandafir, I., & Nour, V. (2017). Phenolic acids and flavonoids profiles of extracts from edible wild fruits and their antioxidant properties. *Int. J. Food Prop. 20*, 3124–3134.

Demir, B., Sayıncı, B., Yaman, M., Sümbül, A., Yıldız, E., Karakaya, O., Alkaya, G.B., & Ercişli, S. (2021). Biochemical composition and shape-dimensional traits of rosehip genotypes. *Folia Hortic. 33*(2), 293–308.

Elmastas, M., Demir, A., Genç, N., Dölek, Ü., & Günes, M. (2017). Changes in flavonoid and phenolic acid contents in some Rosa species during ripening. *Food Chem. 235*, 154–159.

Ercisli, S., & Esitken, A. (2004). Fruit characteristics of native rose hip (Rosa spp.) selections from the Erzurum province of Turkey. *New Zealand J. Crop Hort. Sci. 32*, 51-53.

Ersoy, N., & Salman Özen, M. (2017). Some physico-chemical characteristics in fruits of Rose hip (Rosa Spp.) genotypes from Bolu province in Western part of Turkey. *Agro-knowledge. 17*, 191.

Felsenstein, J. (1993). PHYLIP (Phylogeny Inference Package) Version 3.5c; Distributed by the author; Department of Genetics, University of Washington: Seattle, WA, USA.

Gauch, H.G., & Zobel, R.W. (1990). Imputing missing yield trial data. *Theor Appl Genet. 79*, 753–761.

Gruenwald, J., Uebelhack, R., & Moré, M.I. (2019). Rosa canina—Rose hip pharmacological ingredients and molecular mechanics counteracting osteoarthritis—A systematic review. *Phytomedicine. 60*, 152958.

Gulbagca, F., Ozdemir, S., Gulcan, M., & Sen, F. (2019). Synthesis and characterization of Rosa canina-mediated biogenic silver nanoparticles for antioxidant, antibacterial, antifungal, and DNA cleavage activities. *Heliyon. 5*, e02980.

Guler, E., Bak, T., Karadeniz, T., & Muradoglu, F. (2021). Relationships of fruit characteristics of rosehips (Rosa canina L.) grown in Bolu city center. *J. Inst. Sci. Technol. 11*, 831–838.

Güneş, M., & Dölek, Ü. (2010). Fruit characteristics of promising native rosehip genotypes grown in Mid-north Anatolia region of Turkey. *J Food Agric Environ. 8*(2), 460–3.

Kulaitiene, J., Medveckiene, B., Levickiene, D., Vaitkeviciene, N., Makareviciene, V., & Jariene, E. (2020). Changes in fatty acids content in organic rosehip (Rosa spp.) seeds during ripening. *Plants. 9*, 1793.

Medveckiene, B., Kulaitiene, J., Levickiene, D., & Hallmann, E. (2021). The effect of ripening stages on the accumulation of carotenoids, polyphenols, and vitamin C in rosehip species/cultivars. *Appl. Sci. 11*, 6761.

Medveckiene, B., Levickiene, D., Vaitkeviciene, N., Vaštakaite-Kairiene, V., & Kulaitiene, J. (2023). Changes in pomological and physical parameters in rosehips during ripening. *Plants. 12*, 1314.

Mertoğlu, K., Durul, M.S., Korkmaz, N., Polat, M., Bulduk, I., & Esatbeyoglu, T. (2024). Screening and classification of rosehip (*Rosa canina* L.) genotypes based on horticultural characteristics. BMC Plant Biol. *24*(1), 345.

Munteanu, A.L.**,** Vijan, L.E., Topală, C.M., & Mitrea, R. (2023). Influence of the phytosanitary status, cultivar, and harvest time on the phenolic, chlorophyll, and alkaloid content of Rosa sp. leaves. *Horticulturae. 9*, 1169.

Oargă Porumb, D.P., Cornea-Cipcigan, M., & Corde,a M.I. (2024). Unveiling the mechanisms for the

development of rosehip-based dermatological products: an updated review. *Front. Pharmacol. 15*, 1390419.

Redpath, L.E., Gumpertz, M., Ballington, J.R., Bassil, N., & Ashrafi, H. (2021). Genotype, environment, year, and harvest effects on fruit quality traits of five blueberry (*Vaccinium corymbosum* L.) cultivars. *Agronomy. 11*, 1788.

Rovná, K., Ivanišová, E., Žiarovská, J., Ferus, P., Terentjeva, M., Kowalczewski, P.Ł., & Kacániová, M. (2020). Characterization of Rosa canina fruits collected in urban areas of Slovakia. Genome size, iPBS profiles and antioxidant and antimicrobial activities. *Molecules. 25*, 1888.

Shirdel, M., Motallebi-Azar, A., Matloob, I.M., & Zaare-Nahandi, F. (2013). Effects of nodal position and growth regulators on in vitro growth of dog rose (*Rosa canina*). *Journal of Ornamental and Horticultural Plants. 3* (1), 9–17.

Soare, R., Iancu, P., Soare, M., Bonea, D., Manila, G., & Panita, O. (2014). Morfological and biochemical issues to some Rosa canina L. populations from the spontaneous flora from Southwest Romania. *Micro and Nano Tehnologies advances in biotechnology. 1*, 432-442.

Soare, R., Bonea, D., Iancu, P., & Niculescu, M. (2015). Biochemical and technological properties of *Rosa canina* L. fruits from spontaneous flora of Oltenia, Romania. *Bulletin of the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Horticulture. 72*(1), 182-186.

Stamin, F.D., Vijan, L.E., Topală, C.M., & Cosmulescu, S.N. (2024). The influence of genotype, environmental factors, and location on the nutraceutical profile of *Rosa canina* L. fruits. *Agronomy. 14*, 2847.

Tomljenovic, N., & Pejić, I. (2018). Taxonomic review of the genus Rosa. Agric. *Conspec. Sci. 83*, 139–147.

Winther, K., Vinther Hansen, A.S., & Campbell-Tofte, J. (2016). Bioactive ingredients of rose hips (Rosa canina L) with special reference to antioxidative and anti-inflammatory properties: *in vitro* studies. *Botanics: Targets and Therapy*. *6*, 11-23

Yan, W., & Kang, M.S. (2002). GGE Biplot analysis: A graphical tool for breeders, geneticists, and agronomists, 1st ed.; CRC Press: Boca Raton, FL, USA.

Zobel, R.W., Wright, M.J., & Guach, G.H. (1988). Statistical analysis of yield trial. *Agronomy Journal. 80*, 388–393.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

THE REACTION OF SOME APRICOT CULTIVARS AND SELECTIONS TO THE ATTACK OF SOME PATHOGENS AND FROST IN THE CONTEXT OF CLIMATE CHANGE

Cristina MOALE, Vlăduț Alexandru OPRIȚA, Leinar SEPTAR, Ion CAPLAN, Ioan STOLI

Research Station for Fruit Growing Constanţa, 25 Pepinierei Street, 907300, Valu lui Traian, Constanţa, Romania

Corresponding author email: moalecristina67@gmail.com

Abstract

The main objective of the works was to identify genotypes with increased tolerance or resistance to pathogens under the current climatic conditions for their extension into the culture. The trials on 20 apricot cultivars and selections were carried out in the orchard, during 2020-2022, under natural infection conditions, tracking the frequency and intensity of the attack on different organs of the plants and how they were influenced by current climate changes. These studies demonstrated that the most resistant to Monilinia laxa, Cytospora cincta and Stigmina carpophila were 4 cultivars ('Mamaia', 'Roşii de Mărculeşti', 'Olimp' and 'Elmar'), while 3 other cultivars ('Litoral', 'Pionier' and 'Sirena') had sensitivity to the attack of Cytospora cincta and Stigmina carpophila. The biggest losses caused by frost were recorded during the winter/spring of 2021 in terms of damaged fruit buds: 90% on 'Comandor' 88% on 'Pionier', 85% on 'Roşii timpurii' and 'Sulina', 67% on 'Sirena' and 65% on 'Mari de Mărculeşti'. The nursery has propagated resistant cultivars and selections, while the most valuable genotypes are grown in commercial orchards.

Key words*: Prunus armeniaca, genotypes, diseases, frequency, intensity, warning.*

INTRODUCTION

Apricot (*Prunus armeniaca* L.) is cultivated with good results in the Southeastern region of Romania and is a highly valued fruit tree due to its desirable biological traits, including early fruiting, consistent and abundant yields, ecological adaptability and exceptional fruit quality. However, this species remains highly vulnerable to infections from multiple fungal pathogens, such as *Monilinia laxa* (brown rot), *Stigmina carpophila* (shot hole disease), *Stereum purpureum* (silver leaf), *Gnomonia erytrosthoma* (leaf spot), *Cytospora cincta* (perennial canker), and *Schizophyllum commune*, included among the fungal threats to this species. The genetic resistance of various varieties and selections to diseases plays a crucial role in reducing costs and minimizing environmental impact. These benefits have led to disease and pest resistance becoming a key priority at RSFG Constanța. Multiple studies have previously aimed at improving genetic resistance to diseases and pests, including the work of Trandafirescu Marioara (1989), Trandafirescu M. et al. (2005; 2006) and the creation of apricot varieties Topor E. (1997). Susceptibility and resistance of apricot cultivars and selections to *Monilinia laxa* was studied by Benedek et al. (1990) in Hungary, Komar-Tyomnaya and Richter (2000) in Ukraine, Tzonev and Yamaguchi (1999) in Japan, Balan *et al*. (1999) in Romania, Crossa-Raynaud (1969) in Tunesia, Nicotra et al. (2006) in Italy. In the Mediterranean region, Crossa-Raynaud (1969) identified ‘Hamidi’ as a variety resistant to *Monilinia laxa*. Additionally, both Nicotra et al. (2006) and Balan et al. (1999) reported a high level of resistance in ‘Precoce de Italia’. Research by Komar-Tyomnaya and Richter (2000) at the Nikita Botanical Garden indicated that all studied apricot varieties were vulnerable. In Europe, brown rot of decaying fruit had its first published description in 1796. In the USA it was observed in 1807 for the first time. Identified for the first time in Romania in 1878, this disease has progressively expanded over time (Amzăr and Ivașcu, 2003). European apricot breeding programs primarily focus on developing resistance to major diseases, such as plum pox virus, bacterial infections, brown rot, and chlorotic leaf roll phytoplasma, while also

addressing apricot decline syndrome. Other major aims include boosting adaptability to environmental conditions (such as water stress and temperature shifts), extending the harvesting window, enhancing both productivity and fruit quality, and optimizing tree structure and size (Bassi & Audergon, 2006). Numerous researchers worldwide have studied the adaptability of different varieties and the breeding of new ones tailored to the specific biotic and abiotic factors of their respective countries (Audergon et al., 1995; Bassi et al., 1995; Witherspoon, 1999).

MATERIALS AND METHODS

This study was carried out at the Research Station for Fruit Growing Constanța, situated in the Dobrogea region of Romania. The experimental area is positioned in Valu lui Traian, close to the Black Sea, at coordinates 44°10' N and 28°28' E. Between 2020 and 2022, experiments were performed at RSFG Constanța, Romania, with the goal of analyzing the resistance of various apricot selections and cultivars to pathogen attacks in a changing climate. The study focused on 20 apricot genotypes cultivated in a demonstration plot set up in 2011. The orchard layout consists of rows with 20 trees each, spaced at 4 × 4 meters, resulting in a density of 625 trees per hectare. 'Constanța 14' was used as the rootstock, with trees trained in an improved vase form. The soil type is calcareous chernozem (CZKa), characterized by a loamy texture and an alkaline pH of 8.2. Over the past decade, climate changes have disrupted the natural expression of varietal traits, further influenced by biotic factors and inconsistent cultivation conditions, such as periodic water shortages. Among the genotypes studied are: 'Litoral', 'Comandor', 'Mari de Geoagiu', 'Mamaia', 'Pioner', 'Mărculești 22/4', 'Augustin', 'Roșii de Mărculești', 'Olimp', 'Dulci de Vișani', 'Centenarul Unirii', 'București 500', 'Roșii Timpurii', "Elmar', 'Mărculești 10/5', 'Mari de Aiud', 'Sulina', 'Sirena', 'Mari de Mărculești', 'Saturn'. Observations were conducted to assess the response of various apricot genotypes to infections by major pathogenic agents, particularly *Monilinia laxa* (Aderh. & Ruhl) Honey, *Cytospora cincta* Sacc and *Stigmina carpophila* (Lév.) M.B. Ellis.

Data collection was performed on each row in four replicates per variety/selection, with at least five trees included in each replicate. The research focused on the most sensitive genotypes after a general assessment was carried out, aiming to highlight the most sensitive and tolerant to some pathogens in the context of climate change.

$$\mathbf{F\ [\%] = (n / N) \times 100,}$$

where: F [%] = attack frequency; n = number of diseased organs; N = total number of examined organs.

$$\mathbf{DD\%\ [\%] = [(sum\ (F\% \times I_notes) / n) / 100]}$$

where: DD [%] = damages degree, **F%** = class attack frequency; **I** = attack intensity scored with 0-7 notes; **n** = number of attack classes.

The response of apricot genotypes to infections by major pathogens - *Monilinia laxa* (Aderh. & Ruhl) Honey, *Cytospora cincta Sacc*., and *Stigmina carpophila* (Lév.) M.B. Ellis - was evaluated under natural infection conditions based on the method developed by Crossa-Raynaud (1969). The assessment involved recording both the frequency of infected organs and the severity of symptoms, which were then used to determine varietal resistance.

The study involved recording pathogen attack frequency (F%) and attack intensity (I) on several tree organs, such as leaves, blossoms, young shoots, branches, and fruit. A scale from 0 to 4 was used to assess disease severity. Based on these parameters, apricot genotypes were divided into four resistance classes and further assigned to eight resistance groups, as detailed in Table 1.

Table 1. Classification of Cultivars by Resistance Classes and Groups

Resistance class	Resistance group	Frequency (F%)	Intensity (I%)
1 = tolerant (T)	1	0	0
2 = medium resistance (MR)	2	0.1-11.0	+
	3	11.1-25.0	+
3 = sensitive (S)	4	25.1-34.0	++
	5	34.1-50.0	++
4 = very sensitive (VS)	6	50.1-59.0	+++
	7	59.1-75.0	+++
	8	75.1-100	++++

WA (Without Attack): No infection detected (F% = 0, I = 0).
T (Tolerant): Infection levels remain very low (F% = 0.1–5%, I = +).
WeA (Weakly Attacked): Slight infection observed (F% = 5.1–10%, I = +).
MA (Moderately Attacked): Infection reaches moderate levels (F% = 10.1–25%, I = ++)
S (Sensitive): High infection pressure noted (F% = 25.1–50%, I = ++++).
VS (Very Sensitive): Severe infection recorded (F% = 50.1–100%, I = ++++).

The average temperature for the period 01.03.2020 ÷ 17.03.2020 was between 6.3ºC and 10.5ºC. Average lows ranged from 0.7ºC to 6.1ºC. The absolute minimum was -5.6ºC and the absolute maximum was 17.1ºC. During this period, the amount of precipitation was 3.4 mm. The minimum temperatures recorded on March 15, 16, 17, of -3.4ºC, -5.2ºC and -5.6ºC, respectively, affected the apricot genotypes in full bloom, the resistance of the floral organs decreasing as the vegetation progressed. Between 01.01. and 31.01.2021, the average air temperature fluctuated between -0.8ºC and 7.0ºC. The average lows were between -3.4ºC and 3.5ºC. The absolute minimum was -9.3ºC (17.01.2021) and the absolute maximum was 15.7ºC (23.01.2021). In January, the amount of precipitation was 86.0 mm, above the normal of the area (records stretching over 27 years), respectively 32.9 mm for the month of January. Between 01.02. and 28.02.2021, the average air temperature varied between -1.9ºC and 7.0ºC. The average lows were between -6.5ºC and 3.2ºC. The absolute minimum was -13.4ºC and was recorded on 13.02.2021, and the absolute maximum was 18.9ºC and was recorded on 11.02.2021. The amount of precipitation was 18.8 mm, below the normal of the area, respectively 24.4 mm for the month of February. In the interval 01.03. - 15.03.2021, the average air temperature varied between 3.9ºC and 4.2ºC. The average minimum was between -0.8ºC and -1.0ºC, with an absolute minimum of -5.5ºC (03.07.2021). Average highs varied between 8.7ºC and 10.0ºC, with an absolute high of 18.5ºC (03/05/2021). The amount of precipitation recorded in this time interval was 30.4 mm, a value close to the normal of the area for the month of March, namely 36.9 mm. There were production losses that occurred due to the high temperature amplitude that occurred between February, 10-13. On February 13, the on-board weather station indicated a minimum temperature of -13.4°C, while the previous days saw temperatures above +15.0°C. In the years 2022, the genotypes studied did not register losses. Observations and determinations took place 3-5 days after the recorded climatic accidents. This study examined the response of various apricot varieties and selections to changing climatic conditions in Southeastern Romania during the winters of 2020, 2021, and 2022. Due to recent climate variations, the resistance of these varieties fluctuated from year to year. Throughout the study period (2020-2022), climatic data were collected using an automated WatchDog weather station (Spectrum Technologies Inc., IL, USA) and analyzed in terms of daily mean values. Data collected were periodically moved to a laptop for detailed examination. The occurrence of late frosts and frosts must be taken into account, which due to the climate changes of recent years are making their presence felt more and more often and can completely compromise fruit production. It is a physiological trait of frost resistance of apricot flower buds conditioned by a series of factors such as: absolute temperature, the physiological state of the trees and the temperature evolution from the end of winter dormancy to flowering (Balan et al., 2008). All collected data were stored, organized, analyzed, and visually represented using MS Excel xxx tools. A correlation was determined between the average frost-damaged buds and the mean disease incidence during the 2020-2022 vegetation periods, applying the regression calculation method.

RESULTS AND DISCUSSIONS

Although Southeastern Romania has traditionnally been regarded as highly favorable for apricot cultivation, the species has been increasingly affected by climatic variations over the past decade. The primary challenge has been the fluctuation between extreme low temperatures and sudden warm spells, particularly after the trees have resumed growth.

The influence of temperatures on apricot during the studied period had very varied aspects in correlation with many other factors such as: the variety with its genetic characteristics and properties (early-flowering varieties were sometimes saved over late-flowering ones and even vice versa); agrotechnical soil and plant maintenance (well-maintained and properly developed trees have a greater tolerance to low temperatures); the moment of negative temperatures (after waves of positive temperatures above the biological threshold of

+6.5ºC or alternatively); the topographical position of the parcels, as a result of the fact that negative temperatures manifest themselves differently both on the coordinates of altitude (large variations between the values of soil and air temperatures), as well as on those of latitude and longitude (the downstream part being more or totally affected, and the upstream part less or not at all, sometimes even vice versa; the direction and intensity of the wind, drought, etc. Late frosts in March and April, occurring after a period of relative warmth, pose a greater threat to apricot trees than those during the mandatory dormancy period in December and January. Additionally, the notable climatic shifts in recent years have severely affected flowering onset, fruit set, and, as a result, disease occurrence and total apricot yield.

In 2021, the 'Comandor' variety experienced the highest losses due to late frosts, reaching 90%, despite an abundant initial flowering. Similarly, the 'Pioneer' variety suffered an 88% loss in the same year. In contrast, the lowest percentage of affected fruit was recorded in the 'Litoral' variety, with only 12% damage in 2022 (Figure 1).

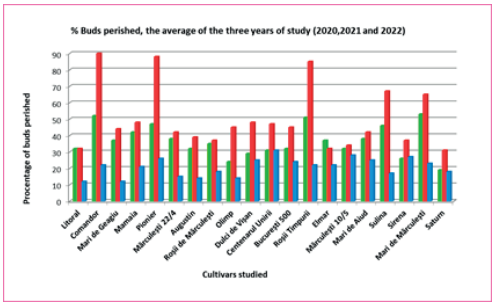


Figure 1. Extent of frost damage to apricot flowering buds during the winters of 2020, 2021, and 2022 in Valu lui Traian, Constanța

The three-year study average indicated that the evaluated apricot varieties and selections demonstrated good resistance to winter frost. The recorded bud loss percentages were as follows: 'Saturn' – 23%, 'Litoral' – 25%, and 'Augustin' and 'Olimp' – 28% (Figure 2).

The minimum temperatures recorded on March 15, 16, 17, of -3.4 ºC, -5.2 ºC and -5.6 ºC, respectively, affected the apricot cultivars ('Comandor' and 'Pioner') in full bloom, the resistance of the floral organs decreasing as the vegetation progressed (Figure 3 a, b).

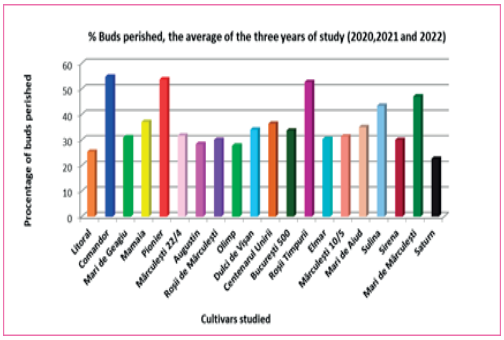


Figure 2. Percentage of apricot tree flowering buds lost to frost (three-year average) in Valu lui Traian, Constanța

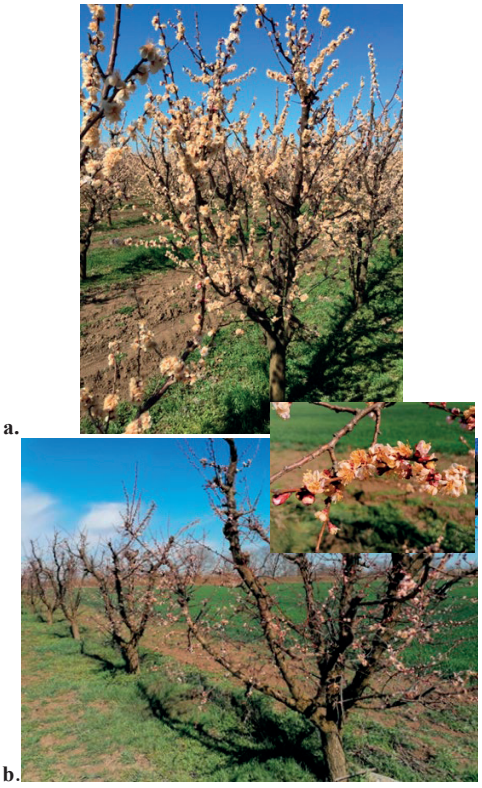


Figure 3. 'Comandor' cultivar 90% affected (a); 'Pioner' cultivar 88% affected (b)

Every year apricot plantations are attacked by a large number of diseases and pests, which produces significant damage from an economic point of view. Practical knowledge of some elements essential biology and ecology of diseases and pests in apricot species as well as the mode of attack on the plants and the effect of the damage, allows the concerned fruit

grower to choose the best decisions to control them rationally (Grigore Mihăescu, 1998). Given that observations were conducted on a large number of apricot varieties and selections, Table 2 presents the behavior of only a selected subset of the extensive biological material studied.

Thus, the genotypes were included in the class with genetic resistance (T) to *Monilinia laxa*, *Cytospora cincta* and *Stigmina carpophila* were 4 cultivars 'Mamaia', 'Roșii de Mărculești', 'Olimp' and 'Elmar', while the other three cultivars 'Litoral', 'Pionier' and 'Sirena', exhibited susceptibility to infections caused by *Cytospora cincta* and *Stigmina carpophila*. In the very sensitive (VS) class, the 'Comandor' and 'Mari de Mărculești' cultivars were highlighted in the 2020 study year and the 'București 500' variety in 2021.

Table 2. The behavior of apricot genotypes when attacked by the main pathogens during 2020-2022

No	Cultivars	Year	*Monilinia laxa*			*Cytospora cincta*			*Stigmina carpophila*		
			F %	I	Resist. class	F %	I	Resist. class	F %	I	Resist. class
1.	Litoral	2020	25	2	S	37.6	2	S	31.3	2	S
		2021	4.6	+2	MR	32.4	2	S	25.4	3	S
		2022	15.6	+2	MR	43.6	3	S	52.4	2	S
2.	Comandor	2020	80.5	4	VS	73.2	3	VS	69.5	3	VS
		2021	78.2	4	VS	80.3	4	VS	73.2	3	VS
		2022	69.1	3	VS	24.2	1	MR	13.2	1	MR
3.	Mari de Geagiu	2020	22.5	2	S	16.4	2	MR	24	1	MR
		2021	76.4	4	VS	0	0	T	19.4	2	MR
		2022	35.2	2	MA	12.6	1	MR	14.6	2	MR
4.	Mamaia	2020	0	0	T	0	0	T	0	0	T
		2021	0	0	T	0	0	T	0	0	T
		2022	0	0	T	0	0	T	0	0	T
5.	Pioner	2020	4.7	1	MR	35.3	2	S	32	2	S
		2021	4.5	1	MR	45.7	3	S	37.3	2	S
		2022	0	0	T	37.4	2	S	22.9	3	S
6.	Mărculești 22/4	2020	44.2	1	MR	65.4	3	VS	0	0	T
		2021	42.4	1	MR	72	3	VS	65.7	3	VS
		2022	47.2	1	MR	21.4	1	MR	24.3	2	MS
7.	Augustin	2020	10.2	0	MR	0	0	T	0	0	T
		2021	12.6	1	MR	0	0	T	0	0	T
		2022	17.4	1	MR	0	0	T	0	0	T
8.	Roșii de Mărculești	2020	0	0	T	0	0	T	0	0	T
		2021	0	0	T	0	0	T	0	0	T
		2022	0	0	T	0	0	T	0	0	T
9.	Olimp	2020	0	0	T	0	0	T	0	0	T
		2021	0	0	T	0	0	T	0	0	T
		2022	0	0	T	0	0	T	0	0	T
10.	Dulci de Vișani	2020	58.7	3	VS	13.5	2	MR	32.2	2	MR
		2021	45.4	3	S	61.6	3	VS	0	0	T
		2022	15.7	1	MR	37.2	2	MR	18	2	MR
11.	Centenarul Unirii	2020	14.2	1	MR	0	0	T	73	4	VS
		2021	17.1	1	MR	12.2	2	MR	39	2	MR
		2022	21.4	1	MR	0	0	T	25.9	3	S
12.	București 500	2020	44.4	2	S	61.7	3	VS	23.7	2	MS
		2021	18.4	1	MR	22.4	1	MR	0	0	T
		2022	24.9	2	MR	24.4	2	MR	24.3	2	MS
13.	Roșii Timpurii	2020	64.5	4	VS	64.5	3	VS	68.4	3	VS
		2021	77.2	4	VS	74.9	3	VS	65.7	3	VS
		2022	32.4	2	MR	16.4	2	MR	24	1	MR
14.	Elmar	2020	0	0	T	0	0	T	0	0	T
		2021	0	0	T	0	0	T	0	0	T
		2022	0	0	T	0	0	T	0	0	T

15.	Mărculești 10/5	2020	26.6	2	S	55.7	3	VS	21.4	2	MS
		2021	13.2	1	MR	27.2	2	MR	17.9	2	MS
		2022	47.2	2	S	22.4	2	MR	21.7	2	MS
16.	Mari de Aiud	2020	0	0	T	63.4	3	VS	0	0	T
		2021	12.1	1	MR	74.5	3	VS	63.2	3	VS
		2022	17.3	1	MR	21.4	1	MR	24.3	2	MS
17.	Sulina	2020	21.4	2	MR	65.7	3	VS	75.3	3	VS
		2021	61.2	3	VS	61.4	3	VS	71.7	3	VS
		2022	13.6	1	MR	18.3	2	MS	0	0	T
18.	Sirena	2020	0	0	T	32.7	2	S	42.7	2	S
		2021	19.3	1	MR	41.5	2	S	32.5	2	S
		2022	0	0	T	44.6	3	S	36.3	2	S
19.	Mari de Mărculești	2020	65.4	3	VS	58.4	3	VS	23.5	2	MS
		2021	59.4	3	VS	65.4	3	VS	78.4	3	VS
		2022	36.4	2	S	19.7	2	MS	62.7	3	VS
20	Saturn	2020	0	O	T	21.2	2	MR	0	0	T
		2021	22.5	2	MR	0	0	T	67.7	3	VS
		2022	0	0	T	64.2	3	VS	19.3	2	MS

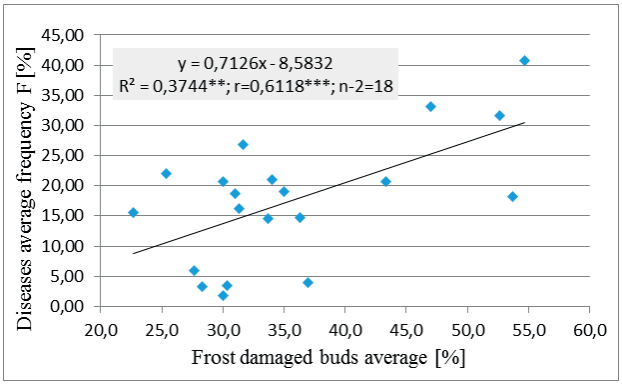


Figure 4. Correlation between the perished buds average damaged by the frost and diseases average frequency during the vegetation season

Assessment of the Figure 4 revealed a strong positive correlation between the buds average percentage damaged by the frost and the average incidence of the diseases occurred on the vegetation periods 2020-2022.

This correlation, is described by the trend of regression equation y=0.7126x-8.5832, and its associated coefficients R^2=0.3744**; r=0.6118***, statistically insured even at 18 liberty degrees (n-2=18).

CONCLUSIONS

In recent years, climate changes have contributed to the increased impact of certain fungal pathogens that pose the greatest economic threat to apricot trees, leading to premature decline.

The most notable ones are *Stigmina carpophila*, *Cytospora cincta*, and *Monilinia laxa*.

The analyzed data highlighted that the late frosts are more damaging nowdays then in the past and we concluded that they are more damaging when they occur at the end of the winter and beginning of the spring, between the bud brake and early flowering. In this period vegetative and generative tissues are developing but are also vulnerable to the piercing action of the ice crystals and contamination with pathogenic fungi.

The cultivars 'Mamaia', 'Roșii de Mărculești', 'Olimp" and 'Elmar" are used as genitors in the breeding activity due to their resistance to the attack of the three pathogens studied.

As a result of the conducted studies, resistant and high-value apricot varieties have been integrated into commercial orchards, playing a significant role in the varietal structure of apricot cultivation in southeastern Romania.

ACKNOWLEDGEMENTS

The authors thank the Romanian Ministry of Agriculture and Rural Development for the financial support (Project: ADER 6.3.22., *The development of innovative technologies of organic fruit growing harmonized with economic and natural resources*) and Romanian Academy of Agricultural and Forestry Sciences "Gheorghe Ionescu-Şişeşti" (Project no. 7940, *The adaptation of some technological solutions to reduce the negative impact of climate change on some stone species from the south-east of the country in order to increase profitability and ensure environmental protection*).

REFERENCES

Amzăr Valentina, Ivascu Antonia (2003). *Ghid de identificare al principalelor boli şi dăunători la speciile pomicole*, Bucureşti, Editura MEDRO, 200 p., ISBN 973-8487-02-1.

Audergon J.M., Duffillol J.M., Gilles F., Signoret V. (1995). Apricot selection in France: New apricot cultivars for French growers. *Acta Horticulturae,* 384.

Bassi, D., & Audergon, J. M. (2006). Apricot breeding: Update and perspectives. *Acta Horticulturae*, 701, 279–294.

Bassi D., Bellini E., Guerriero R., Monastra F., Pennone F. (1995). Apricot breeding in Italy. *Acta Horticulturae,* 384.

Balan, V., Toma, S., Oprea, M. (1999). Reaction of apricot phenotypes from national collection to the *Monilinia laxa* (Aderh. et Ruhl.) Honey inoculum. *Acta Horticulturae*., (ISHS) 488: 667-670.

Balan V., Topor E., Tudor V. (2008). *Caisul şi caisele*. Editura Ceres, Bucureşti, p. 686

Benedek, P., Nyéki, J., Vályi, I. (1990). Csonthéjas gyümölcsfajták érzékenysége a fontosabb kórokozókkal és kártevőkkel szemben - a fajtaspecifikus növényvédelmi technológia kidolgozása. *Növényvédelem*, 26: 12-31.

Crossa-Raynaud, P. H. (1969). Evaluating resistance to *Monilia laxa* (Aderh. & Ruhl.) Honey of varieties and hybrids of apricot and almonds using mean growth rate of cankers on young branches as a criterion of susceptibility. *J. Amer. Soc. Hort. Sci*., 94: 282-284.

Komar-Tyomnaya, L., D., Richter, A., A. (2000). Wild species and distant hybrids of apricot are the sources of resistance to *Monilia laxa* and high content of biologicallyactive substances in fruits. *Acta Hort*., (ISHS) 538: 147-150.

Mihăescu Grigore (1998). *Pomicultura ecologică*. Ed. Ceres, Bucuresti, ISBN 973-40-0439-5, 288 p.

Nicotra, A., Conte, L., Moser, L., Fantechi, P., Barbagiovani, I. (2006). Breeding programme for *Monilia laxa* (Aderh. & Ruhl.) Resistance on apricot. *Acta Hort.,* (ISHS) 701: 307-311.

Tzonev, R., Yamaguchi, M. (1999). Resistance in some *Prunus* species in Japan against blossom blight, caused by *Monilinia laxa* (Ehr.): *Prunus armeniaca* var. ansu Maxim., *Prunus armeniaca* L., *Prunus mume* Sieb. et Zucc. and interspecific hybrids among Prunus species. *Acta Hort.,* (ISHS) 488: 649-654.

Trandafirescu M. (1989). Reactia unor soiuri si hibrizi de cais fata de atacul ciupercii *Monilinia laxa* (Aderh et Ruhl) Honey. Probl.genet.teor.aplic.XXI (2), 75-80.

Trandafirescu M., Teodorescu G. (2005). Utilizarea mijloacelor biologice şi biotehnice în combaterea agenţilor patogeni şi dăunători la piersic şi cais. p. 12-27.

Trandafirescu, M. and Teodorescu, G. (2006). Behaviour of some apricot and hibrids from national collection to the *Monilinia laxa* (Aderh Et Ruhl) Honey Infection. *Acta. Hort.* 701, 371-376. http://dx.doi.org/10.17660/actahortic.2006.701.61

Topor E., Trandafirescu M. (1997). Evaluation of apricot germplasm fund for biological and pomological properties and its use for the breeding program. *Acta Horticulturae,* 488, vol. I., 215-220. http://dx.doi.org/10.17660/actahortic.1999.488.32

Witherspoon M. (1999). Apricot breeding in Australia. *Acta Horticulturae*, 488, vol. I.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

COMPARISON OF QUALITY CHARACTERISTICS OF FRESH AND PROCESSED STRAWBERRIES

Ioana MOLDOVAN[1], Lehel LUKÁCS[1], Gabriela Maria ROMAN[1], Sándor RÓZSA[2], Tincuța-Marta GOCAN[2], Orsolya BORSAI[2]

[1]Horticultural Research Station Cluj-Napoca, 5 Horticultorilor Street, Cluj-Napoca, Romania
[2]University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, 3-5 Calea Mănăștur Street, Cluj-Napoca, Romania

Corresponding author email: orsolya.borsai@usamvcluj.ro

Abstract

Strawberries (Fragaria × ananassa) are highly appreciated for their sensory attributes and nutritional benefits, but their quality can vary significantly between fresh and processed forms. This study aimed to compare the quality characteristics of fresh strawberries of three different varieties, namely, 'Karima', 'Auge' and 'Monterey' with their processed counterparts in the forms of jelly and syrup. Key parameters such as color, texture, total soluble solid content (°Brix), total acidity, vitamin C and phenolic compounds were evaluated. Fresh strawberries exhibited superior color, firmness, and higher levels of bioactive compounds, including vitamin C and phenolics, compared to the processed products. However, jelly and syrup retained substantial soluble solids and demonstrated an extended shelf life, making them suitable alternatives for long-term consumption. Processing led to significant changes in pH and acidity, with jelly maintaining a better balance between sweetness and tartness compared to syrup. Although antioxidant activity decreased during processing, both jelly and syrup still provided notable health benefits. This study highlights the trade-offs between sensory and nutritional quality in fresh and processed strawberries, offering insights into their uses and consumers' preferences.

Key words*: Fragaria x ananassa, jelly, syrup, acidity, TSS, total phenolic content, vitamin C.*

INTRODUCTION

Strawberries (*Fragaria × ananassa*) are among the most consumed fruits worldwide, appreciated not only for their appealing sensory attributes including their vibrant color, juicy texture, and distinct aroma, but also for their high nutritional value (Hannum, 2004).

Strawberries are particularly rich in vitamin C, phenolic compounds and flavonoids (Giampieri et al., 2012; Kumar et al., 2020; Cao et al., 2011).

These bioactive compounds are associated with numerous health benefits, including anti-inflammatory, anti-cancer, cardioprotective and antioxidant effects (Meyers et al., 2003; Tulipani et al., 2008). However, several other fruits contain high levels of antioxidant molecules, a previous study demonstrated an increase in serum antioxidant capacity in humans after including strawberries in their diet (Seyhun, 2008). Strawberry fruits have a high vitamin C content, which ranges between 40-70 mg/100 g. Vitamin C is usually considered as an indicator of nutrient quality during food processing and storage, as it is generally observed that if ascorbic acid is well retained, the other nutrients are also well retained (Sapei & Hwa, 2014).

Due to their high perishability (from 5 to 7 days at 0°C and relative humidity of 95%) strawberries are often subjected to various processing methods such as freezing, drying, and thermal treatments to extend shelf life and facilitate year-round availability while preserving key nutrients (Nunes et al., 2005; Ornelas-Paz et al., 2013; Pereira et al., 2014). However, these processes can induce significant changes in their physicochemical and sensory qualities, including texture, color, flavor, and nutrient content (Patras et al., 2010; Fernández-León et al., 2013).

For example, heat processing may lead to the degradation of thermolabile nutrients like vitamin C, while freezing can affect texture due to ice crystal formation (Karakaya et al., 2011).

Comparative studies which investigated fresh and processed strawberries have provided different findings, with some suggesting minimal losses in quality depending on the method used, while others report significant alterations in both chemical composition and consumer acceptability (Šamec et al., 2016; Huber & Oparka, 2001). Understanding how different processing techniques affect the overall quality characteristics of strawberries is critical for optimizing preservation strategies and ensuring to obtain a healthful, high-quality products to consumers.

Therefore, the main aim of this study was to compare the quality characteristics of fresh strawberries of three different varieties, such as, 'Karima', 'Auge' and 'Monterey' with their processed counterparts as jelly and syrup. By identifying the most favorable preservation techniques, this research seeks to balance shelf-life extension with minimal compromise to nutritional and sensory integrity.

MATERIALS AND METHODS

Experimental site and plant material

In order to carry out this research strawberry plants were grown in the experimental plots of the Horticultural Research Station (46°76′N 23°61′E) of the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. The experimental variants included three everbearing strawberry varieties, namely, 'Karima', also known as 'Aurora Karima', 'Auge' and 'Monterey' (Table 1).

Table 1. Description of strawberry varieties under study

Variety	'Karima'	'Auge'	'Monterey'
Appearance			
Characteristics	High yield, juicy texture, bright fruits, good taste and flavor, long shelf life	Good yield, long-conical and regular shape, high firmness and good flavor	High yield, large, moderately firm fruits with excellent flavor

Strawberry plants were planted in 2023 and grown în greenhouse in hill system (15 cm high, 50 cm wide hills) in two rows, with 30 cm between plants and 1.20-1.60 m between hills, covered with black agrotextile.

Fresh, fully ripened, free from mechanical damage or disease strawberry fruits were randomly harvested in September. In order to determine the physical fruit of each variety 50 fruits/variety were harvested in three replicates and measured to ensure the validity of the data. After harvest, the samples were transported to the laboratory under cooled conditions and stored at 4 °C until further analyses (within 24 hours).

Pomological traits of strawberries

To determine the variety-specific differences between strawberry fruits, different physical parameters were determined including fruit mass (M) and three linear dimensions such as length (L) and width (W) and thickness (T) as presented in Figure 1.

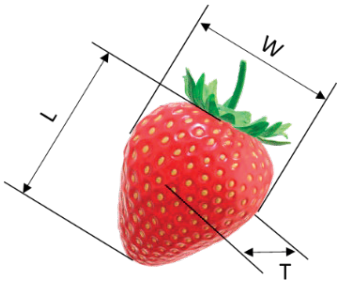


Figure 1. Representation of the three major dimensions of strawberry fruits: length (L), width (W) and thickness (T)

Strawberry mass (M) was measured by using an electronic digital balance (Kern EMB with 0.001 precision). The three linear dimensions namely, the major axis, length, (L), width (W) and thickness (T), were measured as shown in Figure 1, by using a digital vernier caliper (Quatros, accuracy ± 0.01) as suggested by Birania et al. (2022). The aspect ratio (AR) was then calculated according to Megat Ahmad Azman et al. (2020) as AR = L/W.

Sample preparation

Strawberries were washed under running distilled water, dried at room temperature, and homogenized using a blender to obtain a uniform puree. All analyses were conducted in triplicate using freshly prepared homogenates.

Determination of chemical fruit characteristics

To evaluate the chemical properties of the fruits total soluble solid content (TSS), titratable acidity (TA), Vitamin C content, total phenolic content and DPPH antioxidant activity were determined. TSS was measured using a digital hand-held refractometer (Kern Ora 10BA) and expressed as degrees Brix. A few drops of the strawberry juice were filtered through cheesecloth before measurement (Horwitz & Latimer, 2005). Titratable acidity was determined by titrating 10 mL of strawberry juice diluted with 40 mL of distilled water against 0.1 N NaOH using phenolphthalein as an indicator. Results were expressed as a percentage of citric acid equivalents as described by Nour et al. (2010).

Vitamin C (ascorbic acid) content was measured using the 2,6-dichlorophenolindophenol (DCPIP) titrimetric method (AOAC, 2000). A known volume of juice was titrated against a standardized DCPIP solution until a persistent pink endpoint was observed. Results were calculated based on a calibration curve using ascorbic acid standards and expressed as mg ascorbic acid per 100 g fresh weight (FW).

Total phenolic content was determined using the Folin-Ciocalteu method (Singleton & Rossi, 1965). An aliquot (0.5 mL) of fruit extract was mixed with 2.5 mL of 10% Folin-Ciocalteu reagent and 2 mL of 7.5% sodium carbonate. The mixture was incubated in the dark at room temperature for 30 minutes, and absorbance was measured at 765 nm using a UV–Vis spectrophotometer. Results were expressed as mg gallic acid equivalents (GAE) per 100 g FW.

Antioxidant activity was evaluated using the DPPH radical scavenging assay as described by Brand-Williams et al. (1995). A 0.1 mM DPPH solution was prepared in methanol. A 1 mL aliquot of the fruit extract was mixed with 2 mL of DPPH solution and incubated in the dark for 30 minutes. Absorbance was measured at 517 nm. The percentage of DPPH radical inhibition was calculated, and results were expressed as % inhibition or as Trolox equivalents (TE) per 100 g FW, based on a standard curve.

Preservation methods

Strawberry jelly was prepared using fresh, ripe strawberries (*Fragaria* × *ananassa*) following a traditional method with slight modifications. For each batch, 1 kg of whole strawberries was combined with 700 g of granulated sugar. The mixture was allowed to rest at low temperature (refrigerated) until the fruit naturally released juice and a syrup formed. Once syrup formation was evident, 1.5 g of citric acid was added to enhance acidity and preserve color and flavor.

The resulting mixture was then divided into two equal portions: one for making jelly and the other retained as syrup. The jelly portion was heated until the syrup reached a rolling boil. At this stage, the whole strawberries were added back into the boiling syrup and the mixture was simmered for an additional 15 minutes to allow proper infusion and thickening.

After cooking, the hot jelly was immediately poured into sterilized glass jars (Figure 2). The filled jars were then subjected to heat sterilization (e.g., in a water bath) to ensure microbial safety and shelf stability during storage.



Figure 2. Strawberry jelly as the final product

The strawberry syrup was obtained from the liquid portion separated during the strawberry jelly preparation process. The reserved strawberry syrup was first analyzed for its total soluble solids (TSS) using a refractometer. The syrup was then concentrated by boiling until it reached 70% TSS, ensuring a rich consistency and long shelf life. Once the target TSS was achieved, the hot syrup was poured into sterilized glass containers (Figure 3).



Figure 3. Strawberry syrup as the final product

To ensure microbial safety and product stability, a double sterilization process was applied: the filled containers were heat-processed (in a boiling water bath), and then sterilized a second time after 24 hours. This method preserves the flavor, color, and nutritional properties of the strawberries while producing a high-quality, shelf-stable syrup suitable for direct consumption or as a sweetener in various food applications (Kalt, 2005).

Statistical analyses

All the data recorded during this research have been statistically processed and interpreted accordingly. The data presented are means±standard error. To determine the differences between varieties and then fresh and processed variants Tukey's HSD test was performed at $p<0.05$.

RESULTS AND DISCUSSIONS

Pomological traits such as fruit dimensions, mass (weight), and firmness are the most important and critical indicators of strawberry fruit quality, which influence directly consumer preference, marketability, postharvest performance, and processing suitability (Temocico et al., 2019). The current analysis of three cultivars - 'Karima', 'Auge', and 'Monterey' - revealed significant variation in these traits (Table 2), consistent with prior findings that highlight the genetic background and environmental influence on strawberry morphology and texture (Nunes et al., 2020; Kafkas & Oğuz, 2023).

Fruit length, width, and thickness are primary morphological parameters that determine fruit shape and overall visual attractiveness. The results show that 'Auge' exhibited the greatest fruit length (48.21 mm), significantly higher than both 'Karima' and 'Monterey', suggesting a more elongated fruit shape. In contrast, 'Karima' had the largest width (29.96 mm) and thickness (22.80 mm), indicating a more rounded or conical fruit shape. These variations were also reflected in the aspect ratio (length/width), where 'Auge' showed a significantly higher value (1.68), aligning with consumer preferences for uniform and elongated fruit, as noted by Verma et al. (2023). A higher aspect ratio often correlates with a perception of premium quality and is important for fresh market sales (Lewers et al., 2020).

Table 2. Pomological traits of strawberry fruits

Variety	Fruit length (mm)	Fruit width (mm)	Fruit thickness (mm)	Aspect ratio	Fruit mass (g)	Firmness (kgf/cm²)
'Karima'	37.22±1.22 a	29.96±1.64 c	22.80±0.91 c	1.24±0.89 a	16.10±1.18 a	2.24±1.00 c
'Auge'	48.21±0.99 b	28.61±0.82 a	19.53±1.42 a	1.68±0.67 b	15.09±0.77 a	1.94±0.83 b
'Monterey'	37.26±1.03 a	29.28±0.62 b	21.89±1.06 b	1.27±0.72 a	29.50±1.72 b	1.23±1.0 a

*Data shown are means±standard error. Different lowercase letters within the trait indicate statistically significant differences between varieties according to Tukey's HSD test at $p<0.05$ and $n = 30$.

In terms of fruit mass, 'Monterey' significantly outperformed the other cultivars, averaging 29.50 g per fruit - nearly double that of 'Karima' (16.10 g) and 'Auge' (15.09 g). This suggests that 'Monterey' has high potential for commercial production, where larger fruit size often preferred due to its visual impact and economic advantage per unit. Similar high fruit mass has been reported in high-yielding day-neutral cultivars (Nunes et al., 2009). However, this size advantage comes with trade-offs in fruit firmness, which is critical for mechanical handling, transport, and shelf life. Firmness data showed that 'Karima' had the highest value (2.24 kgf/cm²), followed by 'Auge' (1.94 kgf/cm²), while 'Monterey' had the lowest (1.23 kgf/cm²). Firmness is a key determinant of postharvest quality, as it affects resistance to bruising, pathogen susceptibility, and water loss. Laguerre et al. (2023) emphasize that higher firmness contributes to extended shelf-life and improved handling, which is essential in long-distance transport scenarios. Thus, while 'Monterey' may be preferred for size, its reduced firmness may limit its postharvest suitability without advanced

packaging or storage technologies. These findings highlight the importance of balancing size and texture traits when selecting cultivars for specific production or market objectives. For instance, 'Monterey' may be ideal for fresh local markets prioritizing size, while 'Karima' could be better suited for processing or export markets that require firmer fruit with longer shelf-life. Moreover, these trait variations offer valuable data for breeding programs aimed to improve both consumer satisfaction and postharvest resilience (Mezzetti et al., 2018; Predieri et al., 2021).

The total soluble solids (TSS) content, expressed in °Brix, is a crucial quality parameter in strawberries as it reflects the sugar concentration and overall sweetness perceived by consumers (Karakasova et al., 2017). As shown in Figure 4, the TSS values among the three tested strawberry cultivars exhibited no statistically significant differences.

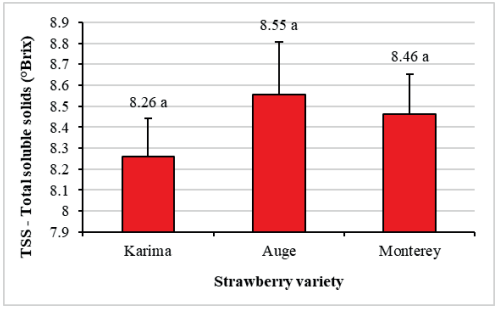


Figure 4. Total soluble solid content of fresh strawberries. Data shown are means±standard error. Same lowercase letters above the bars indicate no statistically significant differences between the varieties according to Tukey's HSD test at $p<0.05$ and n = 30

'Auge' variety recorded the highest TSS value (8.55°Brix), followed by 'Monterey' (8.46°Brix), while 'Karima' had the lowest mean TSS (8.26°Brix). These results are consistent with findings from Temocico et al. (2019), who reported TSS values ranging from 6.30 to 13.20°Brix among several strawberry genotypes. Similarly, Nunes et al. (2020) observed similar values in TSS among certain cultivars ranging between 6.42 and 8.92°Brix grown under similar agro-climatic conditions, highlighting the influence of genotype as well as growing environment and harvest maturity.

From consumer's point of view, TSS levels above 8°Brix are generally considered acceptable for fresh market strawberries, contributing positively to taste and flavor perception (Hancock, 2000). Therefore, all three cultivars evaluated in this study meet or exceed this threshold, suggesting comparable market potential in terms of sweetness.

Beside the total soluble solid content titratable acidity (TA) and the total soluble solids to titratable acidity ratio (TSS/TA) are key determinants of strawberry flavor quality, as they represent the balance between sweetness and sourness, a critical factor in consumer acceptability (Liu et al., 2016; Hancock, 2000). The results presented in Figure 5 show significant differences among the three evaluated strawberry cultivars in both acidity and sugar-acid balance.

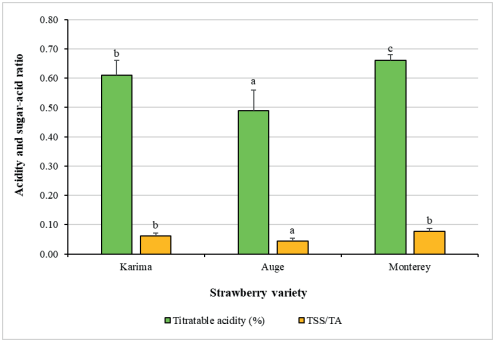


Figure 5. Titratable acidity and sugar-acid ratio in the strawberry varieties analysed. Data shown are means±standard error. Different lowercase letters above the bars indicate statistically significant differences between varieties within the same trait according to Tukey's HSD test at $p<0.05$ and n = 30

'Monterey' exhibited the highest titratable acidity (0.66%), followed by 'Karima' (0.61%), while 'Auge' recorded the lowest acidity (0.49%). These results are consistent with previous findings by Andrade et al. (2015) and Nunes et al. (2020), who noted significant genotypic variation in acidity among strawberry cultivars grown under similar environmental conditions. Higher acidity levels, such as those seen in 'Monterey', can contribute to a sharper taste profile, which may be desirable in processing applications like jelly production.

In contrast, the TSS/TA ratio, a key indicator of perceived sweetness, was the highest in

'Monterey' (0.077), followed by 'Karima' (0.062), while 'Auge' exhibited the lowest ratio (0.044). Despite having the highest acidity, 'Monterey' also maintained a relatively high TSS/TA ratio, suggesting a well-balanced flavor profile. These findings are in accordance with earlier studies indicating that the sugar-acid ratio plays a more decisive role in consumer preferences than sweetness or acidity alone (Kader, 2008). For instance, a study by Scott et al. (2021) reported that cultivars with moderate acidity but high TSS/TA ratios tend to be preferred in sensory evaluations. From this perspective, 'Karima' and 'Monterey' might be more favorable for fresh consumption due to their better sweetness-acidity balance, whereas the lower TSS/TA ratio in 'Auge' may result in a comparatively bland or sour taste. Therefore, due to its lower acidity, may require marketing adjustments or blending with sweeter cultivars for processing purposes.

The chemical composition of strawberries, including vitamin C, titratable acidity, pH, total phenolic compounds, and antioxidant capacity (DPPH), plays a vital role in determining their nutritional quality and health-promoting properties. This study aimed to assess how these chemical parameters vary among strawberry varieties ('Karima', 'Auge', and 'Monterey') in fresh fruits and processed into syrup and jelly.

Processing fruits, including strawberries, often leads to a significant reduction in their bioactive chemical compounds, particularly vitamin C, phenolics, and antioxidants. These compounds are sensitive to heat, oxygen exposure, and changes in pH, which commonly occur during thermal processing methods such as boiling, pasteurization, or jelly preparation (Patras et al., 2010; Tulipani et al., 2009). For example, vitamin C, a water-soluble and heat-sensitive compound, can degrade rapidly during cooking, while phenolic compounds may undergo oxidation or polymerization, reducing their antioxidant capacity (Crespo et al., 2010). Despite these losses, the extent of degradation varies depending on the processing technique, temperature, and duration. Some methods, such as syrup preparation under controlled conditions, may better retain nutritional quality compared to more intense processing like jam or jelly production. Therefore, selecting gentle processing methods and optimizing parameters are crucial for preserving the health-promoting properties of strawberries and other fruits.

The results of this study revealed that the vitamin C content varied significantly among cultivars and processing methods (Table 3). Fresh fruits had the highest levels, with 'Monterey' showing the greatest concentration (23.79 mg/100 g), followed by 'Karima' (23.09 mg/100 g) and 'Auge' (20.34 mg/100 g). These values are within the range reported by Skender et al. (2015) and Gündüz & Özdemir et al. (2014), who noted that vitamin C content in fresh strawberries typically ranges between 20 and 90 mg/100 g depending on cultivar and growing conditions.

Processing substantially reduced vitamin C content, particularly in jelly form. Among all cultivars, jelly had significantly lower vitamin C levels, with reductions ranging from 40% to 60%. This degradation is attributed to the thermal sensitivity of ascorbic acid during cooking and prolonged exposure to oxygen (Patras et al., 2010). The vitamin C content in syrup remained relatively higher than in jelly, particularly in 'Monterey' (24.62 mg/100 g), which suggest shorter processing times and better preservation conditions.

Titratable acidity was the highest in fresh fruits for all cultivars, especially in 'Monterey' (0.66%), aligning with previous results (Šamec et al., 2016). Acid content declined sharply in processed samples, especially in jellies, where values dropped to 0.14-0.17% (Table 3). This reduction may result from acid degradation during heating and dilution during syrup or jelly formulation (Hancock, 2000). Conversely, pH values slightly increased in processed forms, which corresponds with the observed decline in acidity. For example, the pH of 'Monterey' increased from 3.66 in fresh fruit to 3.80 in syrup. This increase might be explained by the syrup formation which usually dilutes natural acids slightly, reducing both TA and sometimes raising the pH.

Table 3. Chemical properties of fresh and processed strawberry fruits

Variety	Fruit sample	Vitamin C (mg/100 g)	Titratable acidity (%)	pH	Total phenolic compounds (mg GAE/100 g)	DPPH (µmol TE/g FW)
'Karima'	fresh	23.09±1.16 bB	0.61±0.01 cB	3.44±0.20 bB	421.07±30.12 cA	17.90±1.33 cA
	syrup	21.33±2.33 bB	0.41±0.03 bB	3.69±0.32 cB	215.82±18.39 bA	14.52±1.02 bA
	jelly	11.40±1.81 aA	0.14±0.02 aA	3.18±0.17 aA	110.97±21.03 aA	12.21±0.98 aA
'Auge'	fresh	20.34±3.45 bA	0.49±0.03 bA	3.22±1.00 bA	511.33±19.87 cB	19.70±0.46 cB
	syrup	19.76±2.72 bA	0.21±0.11 aA	3.39±0.99 cA	236.78±22.33 bB	16.99±1.00 bB
	jelly	12.60±1.02 aB	0.15±0.05 aB	3.11±0.87 aA	150.15±29.41 aB	13.44±0.87 aB
'Monterey'	fresh	23.79±1.99 bB	0.66±0.22 cC	3.66±0.69 bC	609.11±17.42 bC	22.30±0.96 cC
	syrup	24.62±0.76 bC	0.48±0.76 bC	3.80±0.33 cC	239.47±27.21 aB	20.45±1.11 bC
	jelly	13.72±0.99 aC	0.17±0.08 aC	3.49±0.47 aB	180.40±18.33 aC	14.55±1.32 aC

*Data shown are means±standard error. Different lowercase letters within the trait indicate statistically significant differences between fresh and processed fruit samples according to Tukey's HSD test at $p<0.05$ and $n = 30$. Different capital letters show significant differences between varieties within the same chemical trait and sample form (fresh, syrup and jelly).

Phenolic content, a major determinant of antioxidant activity, was the highest in fresh strawberries and decreased significantly with processing. Among fresh samples, 'Monterey' exhibited the highest TPC (609.11 mg GAE/100 g), followed by 'Auge' (511.33 mg GAE/100 g) and 'Karima' (421.07 mg GAE/100 g). These values are comparable to those reported by Tulipani et al. (2008) for high-antioxidant strawberry cultivars.

Jelly production caused the greatest phenolic loss, with values decreasing by more than 60% in all varieties. 'Karima' jelly had the lowest phenolic content (110.97 mg GAE/100 g), indicating its higher susceptibility to degradation. Heat processing, oxygen exposure, and pH changes during jelly preparation can contribute to phenolic degradation (Crespo et al., 2010).

DPPH radical scavenging activity, an indicator of antioxidant capacity, followed a similar trend to total phenolics. Fresh fruits showed the highest antioxidant activity, with 'Monterey' reaching 22.30 µmol TE/g FW. Processing reduced DPPH values in all cases, particularly in jelly samples, where values decreased to 12.21-14.55 µmol TE/g FW. These trends reaffirm the close correlation between phenolic content and antioxidant potential (Scalzo et al., 2005).

Notably, despite reductions, 'Monterey' maintained higher antioxidant activity in both processed forms compared to the other cultivars, making it a promising candidate for functional food products with retained health benefits.

CONCLUSIONS

Processing strawberries into syrup and jelly significantly affects their chemical composition, especially reducing vitamin C, phenolic compounds, and antioxidant activity. Among the studied cultivars, 'Monterey' consistently retained higher levels of bioactive compounds in both fresh and processed forms, suggesting its superiority for both fresh consumption and value-added processing. These findings underscore the importance of cultivar selection and optimized processing techniques to preserve nutritional quality.

ACKNOWLEDGEMENTS

The authors thank to the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca and Horticultural Research Station for supplying all facilities and cooperation to carry out this research.

REFERENCES

Andrade, C. A. W., Miguel, A. C. A., Spricigo, P. C., Dias, C. T. D., & Jacomino, A. P. (2015). Comparison of quality between organic and conventional strawberries from multiple farms. *Rev. Bras. Frutic.*, v. 39, n. 2: (e-045).

AOAC. (2000). Official Method 967.21 – Ascorbic Acid in Vitamin Preparations and Juices. In Official Methods of Analysis (17th ed.). Association of Official Analytical Chemists.

Birania, S., Attkan, A. K., Kumar, S., Kumar, N., & Singh, V. K. (2022). Mass modeling of strawberry (Fragaria x Ananasa) based on selected physical attributes. *Journal of Food Process Engineering*, 45(5), e14023. https://doi.org/10.1111/jfpe.14023

Brand-Williams, W., Cuvelier, M. E., & Berset, C. L. W. T. (1995). Use of a free radical method to evaluate antioxidant activity. LWT-Food science and Technology, 28(1), 25-30.

Cao, G., Sofic, E., & Prior, R. L. (2011). Antioxidant capacity of tea and common vegetables. *Journal of Agricultural and Food Chemistry*, *44*(11), 3426-3431.

Crespo, P., Bordonaba, J. G., Terry, L. A., & Carlen, C. (2010). Characterisation of major taste and health-related compounds of four strawberry genotypes grown at different Swiss production sites. *Food Chemistry*, *122*(1), 16-24.

Fernández-León, M. F., Lozano, M., Ayuso, M. C., & González-Gómez, D. (2013). Impact of drying methods on the quality of functional compounds in strawberries. *Journal of Food Quality*, *36*(1), 1-8. https://doi.org/10.1111/jfq.12003

Giampieri, F., Alvarez-Suarez, J. M., & Battino, M. (2012). Strawberry and human healt: Effects beyond antioxidant activity. *Journal of Agricultural and Food Chemistry*, 60 (23), 6155-6167.

Giampieri, F., Tulipani, S., Alvarez-Suarez, J. M., Quiles, J. L., Mezzetti, B., & Battino, M. (2012). The strawberry: Composition, nutritional quality, and impact on human health. *Nutrition*, *28*(1), 9-19. https://doi.org/10.1016/j.nut.2011.08.009

Gündüz, K., & Özdemir, E. (2014). The effects of genotype and growing conditions on antioxidant capacity, phenolic compounds, organic acid and individual sugars of strawberry. *Food chemistry*, *155*, 298-303.

Hancock, J. F. (2000). Strawberries. In *Temperate fruit crops in warm climates* (pp. 445-455). Dordrecht: Springer Netherlands.

Hannum, S. M. (2004). Potential impact of strawberries on human health: A review of the science. *Critical Reviews in Food Science and Nutrition*, *44*(1), 1-17. https://doi.org/10.1080/10408690490911757

Horwitz, W., & Latimer, G. W. (2005). Official Methods of Analysis of AOAC International (18th ed.). AOAC International.

Huber, D. J., & Oparka, K. J. (2001). Cell wall dynamics in fruit softening and quality and its evaluation by microscopy. *Postharvest Biology and Technology*, *22*(2), 165–174. https://doi.org/10.1016/S0925-5214(01)00185-0

Kader, A. A. (2008). Flavor quality of fruits and vegetables. *Journal of the Science of Food and Agriculture*, *88*(11), 1863-1868.

Kafkas, N. E., & Oğuz, İ. (Eds.). (2023). *Recent studies on strawberries*. BoD–Books on Demand.

Kalt, W. (2005). Effects of production and processing factors on major fruit and vegetable antioxidants. *Journal of food science*, *70*(1), R11-R19.

Karakasova, L., Ampova, J., Babanovska-Milenkovska, F., Durmishi, N., & Stamatovska, V. (2017). Comparison of quality characteristics of fresh and of dried strawberries. *UBT Knowledge Center*, 47-55.

Karakaya, S., Yılmaz, N., & Burdurlu, H. S. (2011). The effects of processing methods on the retention of antioxidants in fruit and vegetables. *Food Reviews International*, *27*(3), 245-260. https://doi.org/10.1080/87559129.2011.563395

Kumar, G., Kaur, A., Yadav, A., & Singh, A. (2020). Strawberry bioactive compounds and their associated healt benefits. *Journal of Food Science & Technology*, *57*(9), 3068-3080

Laguerre, O., Duret, S., Chaomuang, N., Denis, A., Derens-Bertheau, E., Mawilai, P., ... & Pornchaloempong, P. (2023). Influence of long-distance air transport conditions on horticultural product quality: Case study of fresh mango shipment from Thailand to France. *International Journal of Refrigeration*, *152*, 16-25.

Lewers, K. S., Newell, M. J., Park, E., & Luo, Y. (2020). Consumer preference and physiochemical analyses of fresh strawberries from ten cultivars. *International Journal of Fruit Science*, *20*(sup2), 733-756.

Liu, L., Ji, M. L., Chen, M., Sun, M. Y., Fu, X. L., Li, L., ... & Zhu, C. Y. (2016). The flavor and nutritional characteristic of four strawberry varieties cultured in soilless system. *Food Science & Nutrition*, *4*(6), 858-868.

Megat Ahmad Azman, P. N., Shamsudin, R., Che Man, H., & Ya'acob, M. E. (2020). Some physical properties and mass Modelling of pepper berries (*Piper nigrum* L.), variety Kuching, at different maturity levels. PRO, 8(10), 1314.

Meyers, K. J., Watkins, C. B., Pritts, M. P., & Liu, R. H. (2003). Antioxidant and antiproliferative activities of strawberries. *Journal of Agricultural and Food Chemistry*, *51*(23), 6887-6892. https://doi.org/10.1021/jf034506n

Mezzetti, B., Giampieri, F., Zhang, Y. T., & Zhong, C. F. (2018). Status of strawberry breeding programs and cultivation systems in Europe and the rest of the world. *Journal of Berry Research*, *8*(3), 205-221.

Nour, V., Trandafir, I., & Ionica, M. E. (2010). Compositional Characteristics of Fruits of several Apple (Malus domestica Borkh.) Cultivars. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, *38*(3), 228-233.

Nunes, G., Teixeira, F., Schwarz, K., Camargo, C. K., Resende, J. T. V. D., Santos, E. F. D., ... & Novello, D. (2020). Influence of genetic variability on the quality of strawberry cultivars: Sensorial, physical-chemical and nutritional characterization. *Acta Scientiarum. Agronomy*, *43*, e46862.

Nunes, M. C. N., Emond, J. P., Brecht, J. K., Dea, S., & Puschmann, R. (2005). Quality curves for mango fruit (cv. Tommy Atkins and Palmer) stored at chilling and nonchilling temperatures. *Journal of Food Quality*, *28*(4), 360-374. https://doi.org/10.1111/j.1745-4557.2005.00051.x

Nunes, M. C. N., Emond, J. P., Rauth, M., Dea, S., & Chau, K. V. (2009). Environmental conditions encountered during typical consumer retail display

affect fruit and vegetable quality and waste. *Postharvest biology and technology*, *51*(2), 232-241.

Ornelas-Paz, J. D., Cira-Chavez, L. A., Gardea-Bobadilla, V., & Yahia, E. M. (2013). Changes in the bioactive compounds and quality attributes of strawberries during storage. *Food Chemistry*, 138(1), 372-381.

Patras, A., Brunton, N. P., O'Donnell, C., & Tiwari, B. K. (2010). Effect of thermal processing on anthocyanin stability in foods; mechanisms and kinetics of degradation. *Trends in Food Science & Technology*, *21*(1), 3-11. https://doi.org/10.1016/j.tifs.2009.07.004

Patras, A., Brunton, N. P., O'Donnell, C., & Tiwari, B. K. (2010). Effect of thermal processing on anthocyanin stability in foods; mechanisms and kinetics of degradation. *Trends in Food Science & Technology*, *21*(1), 3-11.

Pereira, C., Barros, L., & Ferreira, I. C. (2014). Chemical characterization of strawberry tree fruit (Arbutus unedo L.) and its antioxidant activity measurement. *Food & Function*, *5*(3), 537-544.

Predieri, S., Lippi, N., & Daniele, G. M. (2021, May). What can we learn from consumers' perception of strawberry quality? In *IX International Strawberry Symposium 1309* (pp. 987-994).

Šamec, D., Maretić, M., Lugarić, I., Mešić, A., Salopek-Sondi, B., & Duralija, B. (2016). Assessment of the differences in the physical, chemical and phytochemical properties of four strawberry cultivars using principal component analysis. *Food chemistry*, *194*, 828-834.

Šamec, D., Urlić, B., & Salopek-Sondi, B. (2016). White and red strawberry fruit - A comparison: Anthocyanins, phenolics, antioxidant capacity and allergenicity. *Journal of Functional Foods*, 20, 41-51. https://doi.org/10.1016/j.jff.2015.10.022

Sapei, L., & Hwa, L. (2014). Study on the Kinetics of Vitamin C Degradation in Fresh Strawberry Juices. *Procedia Chemistry*, 9, 62-68.

Scalzo, J., Politi, A., Pellegrini, N., Mezzetti, B., & Battino, M. (2005). Plant genotype affects total antioxidant capacity and phenolic contents in fruit. *Nutrition*, *21*(2), 207-213.

Scott, G., Williams, C., Wallace, R. W., & Du, X. (2021). Exploring plant performance, fruit physicochemical characteristics, volatile profiles, and sensory properties of day-neutral and short-day strawberry cultivars grown in Texas. *Journal of Agricultural and Food Chemistry*, *69*(45), 13299-13314.

Seyhun, Y. (2008). An evaluation of the retention of quality characteristics in fresh and freeze-dried alpine strawberries. *International Journal of Food Science and Technology*, 43, 865-870.

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American journal of Enology and Viticulture, 16(3), 144-158.

Skender, A., Ajdinović, T., & Bećirspahić, D. (2015). The comparison of phenotypic characteristics of current varieties and wild species of Fragaria. Genetika, 47(1), 45-52.

Temocico, G., Sturzeanu, M., Ion, V., & Cristea, S. (2019). Evaluation of strawberry fruit quality for new selections and cultivars. *RomBiotechnolLett*, *24*(4), 742-748.

Tulipani, S., Mezzetti, B., & Battino, M. (2008). Impact of strawberries on human health: Insight into the molecular mechanisms of the antioxidant action. *Journal of Agricultural and Food Chemistry*, *56*(3), 1053-1060. https://doi.org/10.1021/jf072504n

Tulipani, S., Mezzetti, B., & Battino, M. (2009). Impact of strawberries on human health: insight into marginally discussed bioactive compounds for the Mediterranean diet. *Public health nutrition*, *12*(9A), 1656-1662.

Verma, S., Tripathi, V. K., & Dwivedi, A. K. (2023). Effect of gibberellic acid and boron foliar feeding on flowering, fruiting, yield and quality of strawberry (*Fragaria* x *ananassa* Duch.). *Progressive Horticulture*, *55*(2), 148-154.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

IN SITU SELECTION OF LOCAL SOUR CHERRY VARIETIES RESISTANT TO THERMOHYDRIC STRESS

Silvia NICOLAE[1], Corina GAVĂȚ[2], Mihai CHIVU[1], Adelina Ioana STAN[1]

[1]Research Institute for Fruit Growing Pitești - Mărăcineni, 402 Mărului Street, RO 117450, Mărăcineni, Pitești, Argeș County, Romania
[2]Research Station for Fruit Growing Constanța, 25 Pepinierei Street, RO 907300, Valu lui Traian, Constanța, Romania

Corresponding author email: silvia_eu2001@yahoo.com

Abstract

The sour cherry (Prunus cerasus) is a tetraploid tree species native to Central Asia, which is found in semi-spontaneous form in all inhabited areas with favorable conditions for cultivation, especially in hilly areas. The fruits are consumed both fresh, having therapeutic properties, but also in processed form.
The current climate trend of increasing average temperatures and decreasing amounts of precipitation makes the existing varieties suffer to a greater or lesser extent. In order to cope with climate changes, breeding programs also aim to identify valuable genotypes adapted to thermohydric stress conditions.
The research in this paper aims at the in situ identification of some local sour cherry varieties in areas with high temperatures and low rainfall. Such an area is the one in southern Romania, more precisely in the southeast of Dolj county, located at an altitude slightly higher than 100m. Of the 24 selected local varieties, 45.83% have a caliber over 21 mm, the largest being the 24.9 mm at SLV-24-Dj-1.2 selection. In terms of taste and aroma, SLV-24-Dj-2.5 stood out and received the highest score at the tasting.

Key words: *local varieties, resistant, sour cherry, southern Romania.*

INTRODUCTION

The sour cherry (*Prunus cerasus*) is a tetraploid fruit species native to Central Asia, presumed to be the result of a cross between *Prunus avium* and *Prunus fruticosa* Pall (Loescher, 2003). It is smaller in size than the sweet cherry tree, and can reach a height of up to 6 meters. The sour cherry does not grow spontaneously, but because it easily propagates through seeds and suckers, it is found in a semi-wild form in all inhabited areas with favorable growing conditions, especially in hilly areas. It develops normally in most types of soil, especially in loamy, clayey-sandy, sandy soils, and less so in clayey (compact) soils or those rich in sodium salts. It has similar requirements to the sweet cherry regarding environmental factors, although there are differences in thermal and precipitation optima. For sour cherry, the thermal optimum is lower than for sweet cherry, ranging between 15 and 25°C, and it struggles to withstand high temperatures. During the dormant period, it can withstand frosts of up to -29°C. Regarding water requirements, it is a species with high precipitation needs, between 700 and 1200mm/year. In Romania, the counties most favorable for this crop based on climatic and pedological conditions are Sălaj, Vâlcea, Gorj, Maramureș, and Caraș-Severin Counties while the least favorable are Harghita, Tulcea, Brăila, and Covasna Counties (Coman et al., 2014).
The red-purpel fruits, with blackish hues, sweet-sour, reach harvest maturity in Romania from early June to late July or even the beginning of August, depending on the area and climatic conditions. They are appreciated for fresh consumption due to their composition with therapeutic properties. Thus, the antioxidant content contributes to the prevention of cancer and cardiovascular diseases, melatonin regulates the sleep-wake cycle, anthocyanins reduce inflammatory processes (muscle and joint pain), anthocyanins and fibers maintain blood vessel elasticity and reduce cholesterol, and by the slow release of sugars into the blood, it can help manage weight and prevent insulin resistance (Blando and Oomah, 2019). Being a perishable fruit, a

large part of the production is industrialized, resulting in a variety of processed products (soft drinks, cookies, jam, compote, syrups, liqueurs, candies, etc.). In addition, it is also a very good mellifer species, yielding approximately 30-40 kg of honey per hectare of orchard (Bura, 2009).

The largest producer of sour cherries in the world in 2023 is the Russian Federation with a production of 268,256 tons on an area of 41,377 hectares, followed by Turkey with a production of 211,291 tons on an area of 26,000 hectares and Poland with 168,700 tons on an area of 25,300 hectares. In Romania, as of 2023, a production of 29,000 tons was recorded on an area of 2,760 hectares (FAOSTAT data, 2025). Classic sour cherry plantations can reach ages of up to 30-35 years, and 20-25 years for intensive ones. Within the research centers in Romania, especially at RIFG Pitești (located in the south of Romania) and at RSFG Iași (located in the northeast of Romania), there has been interest in selecting local varieties, with which *ex situ* collections of sour cherries have been established. To these, indigenous hybrids and varieties from abroad, both older and newer, were added (Braniște, 2006). In the process of selecting local varieties, vigor, crown type, and fruiting branches of the tree, physical and sensory characteristics of the fruits, tolerance to specific diseases, as well as resistance to pedo-climatic factors were taken into account. The current climatic trend of increasing average temperatures (Lindsey and Dahlman, 2024) and the increase in drought periods causes existing varieties to suffer to a greater or lesser extent. To cope with climate change, breeding programs also aim to identify valuable genotypes adapted to these thermo-hydric stress conditions.

The research in this paper aims to identify *in situ* local varieties of sour cherry in areas with high temperatures and precipitation deficits. Such an area is in the southeastern part of Dolj County, in the region known as the `Sahara of Oltenia` specifically in Daneți, Dobrotești, and Amărăștii de Jos villages situated at an altitude slightly above 100m.

MATERIALS AND METHODS

The southeastern area of Dolj County is characterized by a temperate-continental climate with very hot summers and moderate winters, with multiannual temperatures of approximately 11.5^0C and average annual precipitation amounts totaling up to 500mm.

In the warm season of the year, in the southern part of Dolj County, the higher intensity of winds blowing from the West causes the increased mobility of sand dunes (Ghinea, 2025 February, 3).

The soil, similar to that found in Dăbuleni village (it is located in the southeast of Dolj County), is sandy with low natural fertility, being poorly supplied with nitrogen and phosphorus and moderately supplied with potassium (www.scdcpndabuleni.ro/ro/istoric/4).

The main activity of the locals in the southern part of Dolj County is agriculture, as these areas are favorable for the cultivation of tobacco, watermelons, peaches, apricots, etc. The selection of local sour cherry varieties was done in the households of locals from Daneți, Dobrotești, and Amărăștii de Jos villages, on trees with their own roots.

Following observations on the local varieties of sour cherries from the households of the locals, 24 biotypes were selected. The selection was made at the beginning of June, 2024, on an average sample of 30 fruits.

The studied indicators were vigor, suckering capacity, and productivity in the tree, and for the fruit, skin color, pulp color, fruit weight, stone weight, caliber, firmness, and soluble dry matter.

The assessment of suckering capacity was done on a scale from 1 to 3 as follows: 1-no suckers, 2-weak suckering (1-2 suckers), 3-medium (3-4 suckers). Productivity was estimated on a scale from 1 to 5 as follows: 1 - very low, 2 - low, 3 - medium, 4 - high, 5 - very high. Caliber was determined with an electronic caliper, measuring the larger diameter of the fruit.

Firmness was measured with a nondestructive HPE Qualitest penetrometer, with a 0.25 cm² tip, and is expressed in HPE units.

The soluble dry substance in sour cherry fruits was determined using a portable refractometer with automatic temperature compensation and is expressed in ^{0}Brix. The pulp yield (%) represents the percentage of pulp relative to the weight of the fruit with the peduncle. The dates were processed using the Excel database and SPSS 14 software. The differences between means were highlighted at a probability level of 0.05%

RESULTS AND DISCUSSIONS

In the selection process of sour cherries in this study, the pursued objectives are low to medium tree vigor, tolerance to specific diseases and pests, good fruit quality with a weight over 4g/fruit and good taste, as well as resistance to high temperatures and precipitation unevenly distributed throughout the entire growing season. All the selected types are trees on their own roots, not grafted, with fruits that ripened in early June. They did not show symptoms of specific sour cherry diseases caused by *Monilinia fructigena, Monilinia laxa, Monilinia fruticola, Stigmina carpophila, Xanthomonas campestris pv. pruni, Blumeriella jaapii*, *Capnodium salicinum, Venturia cerasi*, *Apiognomonia erythrostoma, Chondrostereum purpureum*. The selected local varieties have variable vigor and an age ranging from 5 to even over 15 years. In general, the trees had very high productivity, with the fruit skin color varying from red to dark red. More than half of the selected local varieties did not show a tendency to sucker (Table 1).

Table 1. Observations on the external characteristics of the trees and fruits of selected local sour cherry varieties in the southeastern part of Dolj County

Selection code	Tree vigor	Suckering capacity	Productivity	Fruit skin color	Fruit pulp color
SLV-24-Dj-1.1	low	2	5	red	light red
SLV-24-DJ-1.2	high	1	2	dark red	light red
SLV-24-Dj-1.3	low	2	5	red	red
SLV-24-Dj-1.4	high	3	5	red	red
SLV-24-Dj-1.5	low	3	5	dark red	red
SLV-24-Dj-1.6	medium-high	1	5	red	red
SLV-24-DJ-1.7	low-medium	1	5	red	red
SLV-24-Dj-1.8	medium	1	5	red	red
SLV-24-Dj-1.9	low	2	5	red	light red
SLV-24-Dj-1.10	low-medium	1	5	dark red	red
SLV-24-Dj-2.1	high	2	5	red	red
SLV-24-Dj-2.2	high	2	5	dark red	dark red
SLV-24-Dj-2.3	low	-	5	red	red
SLV-24-Dj-2.4	low	1	5	red	red
SLV-24-Dj-2.5	low	3	5	dark red	dark red
SLV-24-Dj-2.6	medium	1	5	red	red
SLV-24-Dj-2.7	high	1	5	dark red	red
SLV-24-Dj-2.8	low	1	5	dark red	dark red
SLV-24-Dj-2.9	low-medium	1	5	dark red	dark red
SLV-24-DJ-2.10	low	1	5	dark red	red
SLV-24-Dj-2.11	low-medium	1	5	dark red	dark red
Selection code	Tree vigor	Suckering capacity	Productivity	Fruit skin color	Fruit pulp color
SLV-24-Dj-2.12	low	1	5	dark red	red
SLV-24-Dj-3.1	high	1	5	dark red	red
SLV-24-Dj-3.2	medium-high	3	5	Red	red

In addition to external observations of the trees and fruits, physicochemical determinations were also made on the fruits, specifically determining pulp yield, fruit size, firmness, and soluble dry matter, with the average values being summarized in Table 2.

Table 2. Physical and chemical indicators monitored in the fruits of selected local varieties in situ from the southeastern area of Dolj County (average values)

Selection code	Pulp yield (%)	Weight of fruit without peduncle (g)	Fruit caliber (mm)	Firmness of the fruit (HPE)	Soluble dry matter (^{0}Brix)
SLV-24-Dj-1.1	90.76	3.67hi	19.99ij	20.23abc	13.60hijkl
SLV-24-Dj-1.2	93.54	7.10a	24.90a	14.83cd	13.43ijkl
SLV-24-Dj-1.3	91.14	4.40defg	20.61fghij	16.60bcd	17.70a
SLV-24-Dj-1.4	91.96	4.77bcde	21.03defghi	14.60cd	13.67ghijkl
SLV-24-Dj-1.5	90.75	3.30i	18.47k	15.77bcd	15.00defghi
SLV-24-Dj-1.6	92.55	4.80bcde	20.87efghij	24.10a	17.07ab
SLV-24-Dj-1.7	91.58	3.90ghi	20.07hij	13.93cd	13.83fghijkl
SLV-24-Dj-1.8	90.83	3.80ghi	19.60j	14.17cd	14.37efghijk
SLV-24-Dj-1.9	94.62	5.23bc	22.46bc	11.33d	12.77l
SLV-24-Dj-1.10	93.87	5.23bc	22.67b	11.83d	13.20jkl
SLV-24-Dj-2.1	91.43	4.70bcde	21.33cdefgh	11.53d	12.67l
SLV-24-Dj-2.2	91.88	5.07bcd	21.91bcde	20.13abc	14.60efghij
SLV-24-Dj-2.3	91.30	4.20efgh	20.74efghij	21.83ab	15.33cdef
SLV-24-Dj-2.4	92.21	4.87bcde	21.57bcdefg	13.97cd	14.03efghijkl
SLV-24-Dj-2.5	94.64	3.83ghi	19.88ij	15.67cd	16.23bcd
SLV-24-Dj-2.6	92.94	5.23bc	22.00bcde	13.97cd	15.13defgh
SLV-24-Dj-2.7	93.32	4.60cdef	20.90efghij	16.23bcd	15.23defg
SLV-24-Dj-2.8	91.73	4.23efgh	20.00ij	19.80abc	16.73abc
SLV-24-Dj-2.9	91.55	4.27efgh	20.71efghij	16.20bcd	12.80kl
SLV-24-Dj-2.10	92.02	3.93fghi	20.39ghij	16.37bcd	14.40efghij
SLV-24-Dj-2.11	91.51	3.97fghi	20.31ghij	15.50cd	15.53cde
SLV-24-Dj-2.12	93.22	5.37b	22.25bcd	12.33d	14.80defghi
SLV-24-Dj-3.1	92.68	6.87a	24.59a	25.33a	13.07jkl
SLV-24-Dj-3.2	92.25	5.00bcd	21.85bcdef	13.93cd	14.00efghijkl

Notes: The values are compared vertically. Means with a different letter are statistically different ($p \leq 0.05$)

The selected varieties, in most cases, have high productivity and did not show symptoms of diseases or viruses. The pulp yield calculated by the ratio between the pulp weight compared to the fruit weight with the peduncle ranged from 94.64% in the `SLV-24-Dj-2.5` selection to 90.75% in the `SLV-24-DJ-1.5` selection.

The selections with the highest average fruit weight without the peduncle are `SLV-24-Dj-1.2` with 7.1g and `SLV-24-Dj-3.1` with 6.87g. On the opposite end is the selection `SLV-24-Dj-1.5`, whose fruit has an average weight of 3.3 g. Out of the 24 local varieties, 70% had an average fruit weight greater than 4 g. Just like the fruit weight, the size was largest in the selections `SLV-24-Dj-1.2` with 24.9 mm and `SLV-24-Dj-3.1` with 24.59 mm, significantly larger than the other local varieties. The smallest fruit caliber was recorded in the selection `SLV-24-Dj-1.5` at 18.47 mm. According to the marketing standard SR 3155:1995, extra quality fruits are those with a minimum caliber of 21 mm, while those in class I are those with a minimum caliber of 18 mm (www.scrigroup.com). From the data obtained from the measurements, 45.83% of the selected varieties fall into the extra class and 54.17% into class I.

The firmness of the fruits varied from 25.33 HPE in the selection `SLV-24-Dj-3.1` to 11.33 HPE in the selection `SLV-24-Dj-1.9`. Observations in the following days showed that the fruits from the selection with the lowest firmness spoiled the fastest.

The soluble dry matter content expressed in ^{0}Brix, was varied between 17.7^{0}Brix for the `SLV-24-Dj-1.3` selection and 12.67^{0}Brix for the `SLV-24-Dj-2.1` selection. The fruits were stored in the refrigerator, and 2-4 days after harvesting, a tasting was organized to assess the organoleptic quality of the fruits in 23 selections. During this tasting, both external

characteristics (ease of peduncle detachment, size, shape, and color of the fruit) and pulp characteristics (texture, stone size, acidity, adherence to the stone, taste, and flavor) were noted. The scoring was done on a scale from 1 to 9 (1-3 unsatisfactory, 4-6 good, and 7-9 very good). There were seven tasters, researchers from RIFG Pitești, aged between 27 and 56 years. The average scores for external characteristics and pulp characteristics for each selection are presented in Table 3.

Table 3. Appreciation of the quality of fruits from selected local sour cherry varieties in the southeast of Dolj County (average scores given by 7 tasters)

Selection code	External characters				Characteristics of the pulp						
	The ease of peduncle detachment	Size	Shape	Fruit color	Texture	Size of the stone	Acidity	Adhesion to the stone	Taste	Flavor	Average
SLV-24-Dj-1.2	7.71a	8.43 a	8.14ab	8.14a	7.29a	6.14a	6.14a	5.71ab	6.29ab	5.43a	6.94a
SLV-24-Dj-1.3	7.57a	7.00 abcdef	7.14abc	7.57a	7.00a	7.14a	7.00a	7.29ab	6.71ab	7.00a	7.14a
SLV-24-Dj-1.4	7.57a	7.43 abcd	7.57abc	7.14a	6.86a	6.71a	6.71a	6.57ab	5.00b	5.29a	6.69a
SLV-24-Dj-1.5	7.43a	5.71 ef	6.57abc	7.00a	6.00a	6.43a	6.14a	6.57ab	6.00ab	5.57a	6.34a
SLV-24-Dj-1.6	7.57a	7.00 abcdef	7.71abc	7.57a	6.71a	6.43a	6.14a	7.43ab	6.00ab	5.57a	6.81a
SLV-24-Dj-1.7	7.57a	5.57 f	6.71abc	6.86a	6.71a	6.57a	6.71a	6.86ab	6.14ab	5.86a	6.03a
SLV-24-Dj-1.8	7.86a	6.00 def	6.57abc	7.14a	7.14a	6.86a	6.57a	7.29ab	6.43ab	6.43a	6.83a
SLV-24-Dj-1.9	8.14a	7.86 abc	7.86abc	7.86a	6.43a	6.43a	6.14a	6.71ab	5.71ab	5.71a	6.89a
SLV-24-Dj-1.10	7.72a	6.81 bcdef	7.30abc	7.45a	6.82a	6.73a	6.37a	7.08ab	6.21ab	5.89a	6.84a
SLV-24-Dj-2.1	7.86a	7.14 abcdef	7.14abc	7.14a	6.43a	6.29a	6.57a	6.14ab	5.86ab	5.43a	6.60a
SLV-24-Dj-2.2	7.86a	7.29 abcde	7.43abc	7.29a	6.71a	6.57a	5.71a	7.29ab	5.71ab	6.14a	6.80a
SLV-24-Dj-2.3	7.43a	6.29 cdef	7.14abc	7.14a	6.29a	6.71a	6.71a	6.14ab	6.57ab	5.71a	6.61a
SLV-24-Dj-2.4	7.43a	7.00 abcdef	6.86abc	6.86a	6.57a	6.86a	6.14a	6.71ab	6.00ab	6.14a	6.66a
SLV-24-Dj-2.5	7.86a	6.14 cdef	6.86abc	7.29a	6.86a	7.29a	7.00a	7.50a	7.71a	7.29a	7.17a
SLV-24-Dj-2.6	8.29a	7.86 abc	7.71abc	7.86a	7.00a	6.86a	7.43a	6.86ab	7.14a	6.43a	7.34a
SLV-24-Dj-2.7	7.57a	7.14 abcdef	7.14abc	7.14a	6.43a	7.14a	6.26a	7.43ab	6.57ab	6.29a	6.91a
SLV-24-Dj-2.8	7.71a	5.57 f	6.43bc	7.29a	6.00a	6.86a	5.86a	6.71ab	6.57ab	6.43a	6.54a
SLV-24-Dj-2.9	7.57a	6.00 def	7.00abc	7.29a	6.86a	6.86a	6.14a	6.00ab	6.29ab	5.43a	6.54a
SLV-24-Dj-2.10	7.86a	6.71 bcdef	6.86abc	6.86a	6.43a	6.43a	6.00a	6.71ab	6.14ab	5.43a	6.54a
SLV-24-Dj-2.11	6.86a	5.86 def	6.14c	6.71a	6.57a	6.57a	6.00a	6,86ab	5.86ab	5.57a	6.30a
SLV-24-Dj-2.12	7.29a	7.71 abc	7.43abc	7.86a	6.57a	6.14a	6.71a	7.00ab	6.57ab	5.57a	6.89a
SLV-24-Dj-3.1	7.71a	8.14 ab	8.29a	7.86a	7.00a	6.00a	5.86a	5.43b	5.86ab	5.71a	6.79a
SLV-24-Dj-3.2	7.57a	7.29 abcde	7.00abc	7.14a	6.43a	6.29a	6.86a	6.71ab	6.43ab	6.14a	6.79a

Regarding the ease of peduncle detachment, there were no significant differences between the local varieties, with average scores ranging from 8.29 for `SLV-24-Dj-2.6` to 6.86 for `SLV-24-Dj-2.11`. In terms of size, the highest score (8.43) was given to the selection `SLV-24-Dj-1.2` (Figure 1) which is also confirmed by the weight measurements (7.1 g) and caliber (24.9 mm) of the fruits. In terms of taste and aroma, the selection `SLV-24-Dj-2.5` (Figure 2.a and 2.b) stood out, receiving the highest scores of 7.71 and 7.29, respectively. Out of the 23 local varieties tasted, 3 selections `SLV-24-Dj-1.3` (Figure 3), `SLV-24-Dj-2.5`, `SLV-24-Dj-2.6` (Figure 4.a and 4.b.) had an average score higher than 7, placing them in the `very good` category, while the rest were rated as `good` with an average tasting score between 6.03 and 6.94.

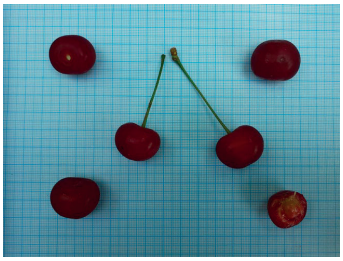


Figure 1. SLV-24-Dj-1.2



a b

Figure 2. SLV-24-Dj-2.5 (a, b)

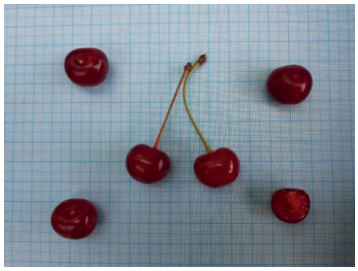


Figure 3. SLV-24-Dj-1.3



a b

Figure 4. SLV-24-Dj-2.6 (a, b)

CONCLUSIONS

Out of the 24 selections, `SLV-24-Dj-1.2` with 7.1g and `SLV-24-Dj-3.1` with 6.87g stood out for fruit size, while `SLV-24-Dj-2.5` stood out for taste and flavor. However, the varieties with an average fruit weight greater than 4g and very good productivity should not be overlooked either. The selected local varieties will be monitored again this year to see if the productivity and quality of the fruits remain consistent.

ACKNOWLEDGEMENTS

This research is part of the project ADER 6.1.1/2023 `Studies on the conservation, evaluation, and breeding of pomological biodiversity represented by species tolerant to thermo-hydric stress` and was supported by Romanian Ministry of Agriculture and Rural Development.

REFERENCES

Branişte N. (2006). *Vişinul. Fondul de germoplasmă la speciile pomicole, de arbuşti fructiferi şi căpşun din colecţiile din România*, Editura Pământul, ISBN: 973-8280-87-7.

Bura M. (2009, april, 8). Plante melifere care înfloresc în aprilie şi mai. *Ferma*. Retrieved January 24, 2025, from https://revista-ferma.ro/plante-melifere-care-infloresc-in-aprilie-si-mai,

Coman Mihai, Chiţu Emil,…, Petre Valeria, Durău Anica (2014). Zonarea speciilor pomicole în funcţie de condiiile pedoclimatice şi socio economice ale României: 69-72, ISBN 978-973-1886-93-0

Criterii de apreciere a calităţii fructelor şi bazele standardizării lor - https://www.scrigroup.com/casa-masina/pomicultura/CRITERII-DE-APRECIERE-CALITATI61414.php

FAOSTAT data (2025, January, 15) Retrieved from https://www.fao.org/faostat/en/#data/QCL

Federica Blando, B. Dave Oomah (2019). Sweet and sour cherries: Origin, distribution, nutritional composition and health benefits - a review. *Trends in Food Science & Technology*, vol. 86: 517-529 https://doi.org/10.1016/j.tifs.2019.02.052

Ghinea D. (2025, February, 3) - Dolj, România geografică. Retrieved from https://romaniadategeografice.net/unitati-admin-teritoriale/judete/judete-d/dolj/

Istoric. SCDCPN Dăbuleni. Retrieved from https://www.scdcpndabuleni.ro/ro/istoric/

Loescher, W. (2003). *Cherries. Encyclopedia of Food Sciences and Nutrition (Second Edition)*, Ed. Academic Press.

Lindsey R. and Dahlman L. (2024, January, 18). Climate Change: Global Temperature. Retrieved February 7, 2025, from https://www.climate.gov/news-features/understanding-climate/climate-change-global-temperature

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

THE ACTION OF PRODUCTS BASED ON NAD, ANA AND BA ON THE FRUIT LOAD CONTROL IN GOLDEN REINDERS APPLE TREES

Ananie PEȘTEANU, Oleg CALESTRU

Technical University of Moldova, 48 Mircești Street, Chisinau, Republic of Moldova

Corresponding author email: ananie.pesteanu@h.utm.md

Abstract

The research was carried out in the plantation of the LLC `Codru ST` enterprise founded in 2006. The efficiency of apple fruits chemical thinning on Golden Reinders variety grafted on the M9 rootstock was studied, under the action of the naphthylacetamide NAD (Geramid-New), naphthylacetic acid ANA (Dirager) and benzyladenine BA (Gerba 4LG), in different doses and thinning periods. Optimum values for the number of fruits in the crown, the average weight of a fruit, the production of fruits per tree and per hectare, as well as the average diameter of a fruit were obtained in the case of treatment with Geramid-New products 2.0 l/ha, Dirager 0.4 l/ha and Gerba 4LG 2.5 l/ha, recording values similar to the manual thinning option. A significant effect was recorded when treating with Geramid-New 2.0 l/ha when 80% of petals fell + 2-3 days, with Dirager 0.4 l/ha when the central fruit diameter was 8-9 mm and Gerba 4LG 2.5 l/ha when the fruit diameter was 10-15 mm.

Key words*: apple, chemical thinning, growth regulators, fruit, average weight, diameter, production.*

INTRODUCTION

In the last decade, the Golden Delicious variety and its clones, both worldwide and in the Republic of Moldova, are the most cultivated yellow apple varieties (Babuc V. et al., 2013; Balan V. et al., 2019; Bucarciuc V., 2022; Cimpoies Gh., 2012; Pește anu A., 2015; Pește anu A. and Calestru O., 2020). The Golden Reinders variety records abundant and regular flowering on all types of fruit branches forming high yields, but in some years a lower quality is recorded (Ambrozic Turk B. and Stopar M., 2010; Pește anu A., 2013; Pește anu A., 2015; Pește anu A. and Calestru O., 2020). In order to exclude this negative phenomenon, it is recommended to intervene by optimizing the fruit load through various thinning methods (manual, chemical) with different growth regulators and in various treatment doses (Greene D., 2002; Dorrigoni A. and Lezzer P., 2007; Ilie A. et al., 2016; Pește anu A., 2013; Pește anu A., 2015; Pește anu A. and Calestru O., 2015; Pește anu A. and Calestru O., 2017).

That is why, at the initial stage, in apple plantations, the fruit load control of the trees is carried out by the chemical method and then, if necessary, the number of fruits can be corrected by the manual method depending on the degree of development and the physiological state of the plants (Vămășescu S. and Balan V., 2014; Dennis F., 2000; Pește anu A., 2017; Stopar M., 2000).

To solve this problem, it is necessary to study the use of different growth regulators whose active substance are NAD, ANA and BA, which allow to carry out treatments from the fall of the petals until the central fruit in the inflorescence is 15 mm diameter (Greene D., 2002; Hiroshi I. et al., 2018; Pește anu A., 2013; Pește anu A., 2015; Pește anu A. and Calestru O., 2020).

MATERIALS AND METHODS

The researches were carried out between 2014 and 2017, in the intensive apple orchard of the SRL "Codru-ST" enterprise, planted near Pauleşti village, Calaraşi district. The plantation was founded in the fall of 2006, with one-year-old trees.

As biological material, the Golden Reinders variety grafted on the M9 rootstock was taken, the crown led according to the improved thin spindle system, the planting distance 3.5 x 1.2 m.

Growth regulators based on NAD, ANA and BA, produced by the company "L. Gobbi" SRL, Italy and established the following experience (Table 1).

Table 1. Scheme of the experience on the method of thinning and the dose of treatment of apple trees with growth regulators

Variants of the experiment	Activ ingredient	Treatment dose, l/ha	Aplication
Without thinning (c)	-	-	-
Hand thinning	-	-	Manual thinning after physiological fall, when the central fruit reaches 15-20 mm in diameter
Geramid New	NAD (44.8 g/l)	1.2	By spraying, when 80% of petals fall +2-3 days, when the central fruit reaches 4-7 mm in diameter
		1.5	
		2.0	
Dirager	ANA (37 g/l)	0.2	By spraying, when the central fruit reaches 8-9 mm in diameter
		0.3	
		0.4	
Gerba 4 LG	6BA (41 g/l)	2.0	By spraying, when the central fruit reaches 10-15 mm in diameter
		2.5	
		3.0	

The research was carried out according to the general methods for experiments with fruit species in the field and laboratory.

The trees were treated with the portable sprinkler in the windless hours, in the morning, from the +18°C temperature. The amount of solution per tree was 0.42 liters, resulting from the number of trees per unit area and the recommended amount of water of 1000 l/ha.

The records for the determination of the number of fruits, the average weight of a fruit, the production per tree, per surface unit and the diameter of the fruits were carried out during the harvest period.

The statistical processing of the main indices was carried out by the method of monofactorial dispersion analysis. The experimental results were processed using the ANOVA test and STATGRAPHICS 18 software package.

RESULTS AND DISCUSSIONS

The number of fruits in a tree results from the share of flowers, the setting coefficient, the fruit load control method and the dose applied within each individual growth regulator.

The data presented in Table 2 show that the number of fruits in the crown of the apple trees was in direct correlation with the factors taken into the study. A higher number of fruits on trees of the Golden Reinders variety was recorded on the variants under study in 2014 and 2016, where they constituted 103-307 and 107-285 pcs./tree, respectively. In 2015 and 2017, the number of fruits in the crown of the trees was the lowest during the research period due to the higher values of fruits in the previous years, constituting 83-130 and 98-128 pcs./tree, respectively.

Further studying how the number of fruits varied during the research, a more destabilized gap was recorded in the control version, where the index under study, in the years 2014 and 2016, recorded the highest values, 307 and 285 pcs./tree, and in 2015 and 2017 no production was recorded due to alternative fruiting. Depending on the number of fruits obtained in the crown of the trees from the control variant, we can mention that the Golden Reinders variety is not completely prone to alternative fruiting, when productions of 58.55 and 54.33 t/ha were recorded in the crown of the trees (Table 2).

Table 2. The influence of growth regulators and treatment dose on the number of fruits in the crown of Golden Reinders apple trees, pcs./tree

Variants of the experiment	Treatment dose (l/ha)	Years				Average 2014-2017
		2014	2015	2016	2017	
Without treatment (c)	-	307	0	285	0	292.5
Hand thinning	-	112	110	113	107	110.5
Geramid New	1.2	154	124	143	128	137.3
	1.5	135	127	115	109	121.5
	2.0	116	107	114	101	109.5
Dirager	0.2	159	130	140	120	137.3
	0.3	140	124	131	114	127.3
	0.4	111	114	117	106	112.0
Gerba 4LG	2.0	139	106	135	115	123.8
	2.5	115	94	110	107	106.5
	3.0	101	83	107	98	97.3
DL 5%	-	7.4	6.9	7.2	6.3	-

The number of fruits on an apple tree of the Golden Reinders variety also differs significantly depending on the method of the fruit load control during the research. In the variant without thinning, the number of fruits per tree after the multi-year average recorded higher values (292.5 pcs./tree), compared to the manual thinning variant (110.5 pcs./tree). The number of fruits in the manual thinning variant was constant throughout the research and did not vary essentially from the multiannual average value, because the goal was to maintain 110-115 pcs./tree in the crown.

The growth regulators used in the chemical thinning of the reproductive organs during the research had their contribution on the average number of fruits in the crown of the apple trees. A higher number of fruits in the crown of apple trees was registered in the case of treatment with the growth regulator Dirager (112.0-131.3

pcs./tree) compared to the product Geramid New (109.5-137.3 pcs./tree), where fruit load control is triggered immediately after petal fall. The lowest number of fruits in the crown of apple trees was obtained when using the growth regulator Gerba 4 LG (97.3-123.8 pcs./tree). The different number of fruits in the apple trees of the Golden Reinders variety is explained by the action of each active substance specifically on blocking the development of the seeds in the seminal chamber of the fruits, their abscission and the climatic conditions that were recorded during the treatment period.

Since it is known that the Golden Reinders variety is part of the varieties where it is more difficult to chemically thin the reproductive organs, various treatment doses were also studied, including those recommended for apple cultivation in the Republic of Moldova.

If, for example, the average number of fruits for the years 2014-2017 in the version treated with the product Geramid New 1.2 l/ha was 137.3 pcs./tree, then in the case of the version Geramid New 1.5 l/ha the value of this index was 121.5 pcs/tree. Increasing the Geramid New product treatment dose to 2.0 l/ha essentially influenced the number of fruits in the tree card, where the index in the study was 109.5 pcs./tree, an approximate value with the manual thinning option.

This is explained by the fact that within some apple varieties, the use of smaller doses of the product can have a negative reaction on the number of fruits obtained in the crown of the trees, a lower influence on the normalization of the fruit load. A. Pește anu and O. Calestru (2020) reached such results, in the conditions of 2018 within the Golden Reinders variety in the Republic of Moldova.

Higher values in the variants where the Dirager product was administered were obtained in the variant treated with a dose of 0.2 l/ha (137.3 pcs./tree). In the Dirager 0.3 l/ha variant, the index in the study decreased (121.5 pcs./tree), while in the case of the Dirager 0.4 l/ha variant, the number of fruits in the crown of the trees during 4 years was 112.0 pcs./tree, more constant value for Golden Reinders apple trees in the respective age period.

In the case of the variants treated with the growth regulator Gerba 4 LG, we register a more essential influence on the index studied, compared to the variants treated with Geramid New and Dirager products. The lowest number of fruits per tree in the variants treated with Gerba 4 LG was obtained in the case of the 3.0 l/ha dose (97.3 pcs./tree), then higher values of the study index were recorded in the variant with the dose of 2.5 l/ha (106.5 pcs./tree), which was approximately at the level of the Geramid New 2.0 l/ha and Dirager 0.4 l/ha variants. A greater number of fruits in the case of the variants treated with the product Gerba 4 LG was registered in the case of administering the dose of 2.0 l/ha (123.8 pcs./tree),

The average weight of a fruit, as an indicator of quality (Table 3), varied greatly during the years studied in the trees of the Golden Reinders variety. The highest average weight of a fruit in the manual thinning variant was recorded in 2017 (171.3 g), and the lowest in 2016 (145.0 g). During the years 2014 and 2015, the average weight of a fruit recorded average values of 160.3 and 167.4 g, respectively. This indicator is largely correlated with the number of fruits obtained within a tree and the weather conditions in the years of reference. Also, the average weight of a fruit in that period decreased essentially due to the large number of fruits in the control version, where the index under study recorded the lowest values, in 2014 - 80.1 g, and in 2016 - 76.8 g.

The average weight of a fruit also changes under the influence of the method of control the fruit load. Lower values were recorded in the variants Geramid New 1.2 l/ha (126.7 g), Dirager 0.2 l/ha (127.8 g) and without thinning (78.4 g) compared with the other variants (137.8-175.1 g). The essential gap between the variants with a lower average weight is explained by the different number of fruits and the reaction of the growth regulator on the variety under study during the years of research. A lower weight of a fruit was obtained in the control variant in the years 2014 and 2016, constituting 80,1 and 76.8 g, respectively, and in the years 2015 and 2017 no harvest was obtained in the respective variants. This phenomenon was recorded due to the alternation of fruiting, which in the Golden Reinders variety is more pronounced than in the trees of other varieties. Within the variant

with manual thinning we record an average weight of 161.0 g, values considered more balanced within the variants studied.

Table 3. The influence of growth regulators and treatment dose on the average weight of a crown fruit of Golden Reinders apple trees, g

Variants of the experiment	Treatment dose (l/ha)	Years				Average 2014-2017
		2014	2015	2016	2017	
Without treatment (c)	-	80.1	0	76.8	0	78.4
Hand thinning	-	160,3	167.4	145.0	171.3	161.0
Geramid New	1.2	120.2	129.7	118.3	138.5	126.7
	1.5	134.7	145.0	146.4	168.2	148.6
	2.0	163.4	170.4	150.8	179.5	166.0
Dirager	0.2	116.8	127.4	120.7	146.3	127.8
	0.3	130.3	137.8	126.9	156.3	137.8
	0.4	159.0	157.1	144.3	173.3	158.4
Gerba 4LG	2.0	131.8	169.3	125.5	160.3	146.7
	2.5	159.2	182.3	148.9	173.4	166.0
	3.0	178.4	188.3	153.4	180.4	175.1
DL 5%	-	5.1	5.4	4.6	5.9	-

The products used for the chemical thinning of the fruits also had an interaction on the index studied in a differentiated way, recording average weights of a fruit according to the active substance used in the treatment and the administered dose.

Comparing the average weight of a fruit according to the growth regulators used for chemical thinning, it was found that as a result of treating the trees with the product Geramid New 2.0 l/ha, a value of 166.0 g was recorded, that is, an increase, compared with the manual thinning option, by 3.1%. Within the Geramid New 1.5 l/ha variant, the studied index was 148.6 g, and the lowest values (126.7 g) were recorded in the case of reducing the dose to 1.2 l/ha. The increase in the average fruit weight is correlated with the amount of product administered within each tree on the variants studied.

In the case of the variants using the Dirager product, the average weight of a fruit at different treatment rates is of interest for modern fruit growing. Thus, if the average weight of a fruit in the Dirager 0.2 l/ha version was 127.8 g, then in the trees treated with the 0.3 l/ha dose, the index in the study increased to 137.8 g. Further increasing the amount of product per surface unit (Dirager 0.4 l/ha), we record higher values of the index taken in the study (158.4 g).

The growth regulator Gerba 4 LG had a more visible effect on the average weight of a fruit, registering lower values in the version treated with the dose of 2.0 l/ha (146.7 g), then in increasing order the version with the dose 2.5 l/ha (166.0 g), and fruits with higher values were recorded in the 3.0 l/ha (175.1 g) version.

Fruit production is the main index by which the efficiency of the technological elements applied to apple culture can be appreciated. Apple fruit production is a complex characteristic, which depends on how the fruit load is managed, the amount of product applied to chemical thinning and how these technological elements interacted with environmental factors (Pește anu A. and Calestru O., 2017; Pește anu A. and Calestru O., 2020).

The data in Table 4 shows that the factors mentioned above had a major impact on fruit production within a tree.

Fruit production within one tree is correlated with the number of fruits on the crown of apple trees and the average weight of a fruit. A higher fruit production in the crown of apple trees with different fruit load management methods without the control variant was obtained in 2014 and 2017 compared to 2015 and 2016.

The fruit load control method during the research influenced the studied index, with higher values being recorded in the Geramid New 2.0 l/ha variant with 18.13 kg/tree, and lower values in the control variant, without thinning (11.85 kg/tree). Approximate values with manual thinning (17.77 kg/tree) were recorded in the version treated with the growth regulator Dirager in the dose of 0.4 l/ha -17.70 kg/tree and Gerba 4 LG 2.5 l/ha - 17.60 kg/tree. Fruit production within a tree in the other variants during the researches recorded lower or insignificantly higher values compared to the manual thinning variant.

Fruit production in the crown of apple trees was also correlated with the dose of product administered during the chemical thinning period. Among the trees of the Golden Reinders variety, higher apple productions were obtained in the Geramid New 1.5 l/ha (17.94 kg/tree) and Geramid New 2.0 l/ha (18.13 kg/ tree). The variant treated at the dose of 1.2 l/ha decreased fruit production (17.32 kg/tree).

Within the variants treated with Dirager 0.2 l/ha, fruit production per tree recorded insignificantly higher or lower values compared to the variants Dirager 0.3 l/ha and Dirager 0.4 l/ha.

Table 4. The influence of growth regulators and treatment dose on fruit production in the crown of Golden Reinders apple trees, kg/tree

Variants of the experiment	Treatment dose (l/ha)	Years				Average 2014-2017
		2014	2015	2016	2017	
Without treatment (c)	-	24.59	0	22.82	0	11.85
Hand thinning	-	17.95	18.41	16.38	18.33	17.77
Geramid New	1.2	18.51	16.08	16.97	17.72	17.32
	1.5	18.18	18.41	16.83	18.33	17.94
	2.0	18.95	18.23	17.19	18.13	18.13
Dirager	0.2	18.57	16.56	16.90	17.55	17.40
	0.3	18.24	17.09	16.62	17.82	17.44
	0.4	17.65	17.91	16.88	18.37	17.70
Gerba 4LG	2.0	18.32	17.95	16.94	18.43	17.91
	2.5	18.31	17.14	16.38	18.55	17.60
	3.0	18.02	15.63	16.41	17.68	16.94
DL 5%	-	0.27	0.35	0.32	0.43	-

Treatments with Gerba 4 LG growth regulator reduced fruit production per tree more significantly compared to Geramid New and Dirager products. The production within a tree of the Golden Reinders variety varied between 16.94 and 17.91 kg/tree. The value of this index for the Gerba 4 LG 2.0 l/ha variant was higher (17.60 kg/tree) than for the Gerba 4 LG 2.5 l/ha variant (17.11 kg/tree). In the case of the Gerba 4 LG 3.0 l/ha variant, production per tree recorded lower values (16.94 kg/tree), i.e. with a decrease of 5.5% compared to the Gerba 4 LG variant 2.0 l/ha.

Fruit production per unit area was not very different from that obtained within a tree. Higher values of fruit production in the variants with chemical thinning was obtained in 2014 (42.02- 45.12 t/ha), then decreasing in 2017 (41.79-44.17 t/ha), 2015 (38.29-43.83 t/ha) and lower values of this index were recorded in 2016 (39.00-40.93 t/ha) (Table 5).

The fruit load rationing method influenced the fruit production per unit area differently. Thus, if, on average, in the fruiting years 2014-2017 for trees of the Golden Reinders variety in the version treated with the product Geramid New 2.0 l/ha, the fruit production was 43.16 t/ha, in the control version, without thinning, the value of the studied index was 28.22 t/ha. Theoretically, the hypothesis would persist that higher fruit production should be recorded in the control variant, without thinning, but based on the studies carried out, we record diametrically opposite results, which is explained by alternative fruiting of the trees of the given variety in 2015 and 2017.

The further study shows that identical values of the index investigated with the manual thinning variant (42.30 t/ha) were recorded in the variant treated with the product Gerba 4 LG 2.5 l/ha (41.30 t/ha). In the other variants, this index was lower or higher compared to the manual thinning variant. The dose of product used in chemical thinning for the growth regulators studied had a direct influence on fruit production. If, for example, fruit production in the case of treating trees with the growth regulator Geramid New 1.2 l/ha was 41.24 t/ha, then the value of this index in the case of the Geramid New 1.5 l/ha variant - 42.61 t/ha. With the increase of the treatment dose, the value of the index in the study decreases, recording in the Geramid New 2.0 l/ha version a production of 43.16 t/ha. At first glance, it seems as if there is no significant difference between the obtained results, but taking into account the average diameter of a fruit, we register a priority of the studied index in the Geramid New 2.0 l/ha variant compared to the other variants (Table 6).

Table 5. The influence of growth regulators and treatment dose on fruit production in the Golden Reinders apple orchard, t/ha

Variants of the experiment	Treatment dose (l/ha)	Years				Average 2014-2017
		2014	2015	2016	2017	
Without treatment (c)	-	58.5	0	54.3	0	28.2
Hand thinning	-	42.7	43.8	39.0	43.6	42.3
Geramid New	1.2	44.0	38.2	40.4	42.1	41.2
	1.5	43.2	43.4	40.0	43.6	42.6
	2.0	45.1	43.4	40.9	43.1	43.1
Dirager	0.2	44.2	39.4	40.2	41.7	41.4
	0.3	43.2	40.6	39.5	42.4	41.4
	0.4	42.0	42.6	40.1	43.7	42.1
Gerba 4LG	2.0	43.6	42.7	40.3	43.8	42.6
	2.5	43.6	40.8	39.0	44.1	41.9
	3.0	42.9	37.2	39.0	42.1	40.3

If in the case of the variants treated with the growth regulator Geramid New, the yield was 41.24-43.16 t/ha, then in the variants treated with the product Dirager the legality registered on the control of the fruit organs is identical, constituting 41.42-42.15 t/ha, but taking into account the average diameter of the fruits, higher values were recorded in the Dirager variant 0.4 l/ha. Insignificantly higher fruit productions per surface unit were recorded in the Dirager 0.4 l/ha (42.15 t/ha) variant, then the Dirager 0.3 l/ha variant (41.48 t/ha), and the last position goes to the Dirager 0.2 l/ha (41.42

t/ha) variant, where the degree of thinning was higher. The influence of the growth regulator Dirager, when the central fruit reached 8-9 mm in diameter, had a more eloquent influence on the studied index.

The Gerba 4 LG product also contributed to fruit production on the variants studied. Thus, the average fruit production during the years 2014-2017 for apple trees of the Golden Reinders variety in the Gerba 4 LG 2.0 l/ha variant was 42.64 t/ha, in the Gerba 4 LG 2.5 l variant/ha was 41.90 t/ha, i.e. a decrease of 6.8% compared to the previous version. The previously mentioned legality is also valid for the variant treated with the Gerba 4 LG 3.0 l/ha growth regulator, where fruit production decreased by 5.4% compared to the Gerba 4 LG 2.0 l/ha variant and by 1.8% compared to the Gerba 4 LG 2.5 l/ha variant. The higher temperature of 25ºC during 4-5 days in 2015, after the treatment, influenced the degree of fruit thinning in the Golden Reinders variety, being at the level of the multiannual average (Gerba 4 LG 2.0 l/ha), or smaller.

The obtained data allow us to highlight that higher productions with chemical thinning of the fruit organs were recorded in the Geramid New 2.0 l/ha variant, in the Dirager 0.4 l/ha variant and when applying the Gerba 4 LG product in the variant treated with 2.5 l/ha.

Fruit quality is a particularly important characteristic when approximately identical productions are recorded per surface unit and it is necessary to make decisions regarding the influence of the product or treatment dose, which is characterized by various morphological, organoleptic, technological, biochemical and, finally, by the food value.

Analyzing the diameter of the fruits, a criterion taken to classify the fruits by quality categories, during the research and the variants studied (Table 6), we note that the index in question is influenced by the method of normalizing the fruit load, by the products used for chemical thinning and the dose administered within each product.

Lower values of the fruit diameter during the years 2014-2017 in the manual thinning variant were recorded in 2016 (70.0 mm). A larger average diameter of the fruits in the respective variant was obtained in 2015 (72.8 mm) and 2017 (73.0 mm), while in 2014 the studied index recorded average values (71.8 mm). The values regarding the average diameter of the fruits during the researches are considered optimal for the Golden Reinders variety.

Table 6. The influence of growth regulators and treatment dose on the quality expressed by the mean diameter of the fruits in the variety Golden Reinders, mm

Variants of the experiment	Treatment dose (l/ha)	Years				Average 2014-2017
		2014	2015	2016	2017	
Without treatment (c)	-	54.3	0.0	53.7	0.0	54.0
Hand thinning	-	71,8	72.8	70,0	73.0	71.9
Geramid New	1.2	65.7	67.2	65.0	68.7	66.7
	1.5	68.2	69.7	69.8	72.8	70.1
	2.0	72.4	72.9	70.4	74.1	72.5
Dirager	0.2	64.7	67.0	65.4	69.8	66.7
	0.3	67.4	68.7	66.8	71.4	68.6
	0.4	71.7	71.5	69.6	73.2	71.5
Gerba 4LG	2.0	66.3	72.9	66.2	72.1	69.4
	2.5	71.9	74.4	70.3	73.2	72.5
	3.0	73.9	75.0	70.9	74.2	73.5

The fruit load rationing method also influences the average diameter of a fruit. Lower values of the given index were recorded in the control variant without thinning (54.0 mm) and in the variants Geramid New 1.2 l/ha (66.7 mm), Dirager 0.2 l/ha (66.7 mm) and Gerba 4 LG 2.0 l/ha (69.4 mm). In the other variants, where chemical thinning was performed, this index evolved more rationally, recording the average fruit diameter greater than 70 mm.

The variant treated with Geramid New 2.0 l/ha and Dirager 0.4 l/ha recorded identical values to the control variant. The growth regulator Gerba 4 LG, which is characterized by a greater degree of thinning of the fruit organs, induced a greater growth on the index studied.

Also, the average diameter of a fruit changes under the influence of the product dose applied to the varieties under study. The study carried out showed that increasing the dose of the product when treated with Geramid New and Dirager led to an increase in the average diameter of a fruit. If, for example, in the Geramid New 1.2 l/ha variant the average diameter of a fruit was 66,7 mm, then, with the dose increased to 1.5 l/ha, it was 70.1 mm, and in the Geramid New variant 2.0 l/ha – 72.4 mm. In the case of treatments with the Dirager product, the larger diameter was recorded in the version with the dose of 0,4 l/ha (71.5 mm), but when applying the Gerba 4 LG product, we note that with the increase in the dose of the product, the index in the study increases.

Maximum values regarding the index under study being entered in the Gerba 4 LG variant 3,0 l/ha (73.5 mm).

In general, it is found that the number of fruits per tree, average fruit weight, fruit production per tree and per unit area, as well as average diameter of a fruit are correlated with fruit load thinning method, growth regulator and the dose administered, as well as the weather conditions during the treatment period, before and after application.

CONCLUSIONS

The growth regulators based on NAD, ANA and BA, in different doses, particularly influence the number of fruits, their average weight, the diameter of the fruits and the productivity of the apple plantation.

For a more efficient regulation of the fruit load in Golden Reinders trees by the chemical method and to exclude the influence of climatic phenomena, it is necessary to use 1-2 growth regulators when regulating the fruit load, depending on the method of action and application period, to be able to develop a fruit thinning strategy.

For the Golden Reinders variety, higher yields and competitive fruit quality were recorded when treated with the product Geramid New 2.0 l/ha at the fall of approx. 80% of petals plus 2-3 days. If the weather conditions are not favorable for treatment with the Geramid New product during this period, the growth regulator Dirager 0.4 l/ha can be applied later, when the central fruit in the inflorescence is 8-9 mm in diameter. If the number of fruit organs in the tree crown is high, it is recommended to treat with Gerba 4 LG 2.5 l/ha, when the size of the central fruit in the inflorescence is 10-15 mm in diameter.

REFERENCES

Ambrozic Turk, B., Stopar, M. (2010). Effect of 6-benzyladenine application time on apple thinning of cv. 'Golden Delicious' and cv. 'Idared'. In: *Acta Agriculturae Slovenica*, vol. *95*(1), pp. 69-73. Available: https://www.researchgate.net/publication/289943227

Babuc, V., Peşteanu, A., & Gudumac, E. (2015). *Conducerea şi tăierea pomilor şi arbuştilor fructiferi*. Chişinău: Editura „Bons Offices", 256 p. ISBN 978-9975-87-021-4.

Balan, V., Vămăşescu, S., Peşteanu, A., Balan, P. (2019). Influenţa fertilizării foliare asupra răririi fructelor şi recoltei la soiul de măr Golden Delicious. In: *Ştiinţa Agricolă*, nr. 1, pp. 47-51. ISSN 1857-0003.

Bucarciuc, V. (2022). *Ameliorarea mărului*. Chişinău, 456 p., ISBN 978-9975-164-70-2.

Cimpoieş, Gh. (2012). *Cultura mărului*. Chişinău: Editura „Bons Offices", 380 p. ISBN 978-9975-80-547-6.

Dennis, F.G. (2000). The history of fruit thinning. In: *Plant Growth Regulation*, *vol. 31*, pp. 1-16. Available: DOI:10.1023/A:1006330009160

Dorrigoni, A., & Lezzer, P. (2007). Chemical thinning of apple with new compounds. In: *Erwerbs-obstabau*, *vol. 49*(3), pp. 93-96. Available: DOI:10.1007/s10341-007-0038-8

Greene, D.W. (2002). Chemicals, timing, and environmental factors involved in thinner efficacy on apple. In: *Hortscience*, *vol. 37*(3), pp. 477-480. Available: DOI: 10.21273/HORTSCI.37.3.477

Hiroshi, I. et al. (2018). A model for representing the relationships among crop load, timing of thinning, flower bud formation, and fruit weight in apples. In: *Scientia Horticulturae*, *vol. 242*, pp.181-187. Available: https://doi.org/10.1016/j.scienta.2018.08.001

Ilie, A., Hoza, D., & Oltenacu, V. (2016). A brief overview of hand and chemical thinning of apple fruit. In: *Scientific Papers. Series B, Horticulture*, *vol. LX*, pp. 59-64. ISSN 2285-5653.

Pesteanu, A. (2013). Efficiency of Fruitlet Thinning Apple "Golden Reinders" by use Naphthylacetamide Acid (NAD). In: *Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Horticulture*, *vol. 70*, no. 1, pp. 180-186. ISSN 1843-5254.

Peşteanu, A. (2013). Fruit thinning by using NAA agent on the Jonagored apple variety. In: *Analele Universităţii din Craiova*, *vol. XVIII*(LIV), pp. 267-272. ISSN 1453 - 1275.

Peşteanu, A. (2015a). Efficiency of fruitlet thinning apple "Golden Reinders" by use NAD and Ethiphon. In: *Analele Universităţii din Craiova, Seria Biologie, Horticultură, Tehnologia Prelucrării Produselor Agricole, Ingineria Mediului*, *vol. XX*(LV), pp. 125-131. ISSN 1453 - 1275.

Peşteanu, A. (2015b). Effect of thinning Idared apple variety using NAD and Ethephon. In: *Lucrări Ştiinţifice. Seria Horticultură, Universitatea de Ştiinţe Agricole şi Medicină Veterinară „Ion Ionescu de la Brad". Iaşi*, *vol. 58*, nr. 1, pp. 237-243. ISSN 1454 - 7376.

Peşteanu, A. (2015c). The influence of thinning agent on base of 6-BA and NAA on productivity and fruit quality of "Golden Reinders" variety. In: *Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Horticulture*, *vol. 72*(1), pp. 151-156. Available: DOI: 10.15835/buasvmcn-hort:10496

Peşteanu, A. (2015d). Normarea încărcăturii de rod la soiul Golden Reinders prin diverse metode de rărire a fructelor. In: *Lucrări Ştiinţifice, UASM. Chişinău: CE UASM, vol. 42*(I): *Horticultură, Viticultură şi*

Vinificaţie, Silvicultură şi Grădini publice, Protecţia plantelor, pp. 109-115. ISBN 978-9975-64-269-9.

Peşteanu, A., & Calestru, O. (2015). Eficienţa răririi fructelor de măr de soiul Renet Simirenko prin utilizarea produselor pe bază de NAD, ANA şi BA. In: *Lucrări ştiinţifice, UASM. Chişinău: CE UASM, vol. 42*(I): *Horticultură, Viticultură şi Vinificaţie, Silvicultură şi Grădini publice, Protecţia plantelor*), pp. 121-125. ISBN 978-9975-64-269-9.

Pesteanu, A. (2017). Influence of growth regulators on productivity of plantation and quality of apple fruits. In: *Annals of the University of Craiova. Biology, Horticulture, Food products processing technology, Environmental engineering, vol. XXII*(LVII), pp. 215-220. ISSN 1453-1275.

Peşteanu, A., & Calestru, O. (2017). Reglarea încărcăturii de rod la pomii de măr de soiul Golden Reinders prin diverse metode de rărire. In: *Ştiinţa Agricolă*, nr. 2, pp. 37-42. ISSN 1857-0003.

Peşteanu, A., & Calestru, O. (2020). Eficacitatea normării încărcăturii de rod la unele soiuri de măr în perioada precoce de dezvoltare a organelor reproductive. In: *Ştiinţa Agricolă*, nr. 1, pp. 46-54. Disponibil: DOI: 10.5281/ZENODO.3884002

Stopar, M. (2000). Comparison of the most frequently used apple thinning compounds for the thinning of 'Jonagold', 'Elstar' and 'Golden delicious' apples. In: *Zbornik Biotehniške fakultete Univerze v Ljubljani*, pp. 89-94. ISSN 1408-340X.

Vămăşescu, S., & Balan, V. (2014) Thinning and foliar fertilization influence on the yield of Idared apple cultivar. In: *Scientific Papers. Series B, Horticulture, vol. LVIII*, pp. 107-110. ISSN 2285-5653.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

THE GROWTH SEASON FOLLOWING BBCH SCALE AND THE GDD REQUIREMENT FOR *RUBUS IDAEUS* VAR. 'PROMYK'. DURING THE ADAPTATION PERIOD, PLACED IN THE URBAN GARDEN

Alin Laviniu POPA[1], Anamaria VÂTCĂ[2], Valentina STOIAN[1], Mădălina TRUȘCĂ[1], Ștefania GÂDEA[1], Sorin VÂTCĂ[1]

[1]Plant Physiology Department, Faculty of Agriculture,
University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca,
3-5 Calea Mănăştur Street, Cluj-Napoca, Romania
[2]Department of Management and Economics, Faculty of Animal Science and Biotechnologies,
University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca,
3-5 Calea Mănăştur Street, Cluj-Napoca, Romania

Corresponding author email: valentina.stoian@usamvcluj.ro

Abstract

*Urban gardening represents high interest within cities due to extended heat waves produced by pronounced climate change. Raspberry has high potential to adapt and develop under urban gardening conditions. The study aimed to assess the adaptation of Rubus idaeus var. 'Promyk' in urban garden from UASVM Cluj-Napoca. The observation on phenotypic features according BBCH (**B**iologische Bundesanstalt, **B**undessortenamt und **CH**emische Industrie) scale was recorded twice a week for the growing season of 2023-2024, together with the range time for each principal growth stage. The corresponding heat units' requirement (GDD-growing degree-days) was computed for each phenophase. Overall, the raspberries presented asynchronous growth and development in the adaptation year. The GDD highlighted differences. Therefore, the plant's development differed at the individual level based on climatic conditions.*

Key words*: adaptation, development, growing degree-days, phenological assessment, raspberry.*

INTRODUCTION

Urban gardens act as incubators for plant growth and development as the climate rises in cities and the heat island effect is increasingly present (Imbroane et al., 2014). Several plants could benefit from and adapt to these changes in this context. These anthropic gardens could sustain multiple interconnected advantages by reducing the negative effects of heat islands, in periods with high temperatures and especially in the summer, through plant evapotranspiration and shade (Wong et al., 2021).

As climate change intensifies, monitoring the impact of climatic factors on crop performance has become a priority in modern agriculture (Croitoru et al., 2020; Rezaei et al., 2023). The ability to ensure optimal environmental conditions directly influences the quality and quantity of potential yields (Bisbis et al., 2018). Key factors such as temperature, photoperiod, soil pH, and moisture must meet the specific needs of each plant species to ensure healthy growth and maximum productivity (Bacelor et al., 2024). Among these factors, temperature emerges as one of the most decisive and restrictive external variables (Raza et al., 2024), significantly shaping the physiological processes that govern plant growth, development, and fruit production (Patel, 2023). Temperature serves as a natural signal for initiating phenological stages (Guesmi et al., 2021). Continuous monitoring of temperature fluctuations is important, as it enables the use of growing degree days (GDD) to measure cumulative heat over time (Miller et al., 2001). These daily records can be transformed into a heat calendar that sustains the prediction of when specific phenological events will occur (Miller et al., 2001). An important indicator is the base temperature (Tb), which represents the minimum temperature required for plants to initiate active growth (Wahid, 2007). This Tb is specific to each species and plays a crucial role in GDD calculations for determining growth dynamics

and developmental stages (Fraisse & Paula-Moraes, 2018).

The raspberry (*Rubus idaeus* L.) is a less demanding crop regarding climatic factors, which has gained increasing popularity in recent years (Brennan et al., 2014). Raspberries thrive in climates characterized by mild winters (Palonen & Buszard, 1997) and moderately dry summers (Swanson et al., 2011), being less sensitive to cold than blackberries (Palonen & Buszard, 1997). However, the crop can be vulnerable to climatic events like freezing winds and early spring frosts, severely affecting growth and fruiting (Waister et al., 1979). Despite these challenges, raspberry cultivation remains widespread due to the fruit's exceptional aroma (Sawicka et al., 2023), rich nutritional profile, and health benefits (Teng et al., 2017). Global raspberry production in 2022 approached almost 950,000 tons, reflecting a growing consumer demand (Ladyzhenskaya et al., 2025). This makes it essential to closely monitor the environmental factors influencing the plant's growth, development, and fruiting potential to ensure quality and sustainability.

Scientific studies reveal that raspberry plants respond differently to climatic variations depending on their variety (Gotame, 2014), with temperature and photoperiod playing a pivotal role in determining growth outcomes (Sønsteby & Heide, 2012). High-temperature stress, for instance, is particularly detrimental, triggering a range of morphological, physiological, and biochemical changes (Guo et al., 2023). The phenological stages of raspberry plants - such as anthesis initiation, flowering (anthesis), and dormancy - are directly influenced by temperature and sunlight exposure (Hodnefjell, 2017). Previous studies have shown that the breaking dormancy, which lasts approximately six weeks (Pruteanu et al., 2024), in red raspberry is triggered by exposure to a Tb of 4°C (Sutherland et al., 2015; Mazzitelli et al., 2007). The phenological stages of budburst and flowering are also highly sensitive to climatic factors and are directly influenced by them (Rusnac, 2021).

To better understand how temperature affects raspberry development, our study aimed to monitor the growth and phenological progression of *Rubus idaeus* L., a promising plant for urban gardening, 'Promyk' primocanes, using the BBCH scale (Meier, 2018). The main study objective was to correlate the plant's growth stages on the BBCH scale with GDD, as the days' interval required to pass from one secondary stage to another, and productivity percentages in the adaptation year. The experiment was conducted in an outdoor urban garden system, highlighting the role of urban agriculture in promoting sustainable urban development and eco-friendly lifestyles. Urban gardens not only offer opportunities for localized food production but also serve as testbeds for innovative agricultural practices, especially in the face of climate variability

MATERIALS AND METHODS

The experiment was installed in the agro-botanical garden of UASVM Cluj-Napoca, thus simulating an urban garden located near km 0 of the city. The selected experimental area was located in the experimental field 46˚45ˈ36ˈˈN and 23˚34ˈ27ˈˈE of the Plant Physiology Department from the Faculty of Agriculture.

Remounted primocanes of raspberry, *Rubus idaeus* L., 'Promyk', were purchased from the Moara Nursery in Suceava County, Romania. This variety was recently launched on the market after extensive research and crossings in Poland (Orzeł et al., 2023), the fourth largest raspberry producer in the world (Adamczuk et al., 2023). Unlike red raspberry varieties, this one lacks anthocyanins (Adamczuk et al., 2023). A total of 27 rooted raspberry shoots were placed in the field with different nutrition surface areas as follows: high nutrition area (SN_max) with 100 cm between shoots, medium nutrition area (SN_med) with 75 cm between shoots, and low nutrition area (SN_min) with 50 cm between rooted shoots. Raspberry crop monitoring took place between 26.02.2024 and 02.01.2025. All the plants were assessed twice a week, each Monday and Thursday, following the BBCH scale (Meier, 2018). Images of plant growth and development were taken with a 48MP camera. The meteorological data were exported from the Meteomanz database from Romania (http://www.meteomanz.com/), where synoptic observations from the Cluj-Napoca station (15120) were selected. Data by days were

further computed for obtaining GDD (growing degree days) and precipitation amount during the raspberry growth season and principal growth stages. The GDD was calculated with a Tb of 4°C with the formula (Vâtcă et al., 2021):

$$GDD=\sum_{1}^{n}\left(\frac{Tmax+Tmin}{2}\right)-Tb$$

The first step consisted in changing the climatic database respectively the negative values were changed in Tb (4). Then, the GDD formula was applied for each day. After exploring the data base, it was found the period of Eco-dormancy which consisted of six weeks of average temperature above 4°C according to Sutherland et al., 2015, that started from 20.02.2024 with only one day of average temperature of 2°C, value not considered. Eco-dormancy is released when the buds burst in the spring. Endo-dormancy happen in the cold period and values between 1-4°C break this dormancy process. Para-dormancy comes after endo-dormancy with chilling and cold temperatures typically run at 4°C. All raspberry canes were assessed individually in the field and for the climatic database, establishing GDD average and days interval for one secondary stage to another. Therefore, all GDD presented values were calculated from one BBCH secondary stage to another identified in order for the entire vegetation period from a complete database without missing data. The GDD values were then made as average for each variant. Together with GDD, the days for setting into a BBCH and from a BBCH stage to the following were noted from the field observations. These days were used to establish a time range specific for a principal or secondary stage to be achieved. BBCH with one digit is describing principal growth stages and BBCH with two digits represent specific secondary growth stages following the newest growth scale proposed by Meier in 2018.

RESULTS AND DISCUSSIONS

The day's range (Table 1) differed between nutrition area variants and was influenced by the climatic conditions, respectively, the GDD and precipitation amount. A wide range of days were found in the endo-dormancy period, for the leaf development stage, flowering, fruiting, and the beginning of senescence. In the first year of the adaptation period, the raspberry did not produce shoots in SN_min variants and produced only very few in SN_max variants.

Table 1. Registered days interval from one secondary stage to the following

BBCH Code	SN_max	SN_med	SN_min
PGS 0	156-213	131-197	69-169
00	128	128	43-128
03	20-43	1-43	19-27
07	1-10	1-22	3-7
09	7-22	1-4	4-7
PGS 1	39-151	35-206	21-108
10	4-36	7-26	7-28
11	1-3	3-54	4-38
12	1-7	3-54	1-3
13	3-7	3-4	1-18
14	4-21	3-4	3-4
15	1-7	3-18	4-14
16	14-42	3-11	1-3
17	1-7	3-14	
18	7-11	3-4	
19	3-10	4-17	
PGS 2	1-38	26-109	
21	1-38	24-88	
22		1-10	
23		1-11	
PGS 5	17-39	9-36	7-22
51	11-28	3-25	3-4
55	3-4	3-7	3-14
59	3-7	3-4	1-4
PGS 6	6-21	6-21	5-10
60	4-14	1-4	
65	1-3	1-3	1-3
69	1-4	4-14	4-7
PGS 7	11-21	10-31	11-17
75	10-17	7-21	7-10
79	1-4	3-10	4-7
PGS 8	5-11	9-12	5-11
81	1-3	3-4	3-4
85	1-4	3-4	1-3
89	3-4	3-4	1-4
PGS 9	66-214	91-190	53-213
91	21-105	21-53	4-122
93	17-67	14-21	24-52
97	28-42	56-116	25-39

The leaf bud development stage (BBCH 0) has different GDD requirements depending on the growth nutrition area (Table 2). Raspberry rooted shoots planted 100 cm apart required the lowest GDD. Planting shoots at small and medium distances implied an increase of 36% and 44% compared to those at SN_max in the raspberry's requirement towards GDD for the BBCH 0 stage initiation and evolution. An increase in the planting area was associated with increased GDD required for leaf growth and development (BBCH 1). Thus, SN_max and SN_med seedlings benefited from a 74% and 72% increase compared to SN_min seedlings in the GDD required to initiate and develop the main stage BBCH 1.

Table 2. Principal growth stages (PGS) and corresponding growing degree days (GDD, in °C) for all three nutrition areas tested for raspberry

No	PGS	SN_max	SN_med	SN_min
1	**BBCH 0**	153	220	208
2	**BBCH 1**	1380	1369	795
3	**BBCH 2**	764	133	0
4	**BBCH 5**	431	313	196
5	**BBCH 6**	221	275	122
6	**BBCH 7**	253	311	245
7	**BBCH 8**	195	175	197
8	**BBCH 9**	591	1061	1652

Interestingly, the development of lateral shoots (BBCH 2) was the most sensitive stage to GDD and different nutritional areas (Table 2). While SN_min buds did not enter the main BBCH stage, the requirement towards GDD of SN_max shoots was six times higher than in SN_med. The initiation of the generative stage in raspberry is associated with the appearance of the flower bud (Hodnefjell et al., 2018). It was observed that SN_min raspberries required a reduced GDD value to enter BBCH 5 compared to the other variants. For this stage, the GDD needs for plants from SN_med and SN_max increased with values in the 117-235 GDD range compared to the minimum recorded in SN_min. The plant's evolution on the BBCH scale in main stages 6 and 7 recorded a similar trend. Raspberries from SN_med had the highest GDD requirements. At the opposite pole were the rooted shoots planted at a distance of 50 cm. Fruit ripening (BBCH 8) occurred under similar conditions of GDD for the SN_max and SN_min variants. On the other hand, SN_med required slightly lower GDD compared to the other variants. The onset of plant senescence (BBCH 9) is associated with increases in GDD proportional to a decrease in the nutrition area. The maximum value, recorded at SN_min, was 1.6 times higher than at SN_med and 2.8 times higher than at SN_max.

Eco-dormancy ends when the average temperature is at least 4°C for at least 6 weeks (Sutherland et al., 2015) (Table 3, Figure 1). From the time the raspberry rooted shoots were planted to when they came out of dormancy, it took 128 days. At SN_ max and SN_min, this physiological phenomenon took only 3 weeks with average temperatures above 4ºC to complete bud swelling (BBCH 03). At SN_med, it took more than 6 weeks with average temperatures above 4°C to observe the beginning of bud burst (BBCH 07).

For better data exploration, GDD is scaled as follows: minimum requirements GDD<50, medium requirements GDD between 50-100, high requirements GDD between 100-150, and very high requirements GDD>150.

Only a few BBCH stages were observed with values less than 50 GDD (Table 3, Figure 1).

Only 10% of the 31 BBCH stages studied in SN_max raspberry plants had GDD requirements lower than 50 (BBCH 07, 11, and 55). Similarly, the same percentage of BBCH stages evaluated at SN_med required GDDs less than 50. On the other hand, several BBCH stages (16% of the total) needed reduced GDD in SN_min, including one related to flower bud development (BBCH 51). When raspberry receives the photoperiod signals and temperature requirements are fulfilled, the flower primordia start broadening its apical meristem and elongate (Hodnefjell, 2017).

For SN_max raspberries, 35% of the BBCH stages evaluated required values in the 50-100 GDDs range for initiation and evolution. Planting the rooted shoots in an average nutrition area influenced the evolution of the plants on the BBCH scale and their GDD requirements. Only 29% of the BBCH stages evaluated required GDD in the 50-100 GDD range. Raspberries planted at a distance of 50 cm recorded 32 percent of the assessed

BBCH stages. It was emphasized that, among the BBCH stages monitored, the fruit-ripening stages showed similar requirements to GDD for all variants (BBCH 81, 85, 89).

For both SN_max and SN_med, it was observed that 19% of the monitored BBCH stages required values in the range 100-150 GDD (Table 3, Figure 1). The difference between the two variants was represented by the specific stages with these requirements. While two-thirds of these stages analyzed at SN_max supported leaf development, at SN_med, only one-third did, and another third was represented by side shoot development. For raspberries with SN_min, only 10% of the monitored stages had requirements in the same range of GDD values.

Table 3. Principal growth stages of raspberry and corresponding GDD values as a response to different nutritional areas

Code	Stage	SN_max	SN_med	SN_min
Principal growth stage 0: Bud development				
00	Winter dormancy or resting period	387/249	387/249	397
03	End of bud swelling			135
07	Beginning of sprouting or bud breaking	39	191	28
09	Bud shows green tips	114	29	45
Principal growth stage 1: Leaf development (main shoot)				
10	First leaves separated	138	153	143
11	First leaves unfolded	34	287	169
12	2 true leaves, leaf pairs, or whorls unfold	70	289	38
13	3 true leaves, leaf pairs, or whorls unfolded	58	43	265
14	4 true leaves, leaf pairs, or whorls unfolded	132	44	51
15	5 true leaves, leaf pairs, or whorls unfolded	81	117	74
16	6 true leaves, leaf pairs, or whorls unfolded	462	80	54
17	7 true leaves, leaf pairs, or whorls unfolded	137	130	
18	8 true leaves, leaf pairs, or whorls unfolded	157	55	
19	9 or more true leaves, leaf pairs, or whorls unfolded	112	170	
Principal growth stage 2: Formation of side shoots/tillering				
21	First side shoot visible; First tiller visible	764	478	
22	2 side shoots visible; 2 tillers visible		128	
23	3 side shoots visible; 3 tillers visible		138	
Principal growth stage 5: Inflorescence emergence (main shoot) /heading				
51	Inflorescence or flower buds are visible	303	166	46
55	First individual flowers visible (still closed)	50	79	95
59	First flower petals visible (in petalled forms)	77	67	55
Principal growth stage 6: Flowering (main shoot)				
60	First flowers open (sporadically)	101	80	
65	Full flowering: 50% of flowers open, first petals may be fallen	56	52	47
69	End of flowering: fruit set visible	64	143	75
Principal growth stage 7: Development of fruit				
75	50% of fruits have reached final size	197	190	147
79	Nearly all fruits have reached final size	56	122	98
Principal growth stage 8: Ripening or maturity of fruit and seed				
81	Beginning of ripening or fruit colouration	50	61	59
85	Advanced ripening or fruit colouration	73	59	65
89	Fully ripe: fruit shows fully-ripe colour, beginning of fruit abscission	71	54	73
Principal growth stage 9: Senescence, beginning of dormancy				
91	Shoot development completed, foliage still green	286	571	1176
93	Beginning of leaf-fall	254	225	266
97	Plant resting or dormant	52	265	210

Note: SN_max - high nutrition area, SN_med - medium nutrition area, SN_min - low nutrition area

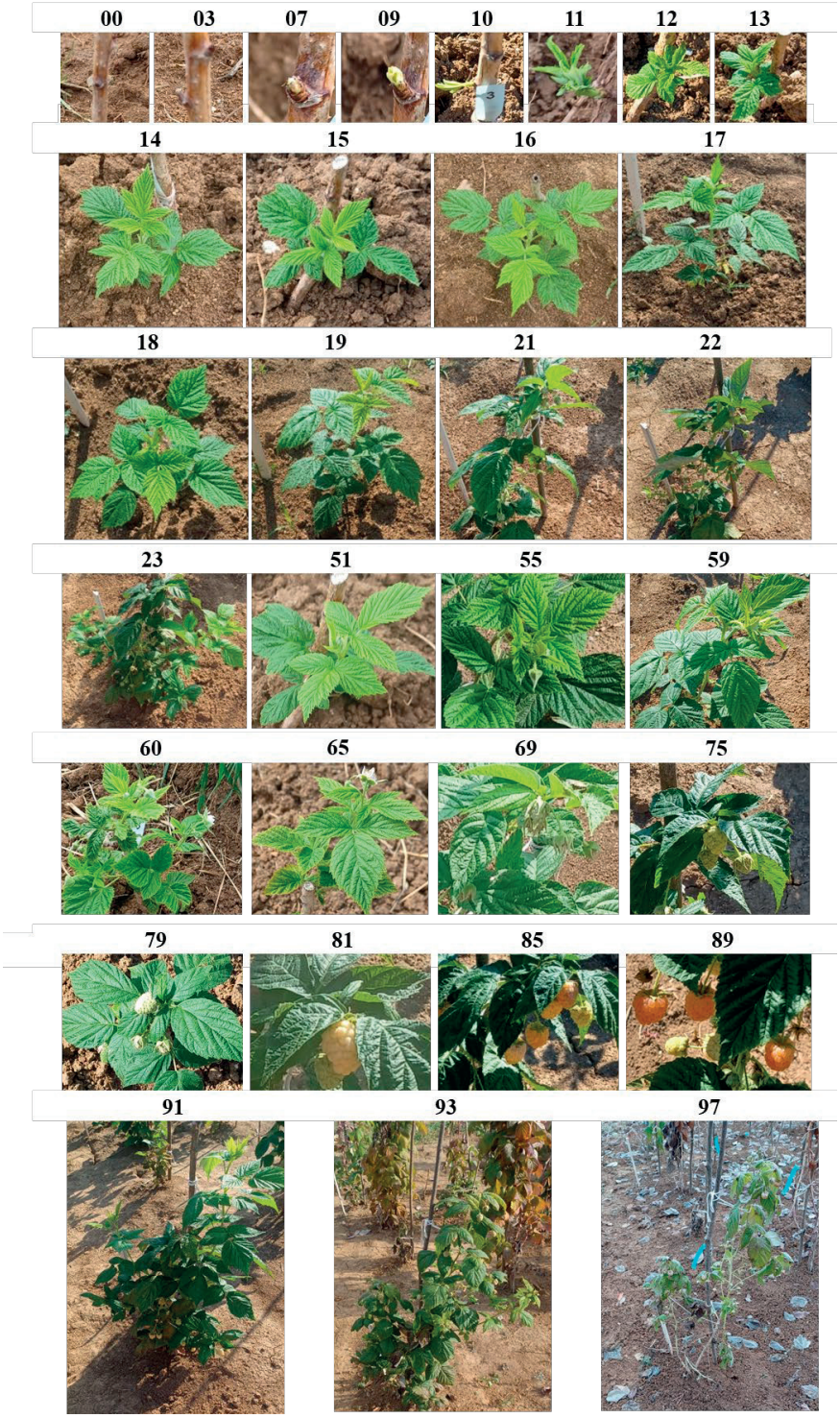


Figure 1. BBCH growth stages at primocanes of raspberry *Rubus idaeus* L., 'Promyk' entire growth period

The experiment also outlined other interesting GDD requirements related to the initiation of BBCH stages and their corresponding GDDs. Such requirements were observed at five large BBCH stages: BBCH 1, 2, 5, 7, and 9, along with 5 BBCH sub-stages related to leaf growth and development, with very high GDD requirements.

The stages from the first unfolded leaf (BBCH 11) to the third unfolded leaf (BBCH 13) required a very high GDD input with values ranging from 169-189 for the minimum and medium nutrition surface (Table 3, Figure 1). On the other hand, the SN_max raspberries also had high GDD requirements for initiating the BBCH 16 stage. Thus, for this variant, the appearance of the sixth unfolded leaf was conditioned by a GDD value higher than the average of the range required for the initiation of BBCH 11, 12, and 13 in the other two variants. Another interesting aspect emerged in all variants for the BBCH 19 stage. The raspberry grown in SN_min did not develop more than six leaves until the appearance of the flower bud (BBCH 51), which means that the plants skipped some growth stages. The other two variants had different requirements for GDD to enter BBCH 19, with a value 1.5 times higher for SN_med than for SN_max. The onset of side shoots was a BBCH step with major requirements for GDD. SN_min did not allow the stumps to develop side shoots, and SN_med had a 1.6-times lower requirement than the large value of growing days that SN_max stumps required. Raspberry developed multiple shoots in SN_med and only a few in SN_max.

Floral bud emergence was triggered under unexpected GDD conditions, with increases in the minimum required value for SN_mic, of 3.6 for SN_med and 6.7 for SN_max. This shows that floral bud emergence fluctuates and has different GDD requirements depending on the growth nutrition area.

Fruit growth to 50% of its final size (BBCH 75) had similar GDD requirements when plants had SN_max and SN_med, whereas SN_min shoots had a 25% decrease in GDD compared to the value recorded at SN_max.

Based on the synthesis of all the observations during the experimentation (Figure 2), the first developmental stage - the leaf plants stage (BBCH 1) - started simultaneously for raspberries that received maximum and minimum surface nutrition. However, in SN_min, the buds stage lasted the longest. As for raspberries planted in a medium nutrient area, they entered BBCH 0 the latest, and the evolution of the stage was very short. The first leaf initiation occurred simultaneously for SN_max and SN_min, but contrary to the evolution observed at BBCH 0, this time, for plants that benefited from SN_max, the period was longer.

The SN_med raspberries showed a similar appearance to the BBCH 0, where they initiated the latest stage (Figure 2). Only SN_med and SN_max plants developed side shoots (BBCH 2), but the stage evolution showed major differences between the two variants. While raspberries planted in a SN_max developed side shoots only at the end of July, those planted in a SN_med required more time to initiate and evolve the main BBCH 2 stage. This stage occurred during all three decades of September and partially overlapped with the main stages of fruit growth and development in SN_med plants. The duration of the BBCH 5 period increased with the increase in the nutrient area. Flower bud emergence and development (BBCH 5) captures interesting aspects in the three tested variants (Figure 2).

The raspberry growth and development evolution for all three nutrient surfaces (Figure 2) showed similar ratios of the BBCH 5 stage with stages 6 and 7. However, notable differences were only observed for the BBCH 8 stage. Stages 5, 6, and 7 start only three, two, and one decade earlier than BBCH 8 at SN_max, and at SN_min, stage 8 starts after the end of BBCH 5. The most interesting aspect was outlined at SN_max, where the BBCH 8 stage ends earlier than 5, 6, 7, emphasizing that either the flowers were not fully developed, the fruits were aborted, or they stopped growing at some point. The decrease in nutritive surface area hastened the entry of plants into senescence, at SN_min noticeably shortening the period of activity. The time of entry into dormancy was the same for all three variants, i.e., at the end of December.

At the maximum nutrition area (SN_max), endo-dormancy runs from the second decade (D2) of October and continues until the second

decade (D2 of February) (Figure 2). Para-dormancy extended from D2 of February to D1 of March. The main growth stage, bud development (BBCH 0), started from D2 of March and ended in D3 of April. Leaf development on the main shoot (BBCH 1) started in D1 of April and ended in D1 of August. The lateral shoots (BBCH 2) were visible in D3 in July. The start of floral emergence (BBCH 5) in the area of maximum nutrition began in D1 of May and ended in D2 of September. Other studies declared that marginal low temperature and short day conditions from September determined a reduction of floral induction (Woznicki et al., 2016). On the main shoot, flowering (BBCH 6) started in D2 of May and finished in D3 of September. Fruit development (BBCH 7) started in D2 of June and finished in D1 of October. Fruit ripening (BBCH 8) started in D2 of June and was completed in D2 of August. Raspberry fruit set began in D1 of September and ended in D3 of December.

On the average nutrient surface (SN_med), endo-dormancy runs from D2 of October to D2 of February (Figure 2). Para-dormancy started in February and went from D2 to D1 in March. Shoot development (BBCH 0) began and ends in the last decade (D3) of April. Leaf development on the main shoot (BBCH 1) began in April in D3 and it was completed in D1 of July.

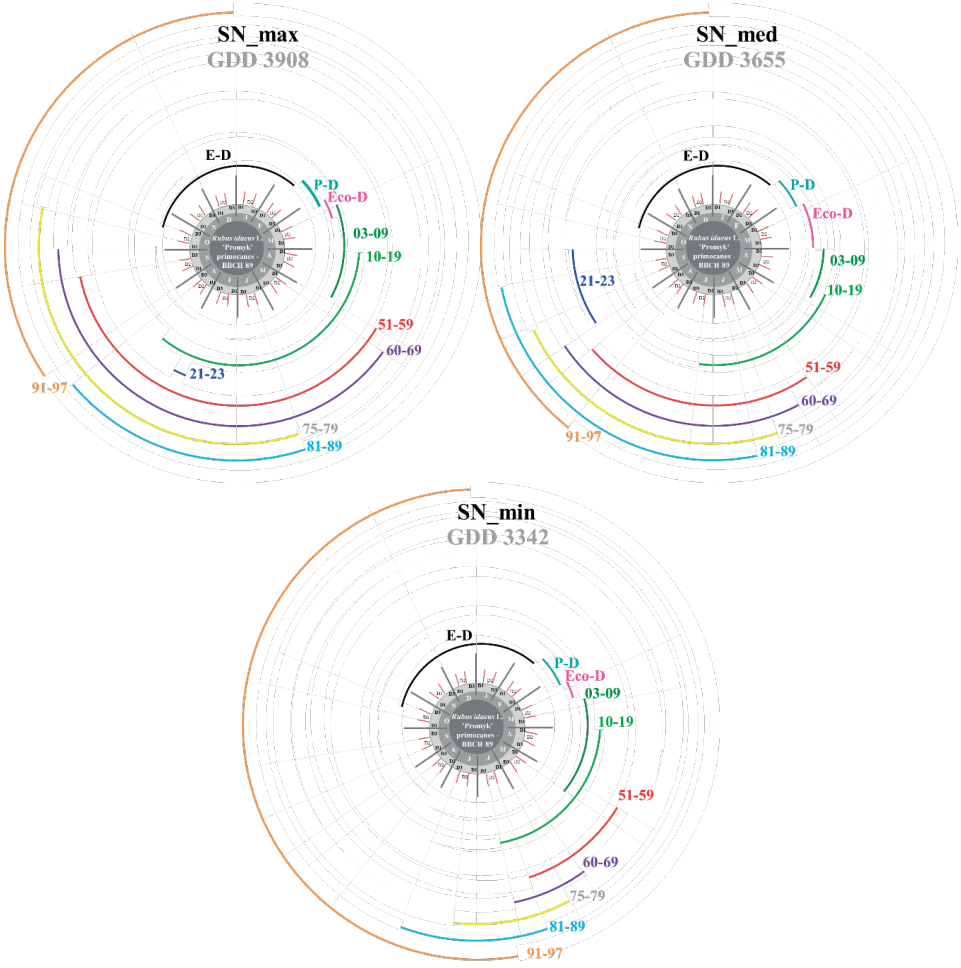


Figure 2. BBCH secondary stages are identified, and the overall growth period depends on the nutrition area

Lateral shoots (BBCH 2) formed between D1 and D3 in September. The BBCH 5 started on D3 of May and ended on D2 of August. Flowering (BBCH 6), on the main shoot, in the minimum nutrition area, started in D1 of June and finished in D3 of August. This results are in accordance with other studies with different temperature and how low temperature can influence the days to anthesis (Woznicki et al., 2016). In a minimum nutrition area, the plants could benefit from higher temperatures, therefore the flowering happens faster and does not last.

BBCH 7, fruit development, started in D2 of June and finished in D1 of September. Fruit ripening (BBCH 8) started in D3 of June and finished in D2 of September. The senescence of raspberry rooted shoots (BBCH 9) occurred between D2 of August and D3 of December. At the minimum nutritive area (SN_min), from the climatic database it was found that endo-dormancy occurred between D2 of October and D2 of February. Para-dormancy started in D2 of February and ended in D1 of March (Figure 2). Shoot development (BBCH 0) took longer, beginning in D2 of March and ending in D1 of May (Figure 2). Leaf development (BBCH 1) started in D1 of April and ended in D2 of June. In the minimum nutrition area, lateral shoot formation (BBCH 2) did not occur. The start of floral emergence (BBCH 5) began in D1 of May and ended in D1 of June. BBCH 6, flowering on the main shoot, began in D3 of May and ended in D2 of June. Fruit development (BBCH 7) started in the D1 of June and ended in the D1 of July. Fruit maturity (BBCH 8) started in D2 of June and ended in D2 of July. The senescence period for raspberry rooted shoots (BBCH 9) was between D3 of June and D3 of December. The leaves started to fall from the end of June at the plants from minimum nutrition area.

The precipitation sums highlighted higher values in the dormancy period in November D2 and lowest in the second decade of October (Figure 3). During the growth period from the raspberry vegetation season, a higher amount of precipitation was registered in June D2 when the fruits developed, fruit maturity was reached, and ripening happened.

Regarding the timing of fruit harvest, the end of main stage 8 and secondary stage BBCH 89, respectively, was staggered differently depending on the nutritional surface available for cutting development (Figure 4).

In the 'Promyk' raspberry planted in the urban garden with a high-nutrition area, the fruit harvest period was carried out over 12 decades with two breaks between July D3-August D1 and October D2.

The fruit harvest began in the second decade of June and lasted until the first decade of November, with a maximum percentage of 37% in D1 of July (Figure 4).

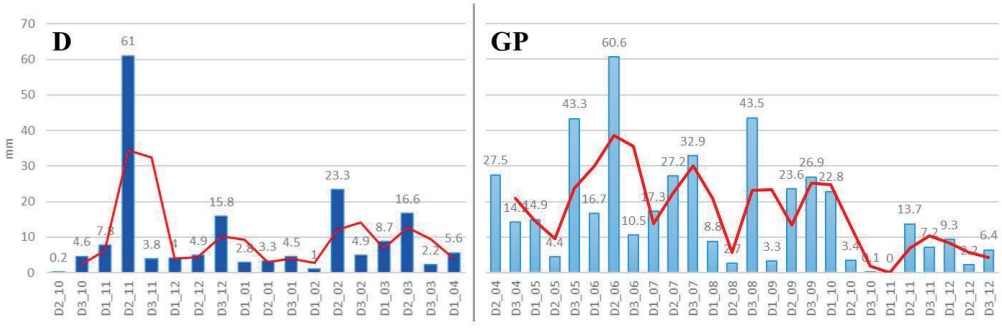
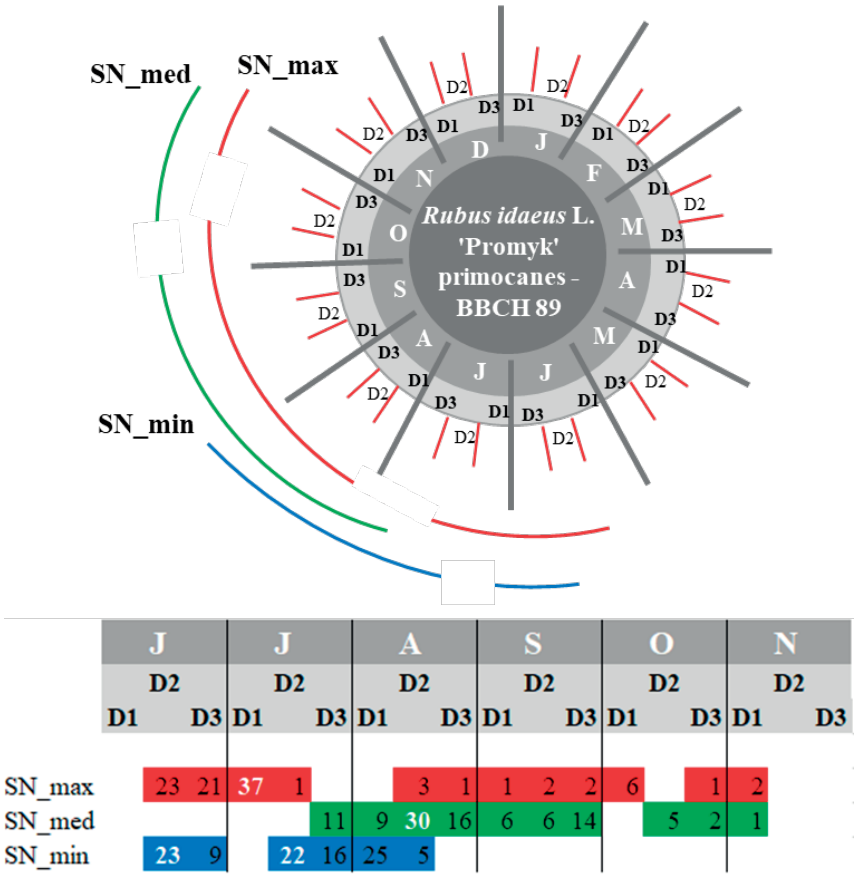


Figure 3. Precipitation amount in the dormancy period (D) and in the active growth period (GP) of raspberry for every decade D1-D3 from all months assessed, the red line represents the moving average trendline



	J		J		A		S		O		N	
	D2		D2		D2		D2		D2		D2	
	D1	D3	D1	D3	D1	D3	D1	D3	D1	D3	D1	D3
SN_max	23	21	37	1	3	1	1	2	2	6	1	2
SN_med				11	9	30	16	6	6	14	5	2	1
SN_min	23	9	22	16	25	5						

Figure 4. Productivity percentages of ripened fruits BBCH 89 in each decade of the entire vegetation period dependent of the nutrition area. Nutrition area was set as SN_max - Maximum, SN_med - Average or medium and SN_min -Minimum

On an average nutrition surface, fruit harvesting was carried out over 10 decades (Figure 4). The beginning of the BBCH 89 stage was observed in July D3 with a maximum harvest value of 30% in August D2 and an interruption during the first decade of October. The ending of fruit harvesting was recorded in the first decade of November and was similar to that in the variant with maximum nutrition surface.

A minimum nutrition surface accelerated the ripening and harvesting period, and this stage was fully achieved over six decades. Fruits started to be harvested similarly to those on a maximum nutrition surface in June D2, with 23%, very close to July D2, with 22%, and ended in August D2 (Figure 4).

CONCLUSIONS

The development of phenological principal and secondary growth stages depend greatly on temperature and precipitation conditions.

The nutrition area produced a specific dynamic of raspberry growth and development. The vegetation period for the raspberry grown in an average nutrition area was the most efficient at a medium GDD. During the entire growth vegetation season, the GDD was proportional to the plant nutrition area; higher GDD was registered for SN_max and lower at SN_min.

After the first year of experiments, it could be recommended to plant at a maximum nutrition area of 100 cm between plants for faster raspberry fruit appearance and ripening.

Raspberry planted at a 75 cm nutrition area provide in the first vegetation year continuous harvesting in time and optimal physiological development. These results would be updated with the next years trends to establish the reason of this results, if the plants were stressed or if this recommendation stand for the stakeholders.

ACKNOWLEDGEMENTS

This paper is part of a PhD study in the thematic area of In-site physiological research on the ecophysiological requirements of *Rubus* spp., conducted by the first author, A.L.P., under the coordination of Sorin Vâtcă (S.V.).

REFERENCES

Adamczuk, N., Ośko, J., Grembecka, M., Konieczyński, P., Migas, P., Orzeł, A., ... & Krauze-Baranowska, M. (2023). Evaluation of the Content of Micro- and Macroelements in Raspberries Depending on the Species, Cultivar Variety, and Geographical Environment. *Nutrients*, *15*(17), 3782.

Bacelar, E., Pinto, T., Anjos, R., Morais, M. C., Oliveira, I., Vilela, A., & Cosme, F. (2024). Impacts of climate change and mitigation strategies for some abiotic and biotic constraints influencing fruit growth and quality. *Plants*, *13*(14), 1942.

Bisbis, M. B., Gruda, N., & Blanke, M. (2018). Potential impacts of climate change on vegetable production and product quality–A review. *Journal of Cleaner Production*, *170*, 1602-1620.

Brennan, R. M., Caligari, P. D. S., Clark, J. R., Brás de Oliveira, P. N., Finn, C. E., Hancock, J. F., ... & Simpson, D. (2014). Berry crops. *Horticulture: Plants for People and Places, Volume 1: Production Horticulture*, 301-325.

Croitoru, A. E., Man, T. C., Vâtcă, S. D., Kobulniczky, B., & Stoian, V. (2020). Refining the spatial scale for maize crop agro-climatological suitability conditions in a region with complex topography towards a smart and sustainable agriculture. Case study: Central Romania (Cluj County). *Sustainability*, *12*(7), 2783.

Fraisse, C. W., & Paula-Moraes, S. V. (2018). Degree-Days: Growing, Heating, and Cooling: ABE381/AE428, rev. 4/2018. *EDIS*, *2018*(2).

Gotame, T. (2014). *Understanding the effects of tempe-razture on raspberry physiology and gene expression profiles*.

Guesmi, B. (2021). Climate as the major factor controlling phenology. In *Agrometeorology*. IntechOpen.

Guo, J., Zhang, R., Cheng, S., Fu, Z., Jia, P., Luan, H., ... & Guo, S. (2023). Physiological and transcriptomic analysis reveal the crucial factors in heat stress response of red raspberry 'Polka'seedlings. *Frontiers in Plant Science*, *14*, 1233448.

Hodnefjell, R. (2017). *Growth and flower initiation i red raspberry (Rubus idaeus* L.*) cultivars* (master's thesis, Norwegian University of Life Sciences, Ås).

Hodnefjell, R., Heide, O. M., Rivero, R., Remberg, S. F., & Sønsteby, A. (2018). Control of growth cessation and floral initiation in red raspberry (*Rubus idaeus* L.) cultivars of diverse origin in controlled and natural environments. *Scientia Horticulturae*, *233*, 412-420.

Imbroane, A. M., Croitoru, A. E., Herbel, I., Rus, I., & Petrea, D. (2014). Urban heat island detection by integrating satellite image data and GIS techniques. Case study: ClujNapoca city, Romania. *Proceedings of the 14th International Multidisciplinary Scientific Geoconference SGEM (14)*, 359-366.

Ladyzhenskaya, O., Aniskina, T., Simakhin, M., Donskih, V., Pashutin, V., Tazin, I., & Kryuchkova, V. (2025). Productivity of Modern Raspberry Varieties and Prospects for Their Selection. *Crops*, *5*(1), 1.

Mazzitelli, L., Hancock, R. D., Haupt, S., Walker, P. G., Pont, S. D., McNicol, J., ... & Taylor, M. A. (2007). Co-ordinated gene expression during phases of dormancy release in raspberry (*Rubus idaeus* L.) buds. *Journal of experimental botany*, *58*(5), 1035-1045.

Meier, U. (2018). *Growth Stages of Mono- and Dicotyledonous Plants*; BBCH Monograph: Braunschweig, Germany.

Miller, P., Lanier, W., & Brandt, S. (2001). Using growing degree days to predict plant stages. *Ag/Extension Communications Coordinator, Communications Services, Montana State University-Bozeman, Bozeman, MO*, *59717*(406), 994–2721.

Orzeł, A., Janik-Wójs, A., Gasparska, M., & Król-Dyrek, K. (2023, July). Recent progress in Polish raspberry and blackberry breeding. In *XIII International Rubus and Ribes Symposium 1388* (pp. 47-52).

Palonen, P., & Buszard, D. (1997). Current state of cold hardiness research on fruit crops. *Canadian journal of plant science*, *77*(3), 399-420.

Patel, M. *Crop Physiology Understanding Plant Growth and Development for Crop Improvements* (Doctoral dissertation, Department of Agronomy, Institute of Agriculture Science, Bundelkhand University).

Pruteanu, A., Botoran, O. R., Vanghele, N. A., Marin, E., & Voicea, I. (2024). Analysis of the phenophases of growth and fruiting of raspberry varieties in the climatic conditions of Bucharest. *Scientific Papers. Series B. Horticulture*, *68*(1).

Raza, A., Bashir, S., Khare, T., Karikari, B., Copeland, R. G., Jamla, M., ... & Varshney, R. K. (2024). Temperature-smart plants: A new horizon with omics-driven plant breeding. *Physiologia Plantarum*, *176*(1), e14188.

Rezaei, E. E., Webber, H., Asseng, S., Boote, K., Durand, J. L., Ewert, F., ... & MacCarthy, D. S. (2023). Climate change impacts on crop yields. *Nature Reviews Earth & Environment*, *4*(12), 831–846.

Rusnac, C. (2021). Declanșarea principalelor fenofaze și calitatea fructelor de zmeur sub influența condițiilor climatice. *Pomicultura, Viticultura și Vinificația*, *85*(1), 9-13.

Sawicka, B., Barbaś, P., Skiba, D., Krochmal-Marczak, B., & Pszczółkowski, P. (2023). Evaluation of the quality of raspberries (*Rubus idaeus* L.) grown in balanced fertilization conditions. *Commodities*, *2*(3), 220-245.

Sønsteby, A., & Heide, O. M. (2012). Effects of photoperiod and temperature on growth, flowering and fruit yield in annual-fruiting red raspberry cultivars (*Rubus idaeus* L.). *European Journal of Horticultural Science*, *77*(3), 97.

Sutherland, L., Pearson, S., & Jennings, N. (2015). A review of dormancy and chilling requirements in raspberries, *Grower report AHDB Horticulture*, 1-11.

Swanson, J. D., Carlson, J. E., Fernández, F. F., Finn, C. E., Graham, J., Weber, C., & Sargent, D. J. (2011). Blackberries and Raspberries. *Genetics, Genomics and Breeding of Berries*, 64-114.

Teng, H., Fang, T., Lin, Q., Song, H., Liu, B., & Chen, L. (2017). Red raspberry and its anthocyanins: Bioactivity beyond antioxidant capacity. *Trends in Food Science & Technology*, 66, 153-165.

Vâtcă, S. D., Stoian, V. A., Man, T. C., Horvath, C., Vidican, R., Gâdea, Ș., ... & Stoian, V. (2021). Agrometeorological requirements of maize crop phenology for sustainable cropping - A historical review for Romania. *Sustainability*, *13*(14), 7719.

Wahid, A., Gelani, S., Ashraf, M., & Foolad, M. R. (2007). Heat tolerance in plants: an overview. *Environmental and experimental botany*, *61*(3), 199-223.

Waister, P. D., Caister, P. D., & Gill, P. A. (1979). Plant hardiness and its significance for the raspberry crop in Scotland. *Scientific Horticulture*, *30*, 59–68.

Wong, N. H., Tan, C. L., Kolokotsa, D. D., & Takebayashi, H. (2021). Greenery as a mitigation and adaptation strategy to urban heat. *Nature Reviews Earth & Environment*, *2*(3), 166-18

Woznicki, T. L., Heide, O. M., Remberg, S. F., & Sønsteby, A. (2016). Effects of controlled nutrient feeding and different temperatures during floral initiation on yield, berry size and drupelet numbers in red raspberry (*Rubus idaeus* L.). *Scientia Horticulturae*, *212*, 148-154.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

IN VITRO PROPAGATION OF THREE CULTIVARS OF BLUE HONEYSUCKLE (*LONICERA CAERULEA* L.)

Ana-Maria RADOMIR, Ramona STAN, Dorin Ioan SUMEDREA

National Research and Development Institute for Biotechnology in Horticulture Ștefănești-Argeș, 37 Bucharest-Pitești Road, 117715, Ștefănești, Argeș, Romania

Corresponding author email: dsumedrea@yahoo.com

Abstract

Lonicera caerulea L. (blue honeysuckle) is a valuable nutraceutical species belonging to the Caprifoliaceae family. This study aimed to develop efficient micropropagation protocols for three blue honeysuckle cultivars: 'Loni', 'Cera', and 'Kami'. The use of 8% calcium hypochlorite for disinfection of explants and a plain Murashige and Skoog (MS) medium for their inoculation proved effective in inducing regenerative processes. In the initiation phase, 'Loni' had the best regenerative potential of the three cultivars studied (between 40 ± 10 and 73.33 ± 11.55% explants that developed shoots). The highest multiplication rate (4.00 ± 0.10 shoots/explant) and the highest average shoot length (7.89 ± 0.50 cm) were obtained for the 'Cera' cultivar on MS medium supplemented with 1 mg/L BAP and 1 mg/L IAA. The most effective culture medium for in vitro shoot rooting proved to be MS½ medium supplemented with 2 mg/L IBA. The 'Loni' cultivar showed the best rooting potential (rooting rate 93.34 ± 5.77%, number of roots/shoot 4.33 ± 0.15, and average root length 2.27 ± 0.06 cm). In vitro regenerated plants were efficiently acclimatized to ex vitro conditions. In vitro rooting can be successfully replaced with in vivo rooting simultaneously with acclimatization, thus shortening the technological flow of obtaining planting material through micropropagation and considerably reducing the unit price of the plant.

Key words: *'Loni', 'Cera', 'Kami', micropropagation, plant growth regulators.*

INTRODUCTION

Currently, the World Health Organization is concerned about the nutrition of the population, especially the lack of dietary fiber, vitamins, and minerals in people's diets (McLennan, 2018; Waterlander et al., 2018). Among foods, fruits and vegetables are of major importance in a healthy diet. In addition to having excellent taste qualities, they are natural sources of bioactive substances with prophylactic and therapeutic properties. Therefore, the demand for these foods is constantly increasing (Chang et al., 2019; Sharma and Lee, 2021).

Among the fruits that contain bioactive compounds with particularly valuable nutritional and therapeutic properties are the fruits of *Lonicera caerulea* L. (blue honeysuckle) (Celli et al., 2014; Caprioli et al., 2016). Blue honeysuckle belongs to the *Caprifoliaceae* family, genus *Lonicera*, which includes about 200 species (Svarcova et al., 2007; Miyashita et al., 2009). *L. caerulea* fruits contain large amounts of vitamins (ascorbic acid, niacin, and tocopherols), minerals (potassium, calcium, and phosphorus), phenolic compounds (protocatechuic, gentisic, ellagic, ferulic, caffeic, chlorogenic, and coumaric acid), and anthocyanins (cyanidin 3-glucoside, peonidin 3-glucoside, cyanidin 3-rutinoside, malvidin 3-arabinoside, petunidin 3-glucoside, and petunidin 3-rutinoside) (Palikova et al., 2008; Wang et al., 2016; Rupasinghe et al., 2018; Grobelna et al., 2019; Grobelna et al., 2020). The health-promoting properties of blue honeysuckle berries include protective effects against cardiovascular and neurodegenerative diseases, osteoporosis, type 2 diabetes, anemia, as well as antimicrobial, anticancer, and anti-inflammatory activity (Park et al., 2005; Kula et al., 2013; Celli et al., 2014; Caprioli et al., 2016; Wang et al., 2016; Cory et al., 2018; Grobelna et al., 2019; Gawroński et al., 2020). These berries can be included in the group of so-called "superfruits" (Bojarska et al., 2019).

Honeysuckle species are traditionally propagated by cuttings. This method, although generally successful, depends largely on the genotype, the age of the mother plant, and the vegetation period (Hui et al., 2012). In contrast, propagation by seeds does not guarantee the regeneration of genetically uniform plants. Also,

Lonicera sp. seeds have a low germination rate and a long germination time.

Intensive honeysuckle cultivation technologies aim to increase production and find the best methods for obtaining high-quality planting material (Debnath, 2007). Thus, to meet the increasing demand for *L. caerulea* fruits and planting material, tissue culture techniques are used as an alternative to traditional propagation methods. *In vitro* multiplication is an effective method to obtain a large number of healthy plants in a relatively short time (Clapa et al., 2023). However, the *in vitro* regenerative potential of plants depends on a number of biotic (species, genotype, and type of explant) and abiotic (culture medium composition, especially plant growth regulators) factors (Gahan and George, 2008). Therefore, there is a need to develop new *in vitro* propagation technologies or optimize existing technologies, especially for less commercialized and less studied species and cultivars.

Research on the *in vitro* multiplication of the species *L. caerulea* is quite limited. In addition, due to the different results obtained, no general recommendations can be made regarding the *in vitro* propagation of this species, each cultivar having a different behavior in *in vitro* culture. Considering the above, the aim of this study was to develop efficient micropropagation protocols for three blue honeysuckle cultivars: 'Loni', 'Cera', and 'Kami'.

MATERIALS AND METHODS

The two major pathways for *in vitro* plant regeneration are organogenesis and somatic embryogenesis. Of these, direct organogenesis is considered the most suitable method for clonal plant multiplication due to the stability and genetic uniformity of the regenerants, but also because the multiplication rate can be maintained at a high level over several subcultures. Starting from this premise, in the research within the present study we opted to implement this method for the *in vitro* propagation of the *L. caerulea* species.

The *in vitro* propagation technique involves four stages: culture initiation, shoot multiplication, shoot rooting, and acclimatization of *in vitro* regenerated shoots to *ex vitro* conditions.

In vitro culture initiation stage

The explants used for initiating *in vitro* cultures consisted of apical and axillary buds taken from shoots harvested from *L. caerulea* mother plants (cultivars 'Loni', 'Cera', and 'Kami') selected from a phytosanitary point of view and for the authenticity of the variety. The studied cultivars were obtained at the Research Institute for Fruit Growing Pitesti, Romania.

The shoots were defoliated, fragmented and first washed with tap water, after which they were sterilized with calcium hypochlorite ($CaCl_2O_2$) in different concentrations: 6%, 8%, and 10%. The sterilization time was 15 minutes. Subsequently, three rinses with sterile distilled water were performed to remove traces of sterilizing agent.

After disinfection of the plant material, the explants were sampled and inoculated under aseptic conditions on MS culture medium (Murashige and Skoog, 1962) without growth regulators.

At this stage of the experiment, as well as in subsequent stages, the culture medium was supplemented with 40 g/L glucose, 32 mg/L NaFeEDTA, and 7 g/L agar.

The culture media were sterilized by autoclaving at 120°C for 20 min. Before autoclaving, the pH of the medium was adjusted to 5.6-5.8 with 1N KOH or 1N HCl.

The cultures were maintained in the growth chamber under controlled conditions of temperature (22-24°C), photoperiod (16 hours light/8 hours dark), and light intensity (2500 lx).

Four weeks after the initiation of cultures, the following parameters were calculated: the percentage of contaminated explants, the percentage of uncontaminated explants that developed shoots, and the percentage of uncontaminated explants that did not develop shoots.

The experiments were organized in 3 repetitions. For each experimental variant, respectively for each repetition, 10 explants were used.

In vitro multiplication stage

The apexes and nodal fragments resulting from the division of regenerated shoots during the *in vitro* culture initiation phase were inoculated on a full-strength MS medium supplemented with different types of cytokinins (BAP -

benzylaminopurine, KIN - kinetin, 2iP - 2 isopentyl adenine) at two concentrations (1 and 3 mg/L) in combination with 1 mg/L IAA (indoleacetic acid) or without the addition of auxin. MS medium without growth regulators was used as a control (Table 1).

Table 1. Experimental variants - *in vitro* multiplication of *L. caerulea* shoots

Variant	Phytohormones (mg/L)			
	BAP	KIN	2iP	IAA
V0	-	-	-	-
V1	1	-	-	-
V2	3	-	-	-
V3	-	1	-	-
V4	-	3	-	-
V5	-	-	1	-
V6	-	-	3	-
V7	1	-	-	1
V8	3	-	-	1
V9	-	1	-	1
V10	-	3	-	1
V11	-	-	1	1
V12	-	-	3	1

The subculturing of the biological material on fresh medium was performed at an interval of four weeks.

Four weeks after subcultivation, the number of shoots per explant and shoot length were evaluated as growth parameters.

The experiments were organized in 3 repetitions. For each experimental variant, respectively for each repetition, 10 explants were used.

***In vitro* rooting stage**

Shoots regenerated on the propagation medium were individualized and cultivated on several variants of rooting medium in which the type of auxin (IBA, NAA, IAA) and its concentration (0.5, 1, 2 mg/L) were varied. The MS½ medium (Murashige and Skoog, 1962) without auxins was used as a control (Table 2).

The development of the rooting process at the level of *L. caerulea* shoots was evaluated, four weeks after the initiation of the experiment, by calculating the following parameters: rooting rate, number of roots/shoot, and average root length.

The experiments were organized in 3 repetitions. For each experimental variant, respectively for each repetition, 30 explants were used.

Table 2. Experimental variants - *in vitro* rooting of *L. caerulea* shoots

Variant	Auxins (mg/L)		
	IBA	NAA	IAA
V0	-	-	-
V1	0.5	-	-
V2	1	-	-
V3	2	-	-
V4	-	0.5	-
V5	-	1	-
V6	-	2	-
V7	-	-	0.5
V8	-	-	1
V9	-	-	2

Acclimatization stage

Acclimatization of in vitro rooted shoots

The *in vitro* rooted shoots were carefully removed from the culture vessels. Their roots were washed in running tap water to remove the agarized medium and eliminate possible sources of phytopathogenic agents. They were then planted in a substrate consisting of a mixture of peat, manure, and perlite in a ratio of 2:1:1 and Jiffy peat pellets.

The plants were placed under glass jars or covered with plastic bags to provide a high-humidity environment and eliminate the risk of dehydration. Two to three weeks after transfer to *ex vitro* conditions, when the plants began to grow, the humidity was gradually reduced by uncovering the cultures, until the plants adapted to natural living conditions. The appearance of the first *ex vitro* leaves marked the end of the acclimatization phase.

Four weeks after the start of the experiment, the acclimatization rate was calculated.

The experiments were organized in 3 repetitions. For each experimental variant, respectively for each repetition, 10 plants were used.

Ex vitro rooting and acclimatization of in vitro regenerated shoots

Shoots of 3-4 cm derived from the propagation medium were transferred to two types of rooting substrate: perlite and Jiffy peat pellets. The shoots were washed with water to remove remaining agar traces, which may favor the occurrence of infections when transferring the shoots to *in vivo* conditions. They were either treated or not with a rooting stimulator (Radi Stim no. 1 - biopreparation for rooting herbaceous cuttings).

During the experiment, the shoots were maintained at a temperature of 22-25^{0}C and high atmospheric humidity (85-90%) by covering them with polyethylene film or jars. Airing and spraying with water were carried out daily, and when the shoots formed new leaves, the cultures were uncovered. After the cultures were uncovered, the photosynthesis process intensified, so that along with rooting, the shoots were also acclimatized.

Four weeks after the start of the experiment, the rooting/acclimatization rate was calculated.

The experiments were organized in 3 repetitions. For each experimental variant, respectively for each repetition, 10 plants were used.

The acclimatized plants were then transplanted into pots in a peat-based mixture for further fortification and development.

Data analysis

Statistical interpretation of data was performed using SPSS 10 for Windows program. Differences between treatments for each cultivar were analyzed with One-way ANOVA - Tukey's HSD posthoc test, being considered significant at $P<0.05$. Values shown are means ± standard deviation (SD).

RESULTS AND DISCUSSIONS

***In vitro* culture initiation stage**

Culture initiation is a basic technological sequence in *in vitro* plant propagation.

One of the essential conditions on which the success of initiating a cell culture depends is the selection of appropriate sterilizing agents for the disinfection of explants. The method of sterilization of biological material varies depending on the origin of the material, the physiological state of the plant, and the type of organ. In general, the selection of a sterilizing agent depends on its effectiveness and the impact on the subsequent development of the explant.

Currently, the most effective preparations are based on mercury, but their toxicity inhibits the further development of plantlets (Mayorova et al., 2016). For example, when 0.10% mercuric chloride ($HgCl_2$) solution was used to sterilize honeysuckle explants (cultivars 'Cheliabinka' and 'Duet'), the regeneration rate was only 65.9% and 64.9% respectively (Dziedzic, 2008). Increasing the concentration to 0.15% resulted in 95% sterile explants, of which 67.5% developed further (Sedlák and Paprštein, 2007). When 0.2% mercuric sulfate ($HgSO_4$) was used, 54.43% explants proliferated, and 4.57% did not develop at all (Krupa-Malkiewicz and Ochmian, 2014). Lisoformin 3000 has also been successfully used for sterilization of explants of 14 species of ornamental and fruit crops (Bludneva et al., 2013). Other sterilizing agents, such as sodium and calcium hypochlorite or hydrogen peroxide did not provide a satisfactory yield of sterile explants. Thus, finding effective and less toxic sterilizing agents to obtain an aseptic honeysuckle culture is a necessity.

In the present study, calcium hypochlorite ($CaCl_2O_2$) was used to sterilize the explants. To optimize the disinfection parameters of the initial biological material, different concentrations of calcium hypochlorite (6%, 8%, and 10%) were tested.

The observations made highlighted the fact that the inclusion of the *L. caerulea* species in the *in vitro* culture system does not pose any particular problems, the use of calcium hypochlorite for sterilizing the explants proving effective.

Overall, for the 3 cultivars studied, the percentage of explants contaminated with fungal and/or bacterial infections after the first four weeks of culture ranged between 10.00 ± 10.00 and 53.33 ± 5.77%. This ranged between 10.00 ± 10.00 and 43.33 ± 5.77% for the 'Loni' cultivar, between 16.67 ± 11.55 and 53.33 ± 5.77% for the 'Cera' cultivar, and between 10.00 ± 10.00 and 53.33 ± 5.77% for the 'Kami' cultivar (Table 3).

The lowest contamination rate was recorded when using 10% $CaCl_2O_2$, but in this case, the percentage of explants that did not develop shoots was higher compared to that recorded when using 8% $CaCl_2O_2$.

Considering that when calcium hypochlorite at a concentration of 8% was used as a disinfectant the explants had the best regenerative potential, we can conclude that this sterilization method was the most effective.

Another important aspect in the initiation stage of *in vitro* cultures is selecting the optimal type of explant to obtain a high percentage of explants that start to grow. In the case of the 3 cultivars studied ('Loni', 'Cera', and 'Kami'), following comparative analyses that targeted the

morphogenetic competence of explants of different origins (apical buds and axillary buds), the results obtained revealed that both types of explants used had the ability to regenerate shoots under *in vitro* conditions, but apical buds had the best morphogenetic potential. The percentage of explants that showed regenerative processes recorded values between 33.33 ± 5.77 and 73.33 ± 11.55% when using apical buds as explants and between 30.00 ± 10.00 and 63.33 ± 5.77% when using axillary buds (Table 3).

The morphogenetic capacity of the explants also differed depending on the cultivar. Of the 3 cultivars studied, 'Loni' had the best regenerative potential (between 40.00 ± 10.00 and 73.33 ± 11.55% of explants developed shoots), followed by 'Kami' (between 36.67 ± 5.77 and 66.67 ± 20.82% of explants developed shoots) and 'Cera' (between 30.00 ± 10.00 and 63.33 ± 15.28% of explants developed shoots) (Table 3).

The use of MS basal medium without growth hormones favored the production of plantlets that were subsequently used to test the morphogenetic response of *L. caerulea* explants on nutrient media with different hormonal formulas (Figure 1 a).

Research by Isac and Mladin (2013) showed that in the case of the cultivars 'Loni' and 'Cera', the best initiation medium was the mixture of Quoirin and Lepoivre (QL) mineral salts (Quoirin and Lepoivre, 1977) and Linsmaier and Skoog (LS) vitamins (Linsmaier and Skoog, 1965), containing 0.1 mg/L GA_3 (gibberellic acid) and 1 mg/L IBA.

Table 3. Influence of explant type and sterilization method on the *in vitro* regenerative potential of *L. caerulea* explants

Cultivar	Explant type	Concentration of $CaCl_2O_2$ (%)	Contaminated explants (%)	Uncontaminated explants that have developed shoots (%)	Uncontaminated explants that have not developed shoots (%)
'Loni'	apical buds	6	43.33 ± 5.77^{b}	53.33 ± 5.77^{ab}	3.33 ± 5.77^{a}
		8	20.00 ± 17.32^{ab}	73.33 ± 11.55^{c}	6.67 ± 5.77^{a}
		10	13.33 ± 11.55^{ab}	46.67 ± 5.77^{ab}	40.00 ± 10.00^{bc}
	axillary buds	6	43.33 ± 11.55^{b}	40.00 ± 10.00^{a}	16.67 ± 5.77^{ab}
		8	16.67 ± 5.77^{ab}	63.33 ± 5.77^{bc}	20.00 ± 10.00^{abc}
		10	10.00 ± 10.00^{a}	46.67 ± 5.77^{ab}	43.33 ± 15.28^{c}
'Cera'	apical buds	6	53.33 ± 5.77^{c}	40.00 ± 0.00^{ab}	6.67 ± 5.77^{a}
		8	26.67 ± 15.28^{abc}	63.33 ± 15.28^{c}	10.00 ± 10.00^{a}
		10	20.00 ± 10.00^{ab}	33.33 ± 5.77^{ab}	46.67 ± 5.77^{bc}
	axillary buds	6	46.67 ± 5.77^{bc}	30.00 ± 10.00^{a}	23.33 ± 5.77^{a}
		8	20.00 ± 10.00^{ab}	53.33 ± 5.77^{bc}	26.67 ± 5.77^{ab}
		10	16.67 ± 11.55^{a}	33.33 ± 5.77^{ab}	50.00 ± 10.00^{c}
'Kami'	apical buds	6	53.33 ± 5.77^{c}	43.33 ± 5.77^{a}	3.33 ± 5.77^{a}
		8	26.67 ± 23.09^{abc}	66.67 ± 20.82^{a}	6.67 ± 5.77^{a}
		10	13.33 ± 11.55^{ab}	40.00 ± 10.00^{a}	46.67 ± 5.77^{c}
	axillary buds	6	46.67 ± 5.77^{bc}	36.67 ± 5.77^{a}	16.67 ± 5.77^{ab}
		8	16.67 ± 15.28^{ab}	56.67 ± 11.55^{a}	26.67 ± 5.77^{b}
		10	10.00 ± 10.00^{a}	40.00 ± 0.00^{a}	50.00 ± 10.00^{c}

Values shown are means ± SD. Different lowercase letters indicate significant differences between treatments for each cultivar, according to Tukey's HSD test ($P<0.05$).

In vitro multiplication stage

The multiplication phase spans several subcultures lasting approximately 4 weeks. The number of subcultures should be limited, any extension of this phase requiring additional checks on the genetic stability of the material. The number of passages should not exceed 10-15 (Kushnir and Sarnatska, 2005).

In this phase, the main goal is to obtain as many plantlets as possible, while maintaining the genetic stability of the material. Increasing the propagation rate can be achieved by stimulating axillary budding, by stimulating shoot elongation, or by both methods simultaneously. At this stage, the composition of the culture medium, especially the phytohormonal balance, plays a decisive role. High multiplication rates are generally achieved by increasing the amount of cytokinins in the culture medium or by a hormonal balance tilted in favor of cytokinins. Phytohormones are considered the most important endogenous substances for

modulating physiological and molecular responses, a critical requirement for plant survival. They act either at their site of synthesis or elsewhere in the plant after their transport (Al-Taey, 2017). Among them, cytokinins are known to significantly improve plant growth by inducing cell division, regulating cellular metabolism, and activating several specific enzymes (Al-Taey and Saadoon, 2012; Al-Taey et al., 2018a; Al-Taey et al., 2018b).

In our experiments, in order to multiply the shoots, the apexes and nodal fragments resulting from the portioning of the regenerated shoots in the initiation phase of *in vitro* cultures were inoculated on MS culture medium (Murashige and Skoog, 1962) supplemented with different types of cytokinins (BAP, KIN, and 2iP) in combination with IAA or without the addition of auxin.

The results obtained showed that shoot regeneration was influenced both by the composition of the culture medium, namely the phytohormonal balance, and by the cultivar. Statistical interpretation of the results obtained using the SPSS 10 program highlighted the fact that, in the case of the 3 cultivars studied ('Loni', 'Cera', and 'Kami'), supplementing the nutrient medium with phytohormones induced an increase compared to the control in both the number of shoots/explant and the length of the shoots, in most variants the increases being significant. Of the three cytokinins used, the most effective in terms of shoot regeneration was BAP, followed by KIN and 2iP, respectively. Increasing the concentration of cytokinins in the nutrient medium from 1 mg/L to 3 mg/L led to a weaker morphogenetic response. The addition of auxin (IAA) to the culture medium supplemented with cytokinins had a positive effect on regenerative processes, regardless of the type or concentration of cytokinin used (Table 4).

The variant in which the culture medium was supplemented with 1 mg/L BAP and 1 mg/L IAA led to the best morphogenetic response, but this was different depending on the cultivar. Of the 3 cultivars studied, 'Cera' presented the best regenerative potential, followed by 'Loni' and 'Kami', respectively. The control variant without phytohormones recorded the lowest values in terms of the number of shoots/explant and shoot length (Table 4).

Table 4. Influence of type, concentration, and combination of phytohormones on the number of shoots/ explant and shoot length in the species *L. caerulea*

Variant	Cultivar					
	'Loni'		'Cera'		'Kami'	
	No. of shoots/ explant	Shoot length (cm)	No. of shoots/ explant	Shoot length (cm)	No. of shoots/ explant	Shoot length (cm)
V0	1.00 ± 0.00^{a}	1.28 ± 0.06^{a}	1.00 ± 0.00^{a}	1.42 ± 0.06^{a}	1.00 ± 0.00^{a}	1.19 ± 0.06^{a}
V1	3.87 ± 0.12^{g}	7.04 ± 0.14^{ij}	3.93 ± 0.15^{g}	7.70 ± 0.49^{hi}	2.47 ± 0.12^{g}	3.46 ± 0.12^{h}
V2	1.60 ± 0.10^{bc}	3.01 ± 0.19^{e}	1.73 ± 0.12^{bc}	2.99 ± 0.13^{de}	1.47 ± 0.15^{bc}	2.44 ± 0.18^{d}
V3	2.93 ± 0.15^{e}	6.47 ± 0.18^{h}	3.07 ± 0.15^{e}	6.87 ± 0.23^{g}	2.17 ± 0.06^{efg}	3.09 ± 0.11^{fg}
V4	1.20 ± 0.10^{a}	2.09 ± 0.30^{cd}	1.23 ± 0.06^{a}	2.16 ± 0.12^{bc}	1.13 ± 0.12^{a}	2.06 ± 0.09^{c}
V5	2.27 ± 0.12^{d}	4.78 ± 0.05^{f}	2.40 ± 0.20^{d}	5.52 ± 0.22^{f}	1.87 ± 0.06^{de}	2.57 ± 0.08^{de}
V6	1.10 ± 0.10^{a}	1.52 ± 0.11^{ab}	1.13 ± 0.06^{a}	1.54 ± 0.06^{ab}	1.03 ± 0.06^{a}	1.44 ± 0.09^{ab}
V7	3.93 ± 0.06^{g}	7.20 ± 0.16^{i}	4.00 ± 0.10^{g}	7.89 ± 0.50^{i}	2.87 ± 0.06^{h}	3.96 ± 0.14^{i}
V8	1.87 ± 0.06^{c}	3.04 ± 0.07^{e}	1.97 ± 0.15^{c}	3.25 ± 0.26^{e}	1.77 ± 0.06^{cd}	2.43 ± 0.08^{d}
V9	3.47 ± 0.12^{f}	6.72 ± 0.15^{hi}	3.53 ± 0.21^{f}	7.07 ± 0.12^{gh}	2.20 ± 0.20^{fg}	3.22 ± 0.12^{gh}
V10	1.30 ± 0.10^{ab}	2.41 ± 0.11^{d}	1.37 ± 0.15^{ab}	2.43 ± 0.14^{cd}	1.27 ± 0.06^{ab}	2.32 ± 0.12^{cd}
V11	2.43 ± 0.15^{d}	5.82 ± 0.13^{g}	2.53 ± 0.15^{d}	6.08 ± 0.18^{f}	2.07 ± 0.15^{def}	2.84 ± 0.14^{ef}
V12	1.13 ± 0.12^{a}	1.79 ± 0.17^{bc}	1.17 ± 0.06^{a}	1.82 ± 0.13^{abc}	1.07 ± 0.06^{a}	1.68 ± 0.11^{b}

Values shown are means ± SD. Different lowercase letters indicate significant differences between treatments for each cultivar, according to Tukey's HSD test ($P<0.05$).

From a qualitative point of view, the biological material regenerated *in vitro* had normal morphology, without aspects of vitrification, necrosis or callus differentiation (Figure 1 b).

Most studies on *in vitro* culture of blue honeysuckle have involved micropropagation by axillary budding in the presence of BAP (Sedlák and Paprštein, 2007; Dziedzic, 2008; Krupa-Małkiewicz and Ochmian, 2014) or the mixture of 2iP and 3-hydroxybenzyladenine (meta-topolin) (Gabryszewska et al., 2016). In research conducted by Isac and Mladin (2013) on two cultivars of *L. caerulea* ('Loni' and 'Cera') the highest multiplication rate was obtained using 1 mg/L BAP and 0.05 mg/L GA_3. In contrast, Fira et al. (2014) obtained a high propagation rate by supplementing the culture medium with 0.7 mg/L *N*-(2-chloro-4-pyridyl)-*N'*-phenylurea (CPPU).

***In vitro* rooting stage**

For effective *in vitro* propagation protocols, it is essential that shoots root in high proportions and that regenerated plants successfully acclimatize to *ex vitro* conditions. Rooting is a difficult process in recalcitrant species, in the absence of roots the survival rate of acclimatized plants being reduced.

It is known that, in *in vitro* cultures, auxins are responsible for stimulating root development and cell elongation. However, high concentrations of auxins added to nutrient media result in callus development and inhibition of root system development (Yankovskaya et al., 2011).

Thus, in order to select the optimal composition of the nutrient medium for the rooting of *L. caerulea* shoots, in the present study several medium variants were tested in which the type of auxin (IBA, NAA, IAA) and its concentration (0.5, 1, 2 mg/L) were varied.

The results obtained showed that *in vitro* rooting of shoots was influenced by both the composition of the culture medium, namely the type and concentration of auxin, and the cultivar.

Statistical interpretation using the SPSS 10 program of the obtained results revealed that supplementing the MS½ nutrient medium with auxins had a stimulatory effect on the rooting rate, number of roots/shoot, and average root length, with significant increases in all variants. The highest values of these parameters were obtained when the culture medium was supplemented with IBA, followed by NAA and IAA respectively. In the case of all auxins used, increasing their concentration led to an increase in the rooting rate, number of roots/shoot, and average root length (Table 5). These results are consistent with those reported by Hartmann et al. (2002).

The variant in which the culture medium was supplemented with 2 mg/L IBA led to the best response in terms of the rhizogenesis process, but this was different depending on the cultivar. Of the 3 cultivars studied, 'Loni' presented the best rooting potential, followed by 'Cera' and 'Kami'.

In the case of all three *L. caerulea* cultivars studied ('Loni', 'Cera', and 'Kami'), shoots did not form roots on the control variant without auxin. These results are consistent with the results reported by Krupa-Małkiewicz et al. (2017) and Wojtania et al. (2018) who, on auxin-free medium, obtained low rooting rates (4-8% in the 'Wojtek' cultivar and 40% in the 'Zojka' cultivar) and a weak root system.

Table 5. Influence of auxin type and concentration on the *in vitro* rooting capacity of *L. caerulea* shoots

Variant	Cultivar								
	'Loni'			'Cera'			'Kami'		
	Rooting rate (%)	No. of roots/shoot	Root length (cm)	Rooting rate (%)	No. of roots/shoot	Root length (cm)	Rooting rate (%)	No. of roots/shoot	Root length (cm)
V0	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
V1	86.67 ± 3.34^{cfg}	3.97 ± 0.15^{de}	2.03 ± 0.06^{ef}	80.00 ± 6.67^{cde}	3.67 ± 0.15^{de}	1.83 ± 0.06^{ef}	73.33 ± 6.67^{de}	2.67 ± 0.15^{cde}	1.33 ± 0.06^{ef}
V2	90.00 ± 3.33^{fg}	4.27 ± 0.12^{ef}	2.10 ± 0.10^{fg}	83.33 ± 3.34^{de}	3.97 ± 0.12^{ef}	1.90 ± 0.10^{ef}	73.34 ± 5.77^{de}	2.97 ± 0.12^{de}	1.40 ± 0.10^{ef}
V3	93.34 ± 5.77^{g}	4.33 ± 0.15^{f}	2.27 ± 0.06^{g}	86.67 ± 3.34^{e}	4.03 ± 0.15^{f}	2.07 ± 0.06^{f}	76.67 ± 3.34^{e}	3.03 ± 0.15^{e}	1.57 ± 0.06^{f}
V4	76.67 ± 6.67^{bcde}	3.63 ± 0.15^{d}	1.73 ± 0.12^{cd}	73.33 ± 8.82^{bcde}	3.33 ± 0.15^{d}	1.57 ± 0.15^{cd}	66.67 ± 3.34^{bcde}	2.37 ± 0.21^{c}	1.07 ± 0.15^{cd}
V5	80.00 ± 6.67^{cdef}	3.77 ± 0.12^{d}	1.87 ± 0.06^{de}	76.67 ± 3.34^{bcde}	3.47 ± 0.12^{d}	1.67 ± 0.06^{de}	70.00 ± 5.77^{cde}	2.47 ± 0.12^{c}	1.17 ± 0.06^{de}
V6	83.33 ± 6.67^{defg}	3.90 ± 0.10^{d}	1.93 ± 0.12^{def}	80.00 ± 6.67^{cde}	3.60 ± 0.10^{d}	1.73 ± 0.06^{de}	73.33 ± 3.34^{de}	2.60 ± 0.10^{cd}	1.23 ± 0.06^{de}
V7	66.67 ± 3.34^{b}	2.77 ± 0.12^{b}	1.47 ± 0.06^{b}	63.33 ± 6.67^{b}	2.47 ± 0.12^{b}	1.30 ± 0.10^{b}	56.67 ± 3.34^{b}	1.47 ± 0.12^{b}	0.80 ± 0.10^{b}
V8	70.00 ± 0.00^{bc}	2.90 ± 0.10^{bc}	1.53 ± 0.06^{bc}	66.67 ± 6.67^{bc}	2.60 ± 0.10^{bc}	1.33 ± 0.06^{bc}	60.00 ± 0.00^{bc}	1.60 ± 0.10^{b}	0.83 ± 0.06^{bc}
V9	73.33 ± 3.34^{bcd}	3.13 ± 0.15^{c}	1.60 ± 0.10^{bc}	70.00 ± 0.00^{bcd}	2.83 ± 0.15^{c}	1.40 ± 0.10^{bc}	63.33 ± 3.34^{bcd}	1.83 ± 0.15^{b}	0.90 ± 0.10^{bc}

Values shown are means ± SD. Different lowercase letters indicate significant differences between treatments for each cultivar, according to Tukey's HSD test ($P<0.05$).

The above-mentioned results confirm that, in the *in vitro* culture of *L. caerulea*, auxins play an important role in achieving high rooting rates (Karhu, 1997; Sedlák and Paprštein, 2007; Dziedzic, 2008; Krupa-Małkiewicz et al., 2017). Similar results have also been reported in other plant species (Soliman et al., 2014).

The results obtained show that IBA at a concentration of 2 mg/L was the most effective auxin for *in vitro* rooting of *L. caerulea* shoots. These results are consistent with those reported by Sedlák and Paprštein (2007), who obtained a 100% rooting rate for the 20/1 and 'Altaj' genotypes on MS medium with macro- and micronutrients reduced to one-third

supplemented with 2.5 mg/L IBA. In studies conducted by Zapolsky et al. (2018) and Kadhim et al. (2019), the highest rooting percentage was obtained using IBA at concentrations of 1 and 1.5 mg/L. Isac and Mladin (2013) obtained a rooting rate of 89.6% for the 'Loni' cultivar on Murashige and Skoog (MS) nutrient medium supplemented with 1 mg/L IBA and 162 mg/L PG (phloroglucinol). According to Dziedzic (2008), WPM medium (Woody Plant Medium) (Lloyd and McCown, 1981) supplemented with 2 mg/L IBA and 5 mg/L IAA was more effective for inducing rhizogenesis in the cultivars 'Czelabinka' (96% rooted shoots) and 'Duet' (92% rooted shoots) compared to MS medium. The superior efficiency of IBA compared to NAA and IAA in the rooting process was also demonstrated by Anuradha et al. (2016) on the strawberry cultivar 'Ofra' and by Koubouris and Vasilakakis (2006) on the *Prunus* species. This may be due to the higher stability of IBA (Nissen and Sutter, 1990).

However, there are studies in which the presence of auxins in the culture medium stimulated unwanted callus formation at the base of rooted shoots, which blocked the formation of new roots and decreased the survival rate of blue honeysuckle transferred under *ex vitro* conditions (Karhu, 1997; Wojtania et al., 2018). On the other hand, according to Dziedzic (2008), Osburn et al. (2009), and Hui et al. (2012), the acclimatization of blue honeysuckle plants regenerated by *in vitro* culture depends largely on the genotype and not on the auxin concentration in the culture medium.

Acclimatization stage

The acclimatization is the final stage of micropropagation. For effective micropropagation protocols, it is essential that *in vitro* regenerated plants successfully acclimatize to *ex vitro* conditions.

Acclimatization of in vitro rooted shoots

The experiments conducted led to the conclusion that the nutrient substrate played an important role in the successful acclimatization of *L. caerulea* vitroplants, the best results being obtained when Jiffy peat pellets were used (100 ± 0.00% acclimatized plants in the case of the 'Loni' cultivar, 93.33 ± 5.77% acclimatized plants in the case of the 'Cera' cultivar, and 86.67 ± 5.77% acclimatized plants in the case of the 'Kami' cultivar). When using the mixture of peat, manure, and perlite in a ratio of 2:1:1, the percentage of acclimatized plants was lower (96.67 ± 5.77% acclimatized plants in the case of the 'Loni' cultivar, 90.00 ± 10.00% acclimatized plants in the case of the 'Cera' cultivar, and 80.00 ± 10.00% acclimatized plants in the case of the 'Kami' cultivar), this substrate however ensuring good fortification of the acclimatized plants. Regardless of the nutrient substrate used, the highest acclimatization rate was recorded for the 'Loni' cultivar, followed by the 'Cera' cultivar and the 'Kami' cultivar (Table 6).

Table 6. Influence of nutrient substrate on the acclimatization capacity of *in vitro* rooted plants to *ex vitro* conditions

Cultivar	Nutritive substrate	Acclimatization rate (%)
'Loni'	peat, manure, and perlite (2:1:1)	96.67 ± 5.77
	peat pellets (Jiffy type)	100.00 ± 0.00
'Cera'	peat, manure, and perlite (2:1:1)	90.00 ± 10.00
	peat pellets (Jiffy type)	93.33 ± 5.77
'Kami'	peat, manure, and perlite (2:1:1)	80.00 ± 10.00
	peat pellets (Jiffy type)	86.67 ± 5.77

Values shown are means ± SD.

Similar results, namely high acclimatization rates, were obtained in other studies on the propagation of the species *L. caerulea* by micropropagation (Wojtania et al., 2020; Liu et al., 2023).

Ex vitro rooting and acclimatization of in vitro regenerated shoots

Observations made four weeks after the start of the experiments demonstrated that, in the studied species, the *in vivo* rooting process took place under optimal conditions, with favorable results. During this period, the shoots showed intense development of the root system, forming a significant number of roots (Figures 1 e and f). Research on the influence of rooting stimulants and rooting substrate on the *in vivo* rhizogenesis process in *L. caerulea* shoots has highlighted the fact that the composition of the substrate influenced the rooting of the shoots, but rooting stimulants played an important role in rooting success, in their absence the shoots rooted in a lower percentage. Treating the base of the shoots with rooting stimulants promotes cell division and the formation of adventitious roots, which allows the rooting period to be shortened and the percentage of rooted shoots to be increased.

The use of Radi Stim as a rooting stimulator and peat pellets as a substrate led to the best results, achieving rooting of 100.00 ± 0.00% of shoots in the case of the 'Loni' and 'Cera' cultivars and 86.67 ± 5.77% in the case of the 'Kami' cultivar. When the shoots were treated with rooting stimulator and perlite was used as the rooting substrate, the percentage of rooted plants *in vivo* was 96.67 ± 5.77% in the case of the 'Loni' cultivar, 93.33 ± 5.77% in the case of the 'Cera' cultivar, and 83.33 ± 5.77% in the case of the 'Kami' cultivar. The lowest values in terms of *in vivo* shoot rooting rate were obtained in the absence of rooting stimulants and when using perlite as a substrate (86.67 ± 5.77% for the 'Loni' cultivar, 83.33 ± 5.77% for the 'Cera' cultivar, and 80.00 ± 0.00% for the 'Kami' cultivar). Of the 3 cultivars studied, the 'Loni' cultivar showed the best rooting potential, followed by the 'Cera' cultivar and the 'Kami' cultivar (Table 7).

High rates of *ex vitro* rooting and acclimatization were also obtained in other studies on *in vitro* propagation of the *L. caerulea* species (max. 96% for the cultivar 'Wojtek' and max. 88% for the cultivar 'Zojka') (Wojtania et al., 2020). Regarding the nutrient substrate, peat or a mixture of peat and perlite/vermiculite has been shown to be a better substrate for the rooting and *ex vitro* growth of blue honeysuckle shoots compared to a mixture of peat and sand (Karhu, 1997; Dziedzic, 2008; Krupa-Małkiewicz et al., 2017; Wojtania et al., 2020).

Table 7. The influence of the culture substrate and the rooting stimulator on the *in vivo* rooting capacity of *L. caerulea* shoots simultaneously with acclimatization

Cultivar	Rooting substrate	Rooting stimulator	Rooting/ acclimatization rate (%)
'Loni'	Perlite	-	86.67 ± 5.77^{a}
	Perlite	Radi Stim no. 1	96.67 ± 5.77^{ab}
	peat pellets (Jiffy type)	-	93.33 ± 5.77^{ab}
	peat pellets (Jiffy type)	Radi Stim no. 1	100.00 ± 0.00^{b}
'Cera'	Perlite	-	83.33 ± 5.77^{a}
	Perlite	Radi Stim no. 1	93.33 ± 5.77^{a}
	peat pellets (Jiffy type)	-	90.00 ± 10.00^{a}
	peat pellets (Jiffy type)	Radi Stim no. 1	100.00 ± 0.00^{a}
'Kami'	Perlite	-	80.00 ± 0.00^{a}
	Perlite	Radi Stim no. 1	83.33 ± 5.77^{a}
	peat pellets (Jiffy type)	-	83.33 ± 5.77^{a}
	peat pellets (Jiffy type)	Radi Stim no. 1	86.67 ± 5.77^{a}

Values shown are means ± SD. Different lowercase letters indicate significant differences between treatments for each cultivar, according to Tukey's HSD test (P<0.05).

Simultaneously with the *in vivo* rooting of the shoots, their acclimatization to *ex vitro* conditions was also performed. The acclimatized plants were subsequently fortified in pots in a peat-based mixture. It should be noted that the *in vitro* regenerated plants retained the morphological characteristics of the donor plants (Figures 1 g and h).

Figure 1 shows aspects of the main stages of *in vitro* culture of *L. caerulea*.

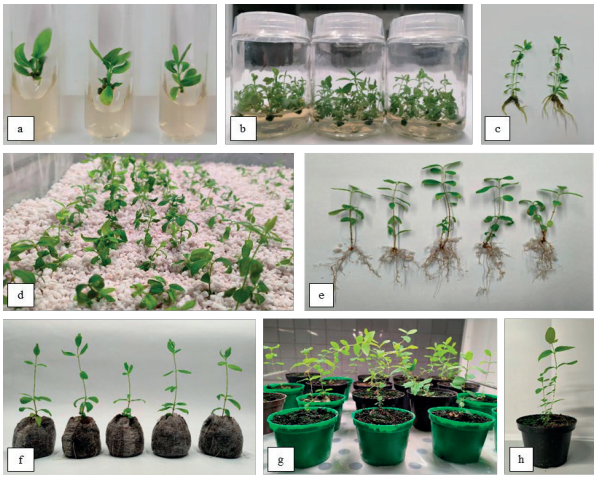


Figure 1. Aspects of *in vitro* culture of *L. caerulea*: a - explants in the initiation phase; b - shoot propagation; c - *in vitro* rooted shoots; d, e - *in vivo* rooting of shoots in perlite; f - *in vivo* rooting of shoots in Jiffy peat pellets; g, h - acclimatized plants fortified in pots

CONCLUSIONS

The use of 8% calcium hypochlorite for disinfection of explants and a plain MS medium for their inoculation proved effective in inducing regenerative processes in the three *L. caerulea* cultivars studied: 'Loni', 'Cera', and 'Kami'. The highest number of shoots per explant and the highest average shoot length were obtained on MS medium supplemented with 1 mg/L BAP and 1 mg/L IAA. The most effective culture medium for *in vitro* shoot rooting proved to be MS½ medium supplemented with 2 mg/L IBA. *In vitro* regenerated plants were efficiently acclimatized to *ex vitro* conditions. *In vitro* rooting can be successfully replaced with *in vivo* rooting simultaneously with acclimatization, thus shortening the technological flow of obtaining planting material through micropropagation and considerably reducing the unit price of the plant. The results obtained show that micropropagation is an effective method of producing large quantities of healthy blue honeysuckle planting material in a short time.

ACKNOWLEDGEMENTS

This work was carried out with the support of the Ministry of Research, Innovation and Digitalization through the Nucleu Program 37N/2023, project PN 23.41.01.02.

REFERENCES

Al-Taey, D. K. A., & Saadoon, A. H. (2012). Effect of treatment of kinetin to reduce the salinity damage by drainage water irrigation on the growth and nitrate accumulation in the leaves of spinach, *Spenacia oleracea* L. *Euphrates J. Agric. Sci.*, 4(4), 11-24.

Al-Taey, D. K. A. (2017). Mitigation of salt stress by organic matter and GA_3 on growth and peroxidase activity in pepper (*Capsicum annum* L.). *Adv. Natural and Appl. Sci.*, 11(10), 1-11.

Al-Taey, D. K. A., Mijwel, A. K., & Al-Azawy, S. S. (2018a). Study efficiency of poultry litter and kinetin in reduced effects of saline water in *Vicia faba*. *Res. J. Pharm. and Tech.*, 11(1), 294-300. http://dx.doi.org/10.5958/0974-360X.2018.00054.9

Al-Taey, D. K. A., Al-Azawi., S. S. M., Al-Shareefi, M. J. H., & Al-Tawaha, A. R. (2018b). Effect of saline water, NPK and organic fertilizers on soil properties and growth, antioxidant enzymes in leaves and yield of lettuce (*Lactuca sativa* var. Parris Island). *Res. Crops*, 19(3), 441-449. https://doi.org/10.31830/2348-7542.2018.0001.14

Anuradha, Sehrawat, S. K., Vijayluxmi, & Bhat Sandhya (2016). Effect of growth regulators on *in vitro* root formation in strawberry. *Res. Environ. Life Sci*., 9(11), 1316-1318.

Bludneva, E. A, Kritskaya, T. A, & Kashin, A. S. (2013). The use of clonal micropropagation for mass production of planting material of ornamental and fruit crops in the botanical garden of Saratov state university. *Bulletin of the Botanic Gardens of Saratov State University*, 11, 119-131.

Bojarska, J. E., Markuszewski, B., Majewska, K. M., Józefowicz, W., & Bieniek, A. (2019). Preliminary studies on the storage of blue honeysuckle (*Lonicera caerulea* L.) berries. In 4 th International Conference "Effects of Pre- and Post-harvest Factors on Health Promoting Components and Quality of Horticultural Commodities", Skierniewice, Poland.

Caprioli, G., Iannarelli, R., Innocenti, M., Bellumori, M., Fiorini, D., Sagratini, G., Vittori, S., Buccioni, M., Santinelli, C., Bramucci, M., Quassinti, L., Lupidi, G., Vitali, L., Petrelli, D., Beghelli, D., Cavallucci, C., Bistoni, O., Trivisonno, A., & Maggi, F. (2016). Blue honeysuckle fruit (*Lonicera caerulea* L.) from eastern Russia: Phenolic composition, nutritional value and biological activities of its polar extracts. *Food Funct.*, 7(4), 1892-1903. https://doi.org/10.1039/C6FO00203J

Celli, G. B., Ghanem, A., & Brooks, M. S. L. (2014). Haskap berries (*Lonicera caerulea* L.) - A critical review of antioxidant capacity and health-related studies for potential value-added products. *Food Bioprocess Technol.*, 7, 1541-1554. https://doi.org/10.1007/s11947-014-1301-2

Chang, S. K., Alasalvar, C., & Shahidi, F. (2019). Superfruits: Phytochemicals, antioxidant efficacies, and health effect - A comprehensive review. *Crit. Rev. Food Sci. Nutr.*, 59(10), 1580-1604. https://doi.org/10.1080/10408398.2017.1422111

Clapa, D., Radomir, A. M., Peticila, A. G., & Harta, M. (2023). Evaluation of biomass production of *Stevia rebaudiana* Bertoni using classical *in vitro* culture and temporary immersion bioreactor system. *Scientific Papers. Series B, Horticulture*, 67(1), 558-565.

Cory, H., Passarelli, S., Szeto, J., Tamez, M., & Mattei, J. (2018). The Role of polyphenols in human health and food systems: A mini-review. *Front. Nutr.*, 5, 87. https://doi.org/10.3389/fnut.2018.00087

Debnath, S. C. (2007). Strategies to propagate *Vaccinium* nuclear stocks for the Canadian berry industry. *Can. J. Plant Sci.*, 87, 911-922.

Dziedzic, E. (2008). Propagation of blue honeysuckle (*Lonicera caerulea* var. *kamtscatica* Pojark.) in *in vitro* culture. *J. Fruit Ornam. Plant Res*., 16, 93-100.

Fira, A., Clapa, D., Cristea, V., & Plopa, C. (2014). *In vitro* propagation of *Lonicera kamtschatica*. *Agricultura - Ştiinţă şi practică*, 1-2, 90-99.

Gabryszewska, E., Góraj-Koniarska, J., Orlikowska, T., Malinowski, T., Markiewicz, M., & Wojtania, A. (2016). Metodyka inicjacji i stabilizacji kultur oraz namnażania pędów jagody kamczackiej (*Lonicera caerulea* L. var. *kamtschatica* Sevast.) *in vitro*. *Research Institute of Horticulture, Skierniewice, Poland*, 13 p.

Gahan, P. B., & George, E. F. (2008). Adventitious regeneration. In *Plant Propagation by Tissue Culture*; George, E. F., Hall, M. A., De Klerk, G.-J., Eds.; Springer: Dordrecht, The Netherlands, pp. 355-366.

Gawroński, J., Żebrowska, J., Pabich, M., Jackowska, I., Kowalczyk, K., & Dyduch-Siemińska, M. (2020). Phytochemical characterization of blue honeysuckle in relation to the genotypic diversity of *Lonicera* sp. *Appl. Sci.*, 10(18), 6545. https://doi.org/10.3390/app10186545

Grobelna, A., Kalisz, S., & Kieliszek, M. (2019). Effect of processing methods and storage time on the content of bioactive compounds in blue honeysuckle berry purees. *Agronomy*, 9(12), 860. https://doi.org/10.3390/agronomy9120860

Grobelna, A., Kalisz, S., Kieliszek, M., & Giurgiulescu, L. (2020). Blue honeyscckle berry (*Lonicera caerulea* L.), as raw material, is particularly predisposed to the production of functional foods. *Carpathian J. Food Sci. Technol.*, 12(3), 144-155. https://doi.org/10.34302/crpjfst/2020.12.3.12

Hartmann, H. T., Kester, D. E., & Geneve, R. L. (2002). Plant Propagation Principles and Practices, 7th edn. Perntice Hall, Inc. New Jersey, USA.

Hui, J. X., Wen, S. C., Hua, Z. Y., & Ming, L. X. (2012). Comparative study on different methods for *Lonicera japonica* Thunb. micropropagation and acclimatization. *J. Med. Plants Res.*, 6(27), 4389-4393. https://doi.org/10.5897/JMPR011.1715

Isac, V., & Mladin, Gh. (2013). Micropropagation of two blue honeysuckle cultivars released in Romania. *Acta Hortic*, 981, 453-459. https://doi.org/10.17660/ActaHortic.2013.981.72

Kadhim, Z. K., AL-Shareefi, J. H. M., & Lateef, S. M. (2019). Effect of growth regulators on *in vitro* micropropagation of blue honeysuckle (*Lonicera caerulea* L.). *Res on Crops*, 20(3), 635-641. http://dx.doi.org/10.31830/2348-7542.2019.093

Karhu, S. T. (1997). Rooting of blue honeysuckle microshoots. *Plant Cell, Tissue Organ Cult.*, 48, 153-159. https://doi.org/10.1023/A:1005768117246

Koubouris, G., & Vasilakakis, M. (2006). Improvement of *in vitro* propagation of apricot cultivar 'Bebecou'. *Plant Cell, Tissue Organ Cult*., 85, 173-180. https://doi.org/10.1007/s11240-005-9066-y

Krupa-Malkiewicz, M., & Ochmian, I. (2014). Propagation of blue honeysuckle (*Lonicera caerulea* L.) in *in vitro* culture. *J. Basic Appl. Sci*., 10, 164-169. https://doi.org/10.6000/1927-5129.2014.10.22

Krupa-Małkiewicz, M., Ochmian, I., Smolik, M., & Ostrowska, K. M. (2017). Comparison of propagation method in *in vitro* and *in vivo* condition of *Lonicera caerulea* L. *Folia Pomer. Univ. Technol. Stetin.*, 334(42)2, 79-88. https://doi.org/10.21005/AAPZ2017.42.2.09

Kula, M., Majdan, M., Radwańska, A., Nasal, A., Hałasa, R., Głód, D., Matkowski, A., & Krauze-Baranowska, M. (2013). Chemical composition and biological activity of the fruits from *Lonicera caerulea* var. edulis 'Wojtek'. *Acad. J. Med. Plants*, 8, 141-148. https://doi.org/10.15413/ajmp.2013.0134

Kushnir, H. P., & Sarnatska, V. V. Microclonal propagation of plants. K.: Naukova dumka, 2005:271 p.

Linsmaier, E. M., & Skoog, F. (1965). Organic growth factor requirements of tobacco tissue cultures. *Physiol Plant*, 18, 100-127. https://doi.org/10.1111/j.1399-3054.1965.tb06874.x

Liu, Y., Zhan, Y., Fu, Q., Li, S., Sun, X., Wang, Y., Yu, M., Qin, D., Huo, J., & Zhu, C. (2023). Plant Regeneration via Somatic Embryogenesis and Indirect Organogenesis in Blue Honeysuckle (*Lonicera caerulea* L.). *Horticulturae*, 9(9), 996. https://doi.org/10.3390/horticulturae9090996

Lloyd, G., & McCown, B. H. (1981). Woody Plant Medium (WPM) - A Mineral Nutrient Formulation for Microculture of Woody Plant Species. *HortScience*, 16, 453.

Mayorova, O. Yu., Hrytsak, L. R., Mazur, D. S., Vitrova, S. A., & Drobyk, N. M. (2016). Microclonal propagation and rooting *in vitro* of rare *Gentiana acaulis* L. species. *Faktory eksperymentalnoi evolutsii orhanizmiv*, 19, 162-166.

McLennan, A. K. (2018). Book review: Food Health. Nutrition, technology, and public health. (Research methods for anthropological studies of food and nutrition (Ed. by Jane Chrzan, John Brett). *Anthropos*, 113, 710-711.

Miyashita, T., Ohashi, T., Shibata, F., Araki, H., & Hoshino, Y. (2009). Plant regeneration with maintenance of the endosperm ploidy level by endosperm culture in *Lonicera caerulea* var. *emphyllocalyx*. *Plant Cell, Tissue Organ Cult.*, 98, 291-301. https://doi.org/10.1007/s11240-009-9562-6

Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.*, 15, 473-497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x

Nissen, S. J., & Sutter, E. G. (1990). Stability of IAA and IBA in nutrient medium to several tissue culture procedures. *Hort. Sci*., 25(7), 800-802.

Osburn, L. D., Yang, X., Li, Y., & Cheng, Z. M. (2009). Micropropagation of Japanese honeysuckle (*Lonicera japonica*) and Amur honeysuckle (*L. maackii*) by shoot tip culture. *J. Environ. Hortic*., 27(4), 195-199. https://doi.org/10.24266/0738-2898-27.4.195

Palikova, I., Heinrich, J., Bednar, P., Marhol, P., Kren, V., Cvak, L., Valentova, K., Ruzicka, F., Simanek, V., & Ulrichova, J. (2008). Constituents and Antimicrobial Properties of Blue Honeysuckle: A Novel Source for Phenolic Antioxidants. *J. Agric. Food Chem.*, 56(24), 11883-11889. https://doi.org/10.1021/jf8026233

Park, E., Kum, S., Wang, C., Park, S. Y., Kim, B. S., & Schuller-Lewis, G. (2005). Anti-inflammatory activity of herbal medicines: inhibition of nitric oxide production and tumor necrosis factor-alpha secretion in an activated macrophage-like cell line. *Am. J. Chinese Med.*, 33(3), 415-424. https://doi.org/10.1142/S0192415X05003028

Quoirin, M., & Lepoivre, P. (1977). Improved media for *in vitro* culture of *Prunus* sp. *Acta Hortic*., 78, 437-442. https://doi.org/10.17660/ActaHortic.1977.78.54

Rupasinghe, H. P. V., Arumuggam, N., Amararathna, M., & De Silva, A. B. K. H. (2018). The potential health benefits of haskap (*Lonicera caerulea* L.): Role of cyanidin-3-Oglucoside. *J. Funct. Foods*, 44, 24-39. https://doi.org/10.1016/j.jff.2018.02.023

Sedlák, J., & Paprštein F. (2007). *In vitro* propagation of blue honeysuckle. *Hort. Sci.*, 34(4), 129-131. https://doi.org/10.17221/1871-HORTSCI

Sharma, A., & Lee, H.-J. (2021). *Lonicera caerulea*: An updated account of its phytoconstituents and health-promoting activities. *Trends Food Sci. Technol.*, 107, 130-149. https://doi.org/10.1016/j.tifs.2020.08.013

Soliman, H. I. A., Metwali, E. M. R., & Almaghrabi, O. A. H. (2014). Micropropagation of *Stevia rebaudiana* Betroni and assessment of genetic stability of *in vitro* regenerated plants using inter simple sequence repeat (ISSR) marker. *Plant biotechnology*, 31(3), 249-256. https://doi.org/10.5511/PLANTBIOTECHNOLOGY.14.0707A

Svarcova, I., Heinrich, J., & Valentova, K. (2007). Berry fruits as a source of biologically active compounds: The case of *Lonicera caerulea. Biomed. Pap. Med. Fac. Univ. Palacky Olomouc Czech Repub*., 151(2), 163-174. https://doi.org/10.5507/bp.2007.031

Wang, Y., Zhu, J., Meng, X., Liu, S., Mu, J., & Ning, C. (2016). Comparison of polyphenol, anthocyanin and antioxidant capacity in four varieties of *Lonicera caerulea* berry extracts. *Food Chem.*, 197, 522-529. https://doi.org/10.1016/j.foodchem.2015.11.006

Waterlander, W. E., Ni Mhurchu, C., Eyles, H., Vandevijvere, S., Cleghorn, C., Scarborough, P., Swinburn, B., & Seidell, J. (2018). Food Future: Developing effective food systems interventions to improve public health nutrition. *Agric. Syst.*, 160, 124-131. https://doi.org/10.1016/j.agsy.2017.01.006

Wojtania, A., Matysiak, B., Góraj-Koniarska, J., Kiszczak, W., Kucharska, D., & Kowalska, U. (2018). Ukorzenianie i aklimatyzacja mikrosadzonek truskawki, maliny, jagody kamczackiej i czosnku w warunkach *ex vitro* – raport z badań wykonanych w 2018 roku. *Research Institute of Horticulture, Skierniewice, Poland*, 16 p.

Wojtania, A., Markiewicz, M., & Góraj-Koniarska, J. (2020). *Ex vitro* Rooting, Acclimatization and Genetic Stability of *Lonicera caerulea* var. *kamtschatica*. *J. Hortic. Res.*, 28(2), 61-70. https://doi.org/10.2478/johr-2020-0019

Yankovskaya, M. B., Shornikov, D. G., Muratova, S. A., & Solovykh, N. V. (2011). Preservation and propagation of valuable forms of berries and ornamental plants by biotechnology methods. *Vestnik IrGSKhA*, 4(44), 160-166.

Zapolsky, Y., Medvedeva, T., Natalchuk, T., & Bublyk, M. (2018). Propagation of edible honeysuckle (*Lonicera edulis* Turcz) in *in vitro* conditions. *Agric. sci. pract.*, 5(2), 18-26. https://doi.org/10.15407/agrisp5.02.018

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

THE EFFECT OF ALTERNATIVE TREATMENT METHODS ON THE EVOLUTION OF THE *PHILAENUS SPUMARIUS* POPULATION IN GOOSEBERRY CULTIVATION

Liliana-Aurora RĂDULESCU (NICHIFOR), Dorel HOZA

University of Agronomic Sciences and Veterinary Medicine of Bucharest,
59 Mărăşti Blvd, District 1, Bucharest, Romania

Corresponding author email: liliana.nichifor@yahoo.com

Abstract

Philaenus spumarius is a polyphagous insect that attacks hundreds of plant species. Initially, it was considered harmless to crops. However, it is now classified as a highly harmful insect because it serves as a vector for polyphagous pathogens - such as Xylella fastidiosa and Phytoplasma solani - which have caused significant damage to Vitaceae, Oleaceae, Rutaceae, Rosaceae, and Solanaceae crops across Europe.
The attacking capacity of Philaenus spumarius is influenced by factors such as the tolerance of the plant variety, the phenological phase at the time of the attack, climatic conditions, and the type of treatment applied.
Field studies conducted on the gooseberry varieties Invicta, Captivator, and Hinnonmaki Red revealed that Philaenus spumarius prefers Hinnonmaki Red. Treatments using Mentha pulegium, Urtica dioica, Thymus serpyllum, and Mentha piperita had varying effects on Philaenus spumarius specimens.
The research was carried out under field conditions.

Key words*: goosberry, Philaenus spumarius, polyphagous pathogens, vector insect, treatment.*

INTRODUCTION

Philaenus spumarius is a polyphagous insect from the order Homoptera, family Aphrophoidae. It damages plants directly by sap absorption and indirectly through the transmission of diseases.
Philaenus spumarius has a wide distribution in the northern hemisphere (Figure 1).

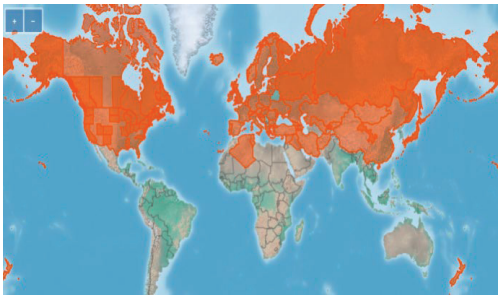


Figure 1. Distribution map of *Philaenus spumarius* (CABI PlantWise Plus, 2022)

In Europe, it was considered not to be a significant pest until it was discovered that it is an important vector of pathogens (Germinara et al., 2017). For this reason, there is limited data on the biology and ecology of *Philaenus spumarius* (Cornara et al., 2018).
Currently, the insect is classified as a serious pest (CABI PlantWise Plus, 2022).
The most important pathogen transmitted is *Xylella fastidiosa*. The bacterium attacks 696 plant species from 88 families (European Food Safety Authority, 2024). *Xylella fastidiosa* causes Pierce's disease in grapevines (PD), the rapid decline syndrome in olives (OQDS), almond leaf scorch (ALS), citrus variegated chlorosis (CVC), and others. The bacterium has caused significant losses in Spain, Italy, Portugal, Germany, France, Israel, Greece, Turkey (Godefroid et al., 2021).
Xylella fastidiosa colonizes the xylem, affects sap transport, and can cause plant death. Currently, there is no effective treatment for plants affected by this bacterium (Godefroid et al., 2021).
Philaenus spumarius also transmits *Phytoplasma solani*, a phytoplasma that causes the disease called stolbur (CABI PlantWise Plus, 2022).
Phytoplasma solani is also found in the scientific literature under the names *Candidatus Phytoplasma solani* and *Stolbur phytoplasma*

(Quaglino, 2017). The pathogen affects plants from the Solanaceae family, but also grapevines (CABI PlantWise Plus, 2022; Quaglino F., 2017).

Philaenus spumarius is a univoltine insect, although in some areas of Greece, it may be bivoltine (Yurtsever, 2000).

In spring, the female *Philaenus spumarius* lays 350-450 eggs in groups of 10-20, in protective foam. The larvae go through five developmental stages. Nymphs surround themselves with protective secretion. Under laboratory conditions, the larval stage lasts 50 days. The development from egg to adult takes place at a GDD of 700-800°C, a sum of temperatures above 5°C (Yurtsever S., 2000).

The color morphism of *Philaenus spumarius* is influenced by climatic factors, as well as by the habitat in relation to altitude (Boucelham et al., 1988).

Research on how substances influence the antennal movements of *Philaenus spumarius* has shown that both sexes respond most actively to octanal, 2-octanol, 2-decanone, (E)-2-hexenyl acetate, and vanillin, depending on the concentration of the substance (Germinara et al., 2017), as well as to α-pinene (Anastasaki et al., 2021). Additionally, the antennae of female *Philaenus spumarius* responded to camphor, limonene, 4-methyl octane, and sabinene (Anastasaki et al., 2021). The studies mentioned were not conducted under field conditions and specifically refer to antennal sensitivity, without indicating how the substance is perceived, whether attractant or repellent.

Some of these substances are found in the composition of plants used in alternative treatments: *Urtica dioica*, *Mentha piperita*, *Thymus serpyllum*, and *Mentha pulegium*.

The pedoclimatic conditions in which the plant grows influence its composition, specifically the substances it contains and their percentage.

Alimoddin et al. (2024) indicated in their article that *Urtica dioica* contains vanillic acid, but did not specify the percentage in its composition. The plant analyzed was grown under the pedoclimatic conditions of India, Kalaburagi district.

In Romania, Dâmbovița County, *Urtica dioica* contains camphor 0.27% (Ilieș et al., 2012).

Mentha piperita contains α-pinene 0.32% and sabinene 0.26% in Iran (Motamedi M. et al., 2012). In Morocco (Chraibi M. et al., 2017), *Mentha piperita* contains: limonene 3.01%, sabinene 1.38%, and α-pinene 0.32%. The cultivar 'Kristinika' contains in Italy (Camele et al., 2021): limonene 4.32% and α-pinene 0.47%. In Spain, *Mentha piperita* contains limonene 0.78% and α-pinene 0.38%, while in Pakistan, it contains limonene 7.58%, α-pinene 3.53%, and sabinene 0.19% (Gholamipourfard et al., 2021).

Mentha pulegium contains limonene 4.293% and α-pinene 0.509% in Algeria (Boukhebti H. et al., 2011). Eftekhari indicated in his 2021 paper that *Mentha pulegium* contains vanillic acid, but did not specify the environment in which the plant was cultivated or the percentage in its composition.

MATERIALS AND METHODS

The research was conducted in the southern part of Romania, at the experimental field in the commune of Domnești, Ilfov County. It was established in 2021, in an area where there are no longer any gooseberry crops.

The experiment was carried out between 2021 and 2024, with three varieties of gooseberries: *Invicta*, *Captivator*, and *Hinnonmaki red*, in five treatment variants with three repetitions, each repetition having 3 plants.

The treatment variants used were infusions of: *Urtica dioica*, *Mentha piperita*, *Thymus serpyllum*, and *Mentha pulegium*. The treatments were applied monthly, from April to July. One row was left as a control.

In order not to diminish the biological reserve of insects, no treatment with horticultural oil or insecticides was applied during the vegetative rest period.

To achieve the proposed goal and objectives, the following general working methods were used: documentary study at the national and international level regarding the current knowledge on the research topic, data analysis and synthesis, field observations in the experimental field, identification of pests, preparation of infusions, comparison, and experimentation.

RESULTS AND DISCUSSIONS

In the period 2021-2022, no specimens of *Philaenus spumarius* or specific foam were observed in the experimental field.
In 2023, *Philaenus spumarius* entered the crop field from the eastern and western edges. Foam was observed on only five gooseberry specimens.
In 2024, foam from *Philaenus spumarius* was observed on 19 gooseberry specimens, of which: 2 gooseberries treated with *Urtica dioica*, 6 gooseberries treated with *Mentha piperita*, 5 gooseberries treated with *Thymus serpyllum*, 2 gooseberries treated with *Mentha pulegium*, and 4 control gooseberries.
In Figure 2 here are images taken under the Mustools G 1200 digital microscope of the foam of *Philaenus spumarius*.

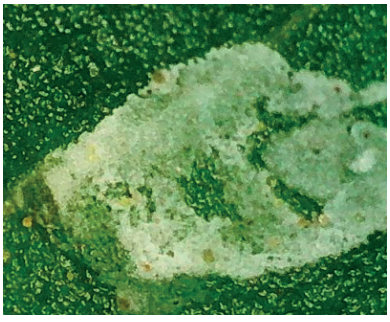


Figure 2. Microscope view of *Philaenus spumarius* foam (10.05.2024) (Source: original)

The gooseberry shrubs treated with *Thymus serpyllum* and *Mentha pulegium* were more attacked than the control specimens (Figure 3).

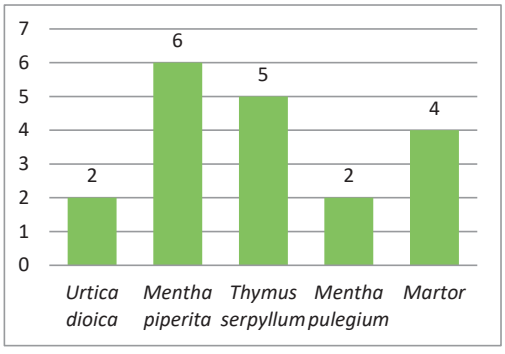


Figure 3 Number of gooseberries on which *Philaenus spumarius* foam was observed, without breakdown by variety, in 2024

The *Hinnonmaki red* variety is the most sensitive to *Philaenus spumarius*, while the *Invicta* variety is the most resistant (Figure 4).

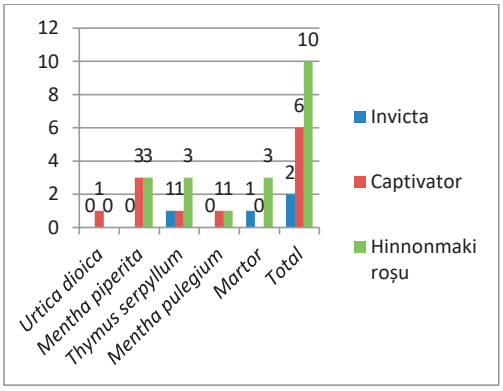


Figure 4. The way *Philaenus spumarius* attacked gooseberry varieties in 2024, depending on the applied treatment

Research conducted by Anastasaki et al. (2021) and Germinara et al. (2017) was not done in the field. It revealed that *Philaenus spumarius* shows olfactory antennal sensitivity to certain compounds, but without specifying how the insect's behavior manifests in response to these compounds.
The substances mentioned in the studies are: octanal, 2-octanol, 2-decanon, (E)-2-hexenyl acetate, vanillin, α-pinene, camphor, limonene, 4-methyl octane, and sabinene.
For the infusions used in the experiment, no laboratory analyses were made in 2023 and 2024.
Thymus serpyllum had an attractive effect on *Philaenus spumarius*.
Nikolić et al. (2014) present the substances found in *Thymus serpyllum*. Correlated with the studies on the substances to which *Philaenus spumarius* shows antennal olfactory sensitivity, it can be observed that *Thymus serpyllum* contains the following substances that influence the behavior of *Philaenus spumarius*: α-pinene, sabinene, and camphor. In natural field conditions, the combination of these substances had an attractive effect. It is specified that the substances and their percentages in *Thymus serpyllum* are determined for plants grown in the pedoclimatic conditions of Serbia.
For *Mentha pulegium* and *Mentha piperita* used in this research, laboratory analyses were

performed at the Applied Chemistry laboratory of the Chemistry Faculty at the Polytechnic University of Bucharest in 2019.

Mentha pulegium had a repellent effect. In the case of *Mentha pulegium*, the only substance found in its composition and noted in the studies is octanol, with a percentage of 2.41%. Although the percentage of substances known to influence the olfactory sensitivity of *Philaenus spumarius* is low, the gooseberry shrubs treated with *Mentha pulegium* were not preferred. It is mentioned that, based on field observations, *Mentha pulegium* creates stress in the gooseberry shrubs, which influences their physiology. In this context, it could be deduced that *Philaenus spumarius* prefers plants with favorable physiology. Future studies will confirm or disprove this assumption. Furthermore, it is possible that *Mentha pulegium* contains other substances with repellent characteristics that have not been identified so far.

Mentha piperita had an attractive effect. In the case of *Mentha piperita*, the substances that were determined to affect the antennal olfactory sensitivity are in very small percentages: limonene (0.09%) and octanol (0.21%). Even though the percentage of limonene and octanol is very low (0.3% of the total components), the number of gooseberries attacked by *Philaenus spumarius* was higher than that of the control gooseberries. The effect of other substances in the composition, for which no specialized studies have been found, cannot be specified.

Urtica dioica had a repellent effect. In the article published by Alimoddin et al. (2024), it is mentioned that *Urtica dioica* contains, among other substances, vanillic acid, without specifying the percentage in the composition. The *Urtica dioica* analyzed was grown under the pedoclimatic conditions of India. In the article by Ilieș et al. (2012), it is mentioned that, among other substances, *Urtica dioica* contains camphor at a concentration of 0.27%. The plant analyzed by Ilieș et al. grew under the pedoclimatic conditions of Romania, Dâmbovița County.

Vanillic acid was not found in the composition of *Mentha* spp. and *Thymus serpyllum*, which suggests that vanillic acid may have a repellent potential. The amount of camphor in *Urtica dioica* indicated in Ilieș's study (0.27%) is lower than the amount of camphor in *Thymus serpyllum* as indicated by Nikolić (0.7%).

For the other substances contained in the infusions used in the treatment, no information was found in the published articles on how they may influence or not influence the behavior of *Philaenus spumarius*.

It cannot be specified how the other substances in the composition influence the positive or negative effect of the substances individually determined in laboratory conditions. The studies on antennal sensitivity do not mention whether experiments were conducted with substances known to have insecticidal or insect-repelling effects (e.g., carvacrol, cineole, etc.) and how *Philaenus spumarius* might have been influenced or not influenced by them.

CONCLUSIONS

The results of the research conducted on gooseberry could be applied to other plants attacked by *Philaenus spumarius*.

Since *Philaenus spumarius* overwinters as an egg, it is important to carry out treatments during the vegetative rest period to reduce the biological reserve.

Philaenus spumarius is more difficult to control because the adults are mobile, and the larvae are protected by a protective foam. In addition, the pest has a highly developed sense of smell.

International research on the antennal sensitivity of *Philaenus spumarius*:

- has been done on a limited number of individual substances;
- has not been conducted under field conditions.

The infusions/decoctions/macerations from plants used in practice contain a set of substances that may have a synergistic effect.

This study was conducted in the experimental field, not in the laboratory. The conclusions of the research reflect the impact of treatments with *Urtica dioica*, *Mentha piperita*, *Thymus serpyllum*, and *Mentha pulegium* on the behavior of *Philaenus spumarius* in its natural environment.

It can be concluded that:

- the treatment with *Urtica dioica* had a repellent effect - only one specimen from the

Captivator variety repetition had foam from *Philaenus spumarius*, and no specimens from the Invicta and Hinnonmaki red varieties had foam;
- the treatment with *Thymus serpyllum* had an attractive effect in the case of the Hinnonmaki red variety, while in the case of the Invicta and Captivator varieties, only one repetition was found with *Philaenus spumarius*;
- the treatment with *Mentha pulegium* had a repellent effect – only one specimen from the repetitions of the Captivator and Hinnonmaki red varieties had foam from *Philaenus spumarius*.
- all repetitions of the Hinnonmaki red and Captivator varieties treated with *Mentha piperita* were found with *Philaenus spumarius*;
- the Hinnonmaki red variety is preferred more by *Philaenus spumarius* than the Invicta and Captivator varieties;
- the most resistant variety to the attack of *Philaenus spumarius* is Invicta.

Considering the major impact that *Philaenus spumarius* has as an insect-vector for dangerous pathogens of economically and socially important crops for European countries, and the mobilization of scientists by the European Food Safety Authority, research on *Philaenus spumarius* will undoubtedly be expanded in the future.

REFERENCES

Alimoddin M. et al. (2024). Pharmacological applications of *Urtica dioica*: a comprehensive review of its traditional use and modern scientific evidence, *Journal of Herbal Medicine*, 48, 100935

Anastasaki E. et al (2021). Electrophysiological responses of *Philaenus spumarius* and *Neophilaenus campestris* females to plant volatiles**,** *Phytochemistry*, 189, 112848

Boucelham M. et.al (1988) - Polymorphism of *Philaenus spumarius* (L.) (Homoptera, Cercopidae) in different latitudes, altitudes and habitats in the USA, *Annales Entomologici Fennici*, 54, No. 2, 49-54

Boukhebti H. et al. (2011). Chemical composition and antibacterial activity of *Mentha pulegium* L. and *Mentha spicata* L. essential oils, *Der Pharmacia Lettre*, 3(4), 267-275

CABI PlantWise Plus (2022). *Philaenus spumarius* (meadow froghopper), avaible online | Plantwise Knowledge Bank

Camele I., Grulová D., Elshafie H. (2021). Chemical Composition and Antimicrobial Properties of *Mentha x piperita* cv. ‚Kristinika', *Plants*, 10, 1567

Chraibi M. et al. (2017). African peppermint (*Mentha piperita*) from Morocco: chemical composition and antimicrobial properties of essential oil.

Cornara D., Bosco D., Fereres A**. (**2018). *Philaenus spumarius:* when an old acquaintance becomes a new threat tu European agriculture, *Journal of Pest Science*, 91, 957-972

Eftekhari A. et al. (2021). Phytochemical and nutra-pharmaceutical attributes of *Mentha* spp.: A comprehensive review, *Arabian Journal of Chemistry*, 14, 103106

European Food Safety Authority – *Xylella fastidosa* (2024), avaible online Xylella fastidiosa | EFSA

Germinara G. S. et al. (2017). Antennal olfactory responses of adult meadow spittlebug, *Philaenus spumarius*, to volatile organic compounds (VOCs), *Food and Chemical Toxicology*, 186, 114562

Gholamipourfard K., Salehi M., Banchio E. (2021). *Mentha piperita* phytochemicals in agriculture, food industry and medicine: Features and applications, *South Africa Journal of Botany*, 141, 183-195

Godefroid M. et al (2021). Climate tolerances of *Philaenus spumarius* shoul be considered in risk assessment of disease outbreaks related to *Xylella fastidiosa, Journal of Pest Science*, 95, 855-868.

Ilieș D.C., Tudor I., Rădulescu V. (2012). Chemical composition of the essential oil of *Urtica dioica, Russian Original*, 48, no. 3.

Motamedi M. et al. (2012). Chemical composition, antifugal and antibiofilm activities of the essential oil of *Mentha piperita*, *ISRN Pharmaceutics*, 10.5402/2012/18645

Nikolić M. et al. (2014). Chemical composition, antimicrobial, antioxidant and antitumor activity of *Thymus serpyllum* L., *Thymus algeriensis* Boiss. and Reut and *Thymus vulgaris* L. essential oils, *Industrial Crops and Products*, 52, 183-190.

Quaglino F. (2017). *Candidatus Phytoplasma solani* (*Stolbur phytoplasma*) , CABI Compendium, 108243.

Yurtsever Selçuk (2000). On the Polymorphic Meadow Spittlebug*, Philaenus spumarius* (L.) (Homoptera: Cercopidae), *Turkish Journal of Zoology*, 24, 447-459

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

VARIABILITY OF BIOMETRIC AND BIOCHEMICAL CHARACTERISTICS IN SOME APPLE VARIETIES

Daniela Nicoleta SCEDEI, Olimpia IORDĂNESCU, Casiana MIHUȚ, Renata SUMĂLAN, Ramona ȘTEF, Carmen DURĂU, Antonela COZMA, Vlad Dragoslav MIRCOV, Maria Mihaela MOATAR, Dacian LALESCU

University of Life Sciences "King Mihai I" from Timișoara,
119 Calea Aradului Street, Timișoara, Romania

Corresponding author emails: lalescu@usvt.ro, mihaela.moatar@usvt.ro

Abstract

Apples, Malus × domestica Borkh., are the third most produced fruit in the world after bananas and watermelons (FAO Statista, 2023) and the most consumed fruit in Romania. The fruit market in Romania is invaded by apple varieties imported from Turkey, Poland, Italy, or Hungary. In recent decades, hybrid varieties obtained in Europe or the United States have also influenced domestic production. For this study, five varieties of apples with different origins were studied, which were purchased from the supermarket (in the year 2024). The varieties studied were Starkrimson, Jonaprince, Jonaprince, Idared, and Golden Delicious. In these varieties, the biometric and biochemical characteristics of the fruits were studied, such as the average weight of the fruit, the height and the diameter (large diameter, small diameter) of the fruits,thefruit firmness,thecontent in soluble dry matter, the percentage of sugar,thefruit acidity and vitamin C. This research focused on the comparisons and linear relationships between the biometric and biochemical characteristics of the studied varieties, followed by principal component analysis (PCA) and cluster analysis.

Key words*: varieties, apples, biometric characteristics, biochemical characteristics, character variability.*

INTRODUCTION

Apples, *Malus* × *domestica* Borkh., are the third most produced fruit in the world after bananas and watermelons (FAO Statista, 2023) and the most consumed fruit in Romania. They are associated with a healthy lifestyle due to their richness in vitamins and antioxidants, such as phenolic acid, which has been shown to reduce cancer risk. With their high adaptability, apples can be grown economically in different environmental conditions, from high altitudes to subtropical regions. In addition to price, other criteria are increasingly important for consumers, such as whether the apples are organically grown or the country of origin. Mainly regarding European countries, regionally produced food is becoming increasingly popular among consumers (Denver & Jensen, 2014). In addition, the taxonomic variety is relevant because it is decisive for the apple's flavor (Siekierzynska et al., 2021). The fruit market in Romania is invaded by apple varieties imported from Turkey, Poland, Italy, or Hungary. In recent decades, hybrid varieties obtained in Europe or the United States have also influenced domestic production. Cultivated in Romania and adapted to pedo-climatic conditions, these varieties created more resistant fruits with a commercial appearance but less tasty.

The nutritional value of apple fruits is well known, and there are variable amounts of ascorbic acid, sugars, organic acids, phenolics, and minerals. The therapeutic value of apples is widely known for curing several diseases. Qualitative parameters such as skin color, fruit firmness, sweetness and acidity, and other organoleptic traits can influence consumer acceptance of specific genotypes (Miloševićet et al., 2023; Mehdi, 2020; Meng, et al., 2014). The biological properties of apple varieties are the essential factors that determine tree growth, production, and the economic effects of their cultivation (Goddrie & Kemp.1991; Ugolik et al.,1994;Wrona et al., 1994;Włodarczyk, 1996; Błaszczyk & Poniedziałek, 1998; Makosz, 2005; Kapłan & Wociór, 2002). Therefore, the main goal of apple breeding programs is to obtain new apple genotypes characterized by high yields and good fruit qualities (shape, size, acidity, juiciness, sweetness, color) and low

susceptibility/resistance of trees to fungal pathogens, as well as adaptation to the climatic conditions of a given area (Lewandowski & Żurawicz, 2007; Laurens, 1998; Sansavini et al., 2004; Kellerhals, 2009; Kumar et al., 2014). Tang et al., 2018, reported that sugar content is one of the most important fruit characteristics to ensure better customer experience repeat purchases and is one of the main attributes in fruit quality classification (Choi et al., 2006). Furthermore (Aprea et al., 2017) demonstrated that the search for increased sweetness in apple breeding programs must consider not only sugar content but also factors such as volatile compounds, textural parameters, and polyphenols.

Although there are over 7,500 apple varieties worldwide, many heritage varieties have been abandoned, despite a resurgence seen in recent years, in favor of mainstream varieties that have emerged from intensive selective breeding programs over the past few decades, leading to the decline of traditional apple orchards in many countries, including the United Kingdom. The reason for this is the drive for sweet, crisp apples that are uniform in size and appearance and have acceptable disease resistance and extended shelf life (Anastasiadi et al., 2017). The fruit content in chemical compounds, respectively the nutritional value, depends on genotype, tissue structure, fruit maturation, pedoclimatic conditions, and the culture technology used but also on harvesting and storing conditions (Drogoudi & Pantelidis, 2011; Stănică et al., 2008; Mitre, 2008; Stopar et al., 2002; Iordănescu et al., 2021).

The nutritional value of apples is well known. It is a variable amount of ascorbic acid, organic sugars, acids, phenolics, and minerals.

The therapeutic value of the apple is widely known to cure several diseases. Qualitative parameters such as skin color, fruit firmness, sweetness and acidity, and other organoleptic features can influence consumer acceptance of specific genotypes (Iordănescu & Olaru, 2014; Sediqi, 2025). In 2023, apple production in Romania was just over 400,000 tons, similar to the previous year's and below the average of the last five years. Our country is the seventh European apple producer, surpassed by Spain, Hungary, Germany, France, Italy, and Poland. According to the National Institute of Statistics data, Romania imported 66,100 tons of fresh apples worth 31.7 million euros in the first five months of last year. The most significant amount came from Poland, which produces around 4 million tons of apples annually.

In 2024, difficult climatic conditions significantly affected apple production in Romania. Summer heat and drought accelerated fruit ripening and reduced fruit size, so many orchards experienced significant losses in production and quality. For example, farmers in Bacău County reported decreases from 80 tons/hectare in 2023 to 60 tons/hectare in 2024, despite irrigation. In addition, the drought led to earlier harvests and poorer fruit quality, with many being diverted to processing instead of being sold for fresh consumption. As a result of the decrease in production, prices increased, with apples sold directly by farmers reaching 2.5-4 lei/kg. In hypermarkets, prices rise to 5-10 lei/kg, even 12 lei for organic apples. In addition, an increase in apple imports is anticipated to meet domestic demand, which is consistently higher than domestic production. According to INS, fruit imports reached almost 1 billion euros in 2023 and generate Romania's second largest trade deficit on the food balance. According to the National Institute of Statistics data, Romania imported in the first five months of the year (2022) 66,100 tons of fresh apples, worth 31.7 million euros, 23.7% more in volume than in a similar period last year. The most significant quantities come from Poland, Moldova, and Germany. The average consumption of apples in Romania is approximately 15-20 kg per year per capita, which underlines the importance of this fruit in the daily diet.

The apple industry faces challenges due to climate change and the need for quantitative production control.

MATERIALS AND METHODS

The biological material

For this study, five varieties of apples with different origins were studied, which were purchased from the supermarket (in 2024). The varieties studied were Starkrimson (Hungary), Jonaprince (Hungary), Jonaprince (Romania), Idared (Poland) and Golden Delicious (Poland). In these varieties, the biometric and

biochemical characteristics of the fruits were studied, such as the average weight of the fruit; height and diameter (large diameter, small diameter) of the fruits; fruit firmness (Sediqi, 2025); content in soluble dry matter; sugar percentage, fruit acidity (pH) and vitamin C.

This research focused on the comparative study between varieties with respect to the characters studied, correlations and regressions, principal component analysis (PCA), and cluster analysis.

The analyzed biometric and biochemical properties

The fruits (25 fruits/variety) were harvested from different parts of the tree. The fruits' diameter and their length have been measured with the help of a caliper. The weight was established by using an analytical balance. Fruit sugar content, expressed as soluble carbohydrates (Brix degree - %), has been obtained with a digital refractometer (Kruss DR 201-95). Fruit flesh firmness was determined with thepenetrometer (Force Gauge PCE-FM 200), and results have been expressed in lbs. The fruit juice acidity was determined using a multiparameter analyzer - pH (Consort C 933). The variability of biometric and biochem ical characteristics in apple varieties iscritical to understanding their quality, nutritional value, and suitability for different uses. This analysis synthesizes findings from multiple studies to provide a comprehensive overview of the differences in biometric traits (diameter, weight, height, firmness) and biochemical traits (Brix, sugar, pH, and Vitamin C) across various apple varieties. The Vitamin C content wasestimatedusing the 2,6-dichlorophenolindophenol titration method. Briefly, 5 g of fresh sample wash omogenized in 50 mL of 0.02 $g \cdot mL^{-1}$ oxalic acid solution and thencentrifuged at 15,000 x for 15 min at 4°C. The supernatant (10 mL) wastitratedto a permanent pink color by 0.1% 2,6-dichlorophenolindophenol (Moldovan et al., 2006). All statistical analyses were done using R Statistical Software [v4.4.1; RCoreTeam, 2024). Comparations (ANOVA and Duncan tests), Pearson's linear correlation coefficients, principal component analysis (PCA), and cluster analysis were performed.

RESULTS AND DISCUSSIONS

A one-way ANOVA was performed to compare the effect of apple variety on large diameter. It was revealed that there was a statistically significant difference in the large diameters between at least two groups (F(4, 45) = 29.28, p < .001; Eta2 = 0.72, 95% CI [0.59, 1.00]). Duncan test for multiple comparisons found that the mean value of large diameter was significantly different between: Idared and Golden (p < .05, 95% C.I. = [4.5890813, 12.328919]); Jonaprince_H and Golden (p < .05, 95% C.I. = [12.9057631, 20.820237]); Jonaprince_H and Idared (p < .05, 95% C.I. = [4.8389112, 11.969089]); Jonaprince_RO and Idared (p < .05, 95% C.I. = [2.857838, 10.3561619]); Starkimson and Idared (p < .05, 95% C.I. = [1.410911, 8.5410888]); Jonaprince_RO and Jonaprince_H (p < .05, 95% C.I. = [11.141081, 18.8809187]); Starkimson and Jonaprince_H (p < .05, 95% C.I. = [9.630838, 17.1291619]) (Table 1). Apple varieties like Scarlet Spur and Starkrimson Delicious were found to have larger diameters, with Scarlet Spur exhibiting the highest fruit weight (289.96 g) and length (32.61 mm) (Ates et al., 2023). Similarly, Starkrimson Delicious had a high fruit width (34.86 mm) (Ates et al., 2023). A one-way ANOVA was performed to compare the effect of apple variety on small diameter. It was revealed that there was a statistically significant difference in the small diameters between at least two groups (F(4, 45) = 46.83, p <.001; Eta2 = 0.81, 95% CI [0.71, 1.00]). Duncan test for multiple comparisons found that the mean value of small diameter was significantly different between: Idared and Golden (p < .05, 95% C.I. = [4.3292496, 10.756750]); Jonaprince_H and Golden (p < .05, 95% C.I. = [14.1937367, 20.766263]); Jonaprince_H and Idared (p <.05, 95% C.I. = [6.9763937, 12.897606]);Jonaprince_RO and Idared (p < .05, 95% C.I. = [1.667394, 7.5886063]); Starkimson and Idared (p <.05, 95% C.I. = [3.207531,9.4344686]);Jonaprince_ROJonaprince_H (p <.05, 95% C.I. = [11.451531, 17.6784686]); Starkimson and Jonaprince_H (p <.05, 95% C.I. = [13.044250, 19.4717504]) (Table 1.).

A one-way ANOVA was performed to compare the effect of apple variety on height. It was revealed that there was a statistically significant difference in the height between at least two groups (F(4, 45) = 13.43, p < .001; Eta2 = 0.54, 95% CI [0.35, 1.00]).Duncan test for multiple comparisons found that the mean value of height was significantly different between: Idared and Golden (p < .05, 95% C.I. = [1.2304709, 11.2735291]); Jonaprince_RO and Golden (p < .05, 95% C.I. = [6.9287323, 17.2952677]);Jonaprince_H and Idared (p <.05,95%C.I.=[4.6757323,15.0422677]);Jonaprince_RO and Idared (p<.05, 95% C.I. = [1.0850135, 10.6349865]); Starkimson and Idared (p <.05, 95% C.I. = [0.6650135, 10.2149865]);Jonaprince_ROand Jonaprince_H (p <.05, 95% C.I. = [10.4187807, 21.0192193]);Starkimson and Jonaprince_RO (table 1.) (p<.05, 95% C.I. = [6.2784709, 16.3215291]) (Table 1.). Fruit height varied significantly across cultivars. For instance, Royal Gala and Starkrimson exhibited taller fruits than Golden Delicious (Zeroual et al., 2024).

A one-way ANOVA was performed to compare the effect of apple variety on weight. It was revealed that there was a statistically significant difference in the weight between at least two groups (F(4, 45) = 22.56, p <.001; Eta2 = 0.67, 95% CI [0.52, 1.00]) (Table 1.). Duncan test for multiple comparisons found that the mean value of weight was significantly different between: Jonaprince_H and Golden (p <.05, 95%C.I.=[87.20551, 156.02849]); Jonaprince_H and Idared (p <.05, 95% C.I. = [79.22729, 145.90271]);Jonaprince_RO and Jonaprince_H (p <.05, 95% C.I. = [93.38807, 163.76393]); Starkimson and Jonaprince_H (p < .05, 95% C.I. = [69.15907, 132.56093]).

A one-way ANOVA was performed to compare the effect of apple variety on firmness. It was revealed that there was a statistically significant difference in the firmness between at least two groups (F(4, 45) = 10.88, p <.001; Eta2 = 0.49, 95% CI [0.29, 1.00]) (Table 1.). Duncan test for multiple comparisons found that the mean value of firmness was significantly different between: Idared and Golden (p<.05, 95% C.I. = [0.049368869,0.8366311]); Jonaprince_H and Golden (p <.05, 95% C.I.=[0.135690392,0.9483096]);Jonaprince_RO-Golden (p <.05, 95% C.I.=[0.776522703, 1.6074773]);Jonaprince_RO-Idared (p < .05, 95%C.I.=[0.355368869,1.1426311]);Jonaprince_RO-Jonaprince_H(p < .05, 95% C.I. = [0.275695022, 1.0243050]);Starkimson and Jonaprince_RO (p < .05, 95% C.I. = [0.4196904, 1.232309608]) (Table 1.). A one-way ANOVA was performed to compare the effect of apple variety on brix. It was revealed that there was a statistically significant difference in the brix between at least two groups (F(4, 45) = 7.22, p <.001; Eta2 = 0.39, 95% CI [0.17, 1.00]).Duncan test for multiple comparisons found that the mean value of brix was significantly different between: Idared and Golden (p < .05, 95% C.I. = [0.82595692, 3.5140431]); Jonaprince_H and Golden (p < .05,95%C.I.=[0.02182595,2.4981741]);
Starkimson and Golden (p <.05, 95% C.I. = [0.19789642, 2.8021036]); Jonaprince_RO and Idared (p <.05, 95% C.I. = [1.4956309, 4.24436913]);Jonaprince_ROand Jonaprince_H (p<.05,95%C.I.=[0.6578964,3.26210358]);Starkimson-Jonaprince_RO (p <.05, 95% C.I. = [0.85595692, 3.5440431]) (Table 1). Starkrimson Delicious and Fuji had high Brix values, with Starkrimson Delicious reaching 14.30% and Fuji reaching 14.27% (Ates et al., 2023) (Yarilgaç et al., 2023).Super Chief and Kala Kulu had lower Brix values, ranging from 11.65% to 10.56% (Ates et al., 2023; Mehdi, 2020). A one-way ANOVA was performed to compare the effect of apple variety on sugar. It was revealed that there was a statistically significant difference in the sugar content between at least two groups (F(4, 45) = 7.22, p<.001; Eta2 = 0.39, 95% CI[0.17, 1.00]). Duncan test for multiple comparisons found that the mean value of brix was significantly different between: Idared and Golden (p <.05, 95% C.I. = [0.87867723, 3.7353228]); Jonaprince_H and Golden (p <.05, 95% C.I. = [0.02418489,2.6558151]); Starkimson-Golden (p <.05, 95% C.I. = [0.21024659, 2.9777534]); Jonaprince_RO and Idared (p <.05, 95%C.I. = [1.5884495,4.50955046]); Jonaprince_RO and Jonaprince_H (p < .05, 95% C.I. = [0.6982466, 3.46575341]); Starkimson-Jonaprince_RO (p < .05, 95% C.I. = [0.90767723, 3.7643228]) (Table 1.). A one-way ANOVA was performed to compare the effect of apple variety on Ph. It

was revealed that there was a statistically significant difference in the pH between at least two groups (F(4, 10) = 7.66, p = 0.004; Eta2 = 0.75, 95% CI[0.36, 1.00]). Duncan test for multiple comparisons found that the mean value of pH was significantly different between:Starkimson and Golden (p <.05, 95% C.I. = [0.05456671 0.54543329]); Starkimson and Idared (p<.05, 95% C.I. = [0.24614903,0.75385097]); Starkimson and Jonaprince_H (p <.05, 95% C.I. = [0.24614903 0.75385097]); Starkimson and Jonaprince_RO (p <.05, 95% C.I. = [0.03180021, 0.50153312]) (Table 1.). A one-way ANOVA was performed to compare the effect of apple variety on vitamin C content. It was revealed that there was a statistically significant difference in the vitamin C content between at least two groups (F(4, 10) = 9.07e+05, p < .001; Eta2 = 1.00, 95%CI [1.00, 1.00]).Duncan test for multiple comparisons found that the mean value of vitamine C content was significantly different between: Starkimson and Golden (p < .05, 95% C.I. = [5.6204519,5.6555481]); Jonaprince_H and Golden(p<.05, 95% C.I .= [3.8622075, 3.8957925]); Jonaprince_P and Golden (p < .05, 95% C.I. = [7.9394519, 7.9745481]); Starkimson and Golden (p<.05, 95% C.I. = [0.1832075,0.2167925; Jonaprince_H and Idared (p < .05, 95% C.I. = [9.4990072, 9.5349928]); Jonaprince_RO and Idared (p < .05, 95% C.I. = [13.5767228, 13.6132772]); Starkimson and Idared(p < .05, 95% C.I. = [5.4212075, 5.4547925]); Jonaprince_RO and Jonaprince_H(p < .05, 95% C.I. = [4.0612075, 4.0947925]); Starkimson and Jonaprince_H (p < .05, 95% C.I. = [4.0614519, 4.0965481]); Starkimson and Jonaprince_RO (p <.05, 95% C.I. = [8.1390072, 8.1749928]) (Table 1.). Apples grown in the Moscow region had 3-5% higher antioxidant activity, including Vitamin C, than imported varieties (Motyleva & Borisova, 2019; Bogdanescu et al., 2018). In order to understand and measure the strength and direction of the linear relation ships between biometric and biochemical parameters, Pearson's correlation coefficients were calculated. The Pearson's product-moment correlation between L_diameter and S_diameter is positive, statistically significant, and very large (r = 0.98, 95% CI [0.76, 1.00], t(3) = 9.27, p= 0.003); between L_diameter and Weight is positive, statistically significant, and very large (r = 0.91, 95% CI [0.13, 0.99], t(3) = 3.76, p =0.033); between S_diameter and Weight is positive, statistically significant, and very large (r = 0.90, 95% CI [0.10, 0.99], t(3) = 3.62, p =0.036); between Brix and Vit_C is positive, statistically significant, and very large (r = 0.77, 95% CI [0.35, 0.98], t(3) = 2.10, p =0.047); between Sugar and Vit_C is positive, statistically significant, and very large (r = 0.77, 95% CI [0.35, 0.98], t(3) = 2.10, p =0.047), and between Sugar and Brix is positive, statistically significant, and very large (r = 1.00, 95% CI [1.00, 1.00], t(3) = 2839.52, p < .001) (Figure 1.).

Table 1. Statistical comparisons between the biometric and biochemical characters obtained in the studied apple varieties

Variety	Large diameter (mm)	Small diameter (mm)	Height (mm)	Weight (g)	Firmness	Brix (%)	Sugar (%)	Ph	Vitamin C (mg/100 g)
Starkimson_H	73.862 ± 4.335 c	68.196 ± 1.785 c	73.500 ± 4.460 a	180.527 ± 29.939 b	3.135 ± 0.512 ab	13.13 ± 0.583 a	11.451 ± 0.619 a	3.966 ± 0.057 a	17.673 ± 0.002 d
Jonaprince_H	87.242 ± 4.458 a	84.454 ± 4.741 a	77.919 ± 8.614 a	281.387 ± 65.223 a	2.959 ± 0.211 b	12.89 ± 1.679 a	11.197 ± 1.784 a	3.466 ± 0.208 b	21.552 ± 0.003 c
Idared_PL	78.838 ± 4.306 b	74.517 ± 4.214 b	68.06 ± 3.904 b	168.822 ± 26.984 b	3.058 ± 0.284 b	13.80 ± 1.858 a	12.164 ± 1.975 a	3.466 ± 0.152 b	13.595 ± 0.002 e
Jonaprince_RO	72.231 ± 2.295 c	69.889 ± 1.904 c	62.200 ± 3.559 c	152.811 ± 9.935 b	2.309 ± 0.349 c	10.93 ± 0.266 b	9.115 ± 0.282 b	3.700 ± 0.100 b	27.190 ± 0.020 a
Golden_PL	70.379 ± 3.979 c	66.974 ± 2.638 c	74.312 ± 4.300 a	159.77 ± 14.694 b	3.501 ± 0.594 a	11.63 ± 1.662 b	9.857 ± 1.765 b	3.666 ± 0.057 b	21.752 ± 0.003 b

Different lower-case letters indicate a statistically significant difference in the biometric and biochemical characteristics of studied apple varieties.

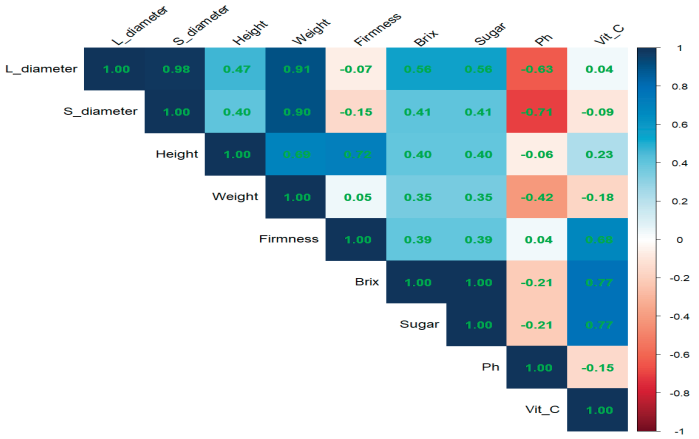


Figure 1. Correlation between the biometric and biochemical characteristics of studied apple varieties

The inertia of the first dimension shows if there are strong relationships between variables and suggests the number of dimensions that should be studied. The first two principal components of analysis express 78% of the total dataset inertia; that means that 78% of the individual's cloud total variability is explained by the plane spanned by the first two principal components (Figure 2).

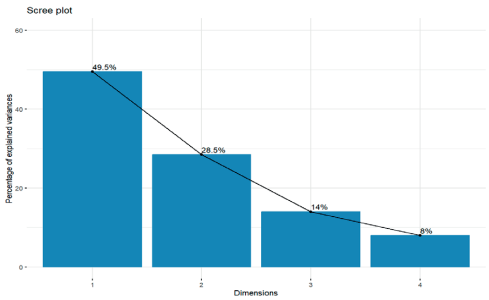


Figure 2. Scree plot of PCA

This percentage is very high; thus, the first plane represents the data variability very well. The variability explained by this plane is thus highly significant (the reference value is the 0.95-quantile of the inertia percentages distribution obtained by simulating 6543 data tables of equivalent size based on a standard distribution). The first principal component factor is significant: it expresses itself 49.5% of the data variability. This axis presents an amount of inertia greater than those obtained by the 0.95-quantile of random distributions. This observation suggests that this axis carries great factual information. The most important contribution for this component comes from the following variables: L_diameter, S_diameter, Sugar, Brix, and Weight (which are all highly correlated) (Figure 3 and Figure 5).

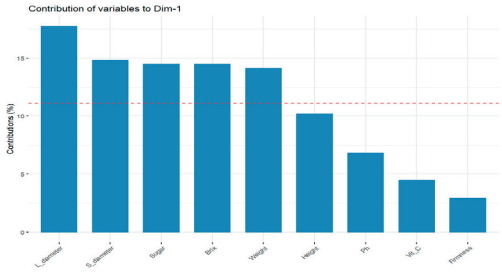


Figure 3. Contribution of variables to the first dimension of PCA

The second principal component expresses itself 28.5% of the data variability. The most important contribution for this component comes from the variables Vit_C, Firmness, and S_diameter (Figure 4 and Figure 5).

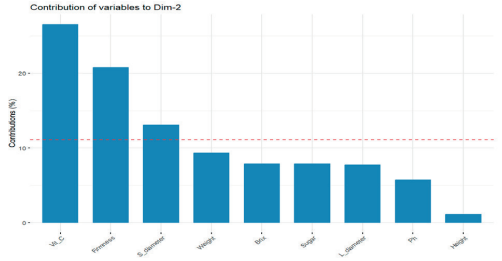


Figure 4. Contribution of variables to the second dimension of PCA

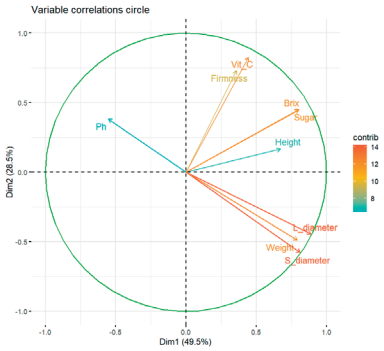


Figure 5. Projection of the variables on the plane spanned by first and second components

Ascending Hierarchical Classification of the individuals.The classification made on individuals reveals 3 clusters. Cluster 1comprises Jonaprince_RO; cluster 2 comprises the following individuals: Golden_PL, Starkimson_H, and Idared_PL, and cluster 3 comprises Jonaprince_H (Figure 6 and Figure 7).

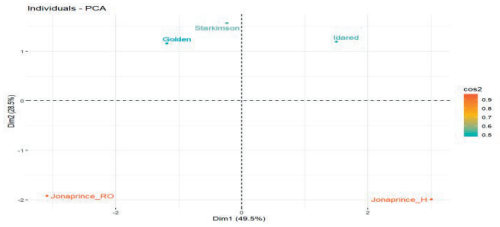


Figure 6. Projection of the observables on the plane spanned by first and second components

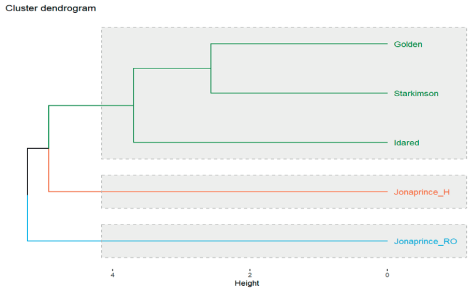


Figure 7. Cluster dendrogram of the population of studied apple varieties

CONCLUSIONS

From the results obtained, it can be observed that there were significant differences in the biometric characters between the same variety of apple with different origins, that is, the Jonaprince_H variety presented higher values in terms of the large diameter (87,242 mm), the small diameter (84,454 mm) and the average height of the fruits (77,919 mm) compared to the Jonaprince_Ro variety with the large diameter of 72,231 mm, 69,889 mm small diameter and average height of 62,200 mm. The content of soluble dry substance (Brix-%) of apple fruits had values between 10.93% (Jonaprince_RO) and 13.80% (Idared_PL), and the values of ascorbic acid varied between 13.595 mg/100 g and 27.190 mg/100 g, the dynamics is seen as soon as the amount of dry substance content decreases ascorbic acid in the fruits apple grows immediately.

REFERENCES

Anastasiadi, M., Mohareb, F., Redfern, S.P., Berry, M., Simmonds, M. S.J., Terry, L. A. (2017). Biochemical profile of heritage and modern apple cultivars and application of machine learning methods to predict usage, age, and harvest season. *Journal of Agricultural and Food Chemistry*, 65(26), 5339–5356.

Aprea, E., Charles, M., Endrizzi, I.,Corollaro, L.M.,Betta, E.,Biasioli, F.,Gasperi, F. (2017). Sweet taste in apple: The role of sorbitol, individual sugars, organic acids and volatile compounds. *Sci. Rep.* 2017, *7*, 44950.

Ates, U., Öztürk, B., Uzun, S. (2023). Characterization of Bioactive Contents and Fruit Traits in Some Apple Cultivars Grown in Kayseri.*Black Sea Journal of Agriculture*.

Błaszczyk, J., & Poniedziałek, W. (1998). Growth and Yielding of 20 Apple Varieties in the Kraków Region. In *Proceedings of the 37 Polish National Scientific Conference of Judical Institute of Pomology and Floriculture, Skierniewice*, Poland, 25–27 August 1998; pp. 363–365.

Bogdanescu, D., Bordean, D.-M., Poiana, M. A., Hadaruga, N., Tataru, O., Rivis, A. (2018). Characterization of the main Romanian apple varieties based on pH, refractometric index and moisture content. *JournaL of Horticulture, Forestry and Biotechnology*, Volume 22(2), 95- 99.

Choi, D., Cho, H.-T., Lee, Y. (2006). Expansins: Expanding importance in plant growth and development. *Physiol. Plant.* 2006, *126*, 511–518.

Denver, S.& Jensen, J.D. (2014).Consumer Preferences for Organically and Locally Produced Apples. *Food Quality and Preference*, 31, 129-134.

Drogoudi, D.P., & Pantelidis, G. (2011). Effects of position on canopy and harvest time on fruit physico-chemical and antioxidant properties in different apple cultivars.*Scientia Horticulturae*. No.129, 752-760.

Goddrie, P.W., & Kemp, H. (1991). Cultivar testing with apple. *Wilherminadorp Res. St Ann. Rep.* , 48–54.

Iordănescu, O.A., & Olaru, D.N. (2014). *Pomologie*, Ed. Agroprint, Timişoara.

Iordănescu, O.A., Iuga, C., Hădărugă, N., Becherescu, A., Drăgunescu, A., Băla, M., Scedei, D. (2021). Research on fruit quality of several apple cultivars from different locations in the area of western Romania.*Scientific Papers.Series B, Horticulture*, Vol. LXV, No. 1.

Kellerhals, M. (2009). Introduction to Apple (*Malus* × *domestica*). In *Genetics and Genomics of Rosaceae*; Folta, K.M., Gardiner, S.E., Eds.; Springer: New York, NY, USA, 2009; pp. 73–84. ISBN 978-0-387-77490-9.

Kumar, S., Volz, R.K., Chagné, D., Gardiner, S. (2014). Breeding for apple (*Malus* × *domestica* Borkh.) fruit quality traits in the genomics era. In *Genomics of Plant Genetic Resources*; Tuberosa, R., Graner, A., Frison, E., Eds.; Springer: Dordrecht, The Netherlands, 2014; pp. 387–416. ISBN 978-94-007-7574-9.

Laurens, F. (1998). Review of the current apple breeding programmes in the world: Objectives for scion cultivar improvement. *ActaHortic.* 1998, *484*, 163–170.

Lewandowski, M., & Żurawicz, E. (2007). Effect of genotype on germination of *Malusdomestica* seeds. In *Natural and Induced Variability in the Genetic Improvement of Horticultural Plants*; UTP in Bydgoszcz: Bydgoszcz, Poland, 2007; pp. 73–80.

Makosz, E. (2005). The Vision of the Fruit Market in Poland. In Proceedings of the 44 *Congress of the Fruit-Growers' Market Fruit, Skierniewice*, Poland, 27 October 2005; pp. 5–13.

Mehdi, A. (2020). Assessment of physico-chemical and phyto-chemical properties of six apple varities cultivated in district Nagar and Hunza Gilgit Baltistan, Pakistan. *Pesquisa Agropecuaria Brasileira*.

Meng, X. J., Gong, L. Y., Huang, G. H., Liu, N. Q., Bi, J., & Li, B. (2014). Physical and Chemical Compositional Characterization of Six Apple Cultivars.*Advanced Materials Research*.

Milošević, T., Milošević, N. T., Miletic, N. (2023). Segregation of apple cultivars on the basis of main fruit physical and chemical properties and antioxidant activity. *Turkish Journal of Agriculture and Forestry*.

Mitre, V. (2008). *Pomologie*, Edit. Todesco, ClujNapoca.

Moldovan, P., Toşa, M.I., Leţ, D., Majdik, C., Paizs, C., Irimie, F. D. (2006). *Aplicaţii pentru laboratorul de biochimie*, Editura Napoca Star, Cluj Napoca, 110-115.

Motyleva, S. M., & Borisova, A. A. (2019). Comparative biochemical composition of apple fruits of domestic and foreign varieties.*Horticulture and viticulture*, № 6, p. 12-18.

RCoreTeam. (2024). R:ALanguage and Environment for Statistical Computing. *R Foundation for Statistical Computing*: Vienna, Austria. Available online: https://www.R-project.org/.

Sansavini, S.,Donati, F., Costa, F.,Tartarini, S. (2004). Advances in apple breeding for enhanced fruit quality and resistance to biotic stresses:new varieties for the european market. *J. Fruit Ornam. Plant Res.*, *12*, 40.

Sediqi, A. S. (2025). Physiological changes in apple fruit in response to physical damage received during post-harvest handling practices. *ESRJ*, *1*(1), 144–163.

Siekierzynska, A., Piasecka-Kwiatkowska, D., Myszka, A. B., Sozanska, B., Sozanski, T. (2021). Apple allergy: Causes and factors influencing fruits Marta allergenic properties–Review. *ClinTransl Allergy*.

Stănică, Fl., Peticilă, A., Dumitrascu, M. (2008). Influence of weather conditions on apple flowering. Proceedings of International Scientific Conference, Jurmala-Dobele. *Sustainable Fruit Growing: From Plant to Product.*173-176.

Stopar, M., Bolcina, U., Vanzo A., Vrhovsek, U. (2002). Lower crop load for Cv. Jonagold apples (Malus x domestica Borkh.) increases polyphenol content and fruit quality. *Journal of agricultural and food chemistry*. Vol. 50/Issue 6, 1643-1646

Tang, C., He, H., Li, E., Li, H. (2018).Multispectral imaging for predicting sugar content of 'Fuji' apples. *Opt. Laser Technol.* 2018, *106*, 280–285.

Ugolik, M.,Kantorowicz-Bąk, M.,Ugolik, D.(1994). Evaluation of the Growth and Yield of Several New Apple Varieties on the M.9 Rootstock. In Proceedings of the 33 Polish National Scientific *Conference of Institute of Pomology and Floriculture, Skierniewice*, Poland, 30 August-1 September; pp. 86–87.

Włodarczyk, P. (1996) Selected Factors Affecting the Early Start Fruiting Apple Trees.*AkademiaRolnicza Lublin: Lublin*, Poland.

Yarilgaç, T., Uzun, S., Karakaya, O., Ates, U., Öztürk, B. (2023). Fruit Characteristics of Some Standard Apple Cultivar/Rootstock Combination in Ordu Ecology. *Journal of the Agricultural* Faculty of Gaziosmanpaşa University.

Zeroual, I., Benaziza, A., Adjal, F., Kheloufi, A. (2024). Evaluation of Morphological Characteristics in Some Apple Varieties *(Malus Pumila Mill.)* from Northeastern Algeria. The *Annals of "Valahia" University of Targoviste*.

*https://revista-ferma.ro/polonia-bate-mar-romania-la-productia-de-mere-cat-costa-kilogramul-la-noi-si-de-ce-vom-importa-si-mai-multe-fructe/

*https://stat.gov.pl/en/topics/agriculture-forestry/agricultural-and-horticultural-crops/.

*https://www.economica.net/romania-produce-11-din-merele-ue-insa-cantitatile-stagneaza-din-2022-nici-productia-de-pere-nu-a-fluctuat-exista-premisa-cresterii-preturilor_691515.html

*https://www.fao.org/statistics/en

*https://www.researchgate.net/publication/283329319_Titrimetric_Determination_of_Ascorbic_Acid_Contents_in_Plant_Samples_by_2_6Dichlorophenolindophenol_Method.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

BLOSSOMING PHENOPHASE AT SOME APRICOT AND PEACH CULTIVARS DEPENDING ON AIR TEMPERATURE IN SOUTH-EASTERN AREA OF ROMANIA

Leinar SEPTAR, Corina GAVĂȚ, Vladut Alexandru OPRIȚA, Cristina MOALE, Ion CAPLAN, Ioan STOLI

Research Station for Fruit Growing Constanța, 25 Pepinierei Street, Valu lui Traian, Constanța, Romania

Corresponding author email: septarleinar71@gmail.com

Abstract

Phenological phenophases (such as blossoming) are highly responsive to air temperature and are influenced by the dormancy period at fruit trees. The paper presents the ten-year results of the effect of air temperature on the flowering of four cultivars of apricot and four cultivars of peaches under the conditions of Valu lui Traian commune, Constanta county, Romania country. It was established that there were notable variations in the start, course, and length of flowering between the years. During the ten-year study period, the beginning of flowering was triggered at the earliest on March 13 in the year 2020 for the apricot (Tudor cultivar) and on March 20 in the year 2020 for the peach (Raluca cultivar). Apricot flowering lasted a maximum of 16 days (year 2019 - Olimp cultivar) and a minimum of 5 days (year 2020 -Tudor cultivar). In the case of peaches, flowering lasted a maximum of 22 days (year 2020 - Raluca cultivar) and a minimum of 6 days (year 2019 - Mimi cultivar). In years with an earlier flowering date, the differences in the start of flowering between of the cultivars were more noticeable.

Key words*: onset and end of blossoming, Prunus armeniaca, Prunus persica.*

INTRODUCTION

The region of Dobrogea is very famous for apricot and peach production. The south-eastern area with a specific climate and favourable agro-ecological conditions allow good results in apricot and peach cultivation and relatively stable yields. Despite the favourable conditions in the specified area, due to the early blossoming of diverse apricot and peach cultivars there are noteworthy contrasts within the course of the blossoming phenophase among the cultivars, as well as large differences in different years. Blossoming is one of the foremost phenophases for all fruit trees species. Studying the genotypes of apricots and peach when they bloom is also crucial for breeding, and effective production (Bellini, 2007). It is an essential element of the precise cultivar description (Ledbetter, 2008; Campoy et al., 2011). This phenophase is very important for apricot trees since they blossom early. One of the main reasons for the fluctuation in yields of apricot in the south-eastern area of Romania is the freezing of blossoms or unopened flower buds caused by low spring temperatures and late frosts (Moale C. and Asanica A. 2017). Flowering buds are more susceptible to frost because they have a shorter rest time and emerge from this state sooner, which is impacted by both winters, heavy frosts, and early spring when the trees have lost their hardening condition (Abrahma M.G. and Savage M.J. 2008). Plant growth and fructification can be positively or negatively impacted by global warming. The writings of a few authors included information about the consequences of climate change: Falloon P. and Betts R., 2010; Abrahma M.G. and Savage M.J., 2008; Challinor A.J. et al., 2007; Challinor A.J. et al., 2005. Phenology investigation can be used in horticulture to choose cultivars that are climatic accident-resistant, able to adjust to current changes in the climate, and not always cause harvest loss (Cosmulescu S. et al., 2015). In many regions of the world, the redistribution of species has already been caused by climate change. (Thuiller W. et al., 2005). Phenological phases can be used as markers of climate change because phenology and climate are closely associated (Kalvane et al., 2009). The regulation of phenological processes both prior to and during blooming is significantly influenced by

temperature (Lakatos et al., 2008; Rodrigo and Herrero, 2002).
Using four cultivars of apricot and four cultivars of peach, the work aims to analyse the impact of air temperature before and throughout the blossoming phase over a ten-year period.

MATERIALS AND METHODS

The investigations were carried at the Research Station for Fruit Growing Constanta, Romania, in the Valu lui Traian village, located at the 44°10' northern latitude and 28°28' eastern longitude. The area under discussion is around 13-14 km west of the Black Sea and has an average altitude of 72 m above sea level.
The experiment included the following cultivars for apricots: Elmar, Tudor, Olimp, Goldrich and for peaches: Raluca, Filip, Mimi, Redhaven. The apricot trees were grafted on the Constanta 14 rootstock and planted at a distance of 5 × 4 m (500 trees ha^{-1}) and the peach trees were grafted on the Tomis 1 rootstock and planted at a distance of 4 × 4 m (625 trees ha^{-1}). The canopy shape was a classic vase and standard cultural practices were applied. The air temperature was recorded by an automatic weather station (iMetos, IMT 300, Pessl Instruments, Austria) by a 1-h step. The study allowed finding some characteristics of the blossoming phenological phase (beginning, duration and end), respectively BBCH 61 - the date of the beginning of flowering, when 10% of the flowers opened, and BBCH 69 - the end date of flowering, when 90% of the flowers fell from the crown (Meier U., 2001).
To examine the relationship between Year of Calendar and beginning of blossoming date (DOY), the Pearson correlation coefficient was calculated. Pearson correlation analysis and scatter plot generation were performed using IBM SPSS Statistics version 26.

RESULTS AND DISCUSSIONS

The area has a semi-arid, continental climate with hot, dry summers, regular dry winds all year round, and mild winters that typically don't include any snow. For the 2013-2024 (November-April) period, the air temperature was monitored (mean air temperature at the height of 2 m, maximum and minimum air temperature, mean and absolute values). Compared to the multiannual monthly values of the mean air temperature, it was observed that the positive differences varied between 0°C (January 2015, 2020) and 4.0°C (April 2024), and the negative ones between -5.7°C (January 2017) and -0.2°C (December 2021). There were 39 positive differences and 27 negative differences, indicating a warming trend for the area where the studies were conducted (Figure 1). Mean values of the maximum air temperature varied between -0.2°C (January 2017) and 21.3°C (April 2018, 2024). Compared to the multiannual values of the mean maximum air temperature, 35 positive differences and 31 negative differences were obtained, which signifies a climate warming trend (Figure 2).
Mean values of the minimum air temperature varied between -7.6°C (January 2017) and 8.3°C (April 2024). Compared to the multiannual values of the mean minimum air temperature, 34 positive differences and 32 negative differences were obtained (Figure 3). Regarding the absolute values of the air temperature during the period under study, the highest temperature was recorded in April 2016, the 32.8°C, respectively, and the lowest was -17.6°C and was recorded in January of 2014 and 2017 (Figure 4).

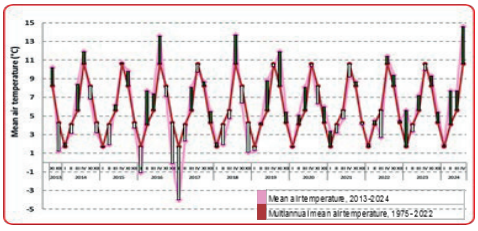


Figure 1. Mean air temperature (°C), 2013-2024 (November-April) comparative with multiannual mean air temperature (°C), 1975-2022 (November-April)

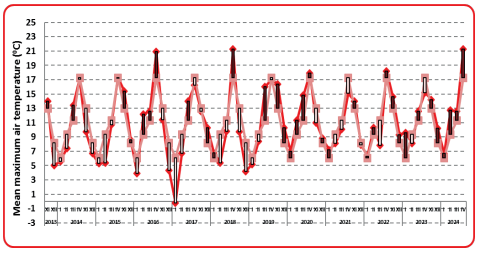


Figure 2. Mean maximum air temperature (°C), 2013-2024 (November-April) comparative with multiannual mean maximum air temperature (°C), 1975-2022 (November-April)

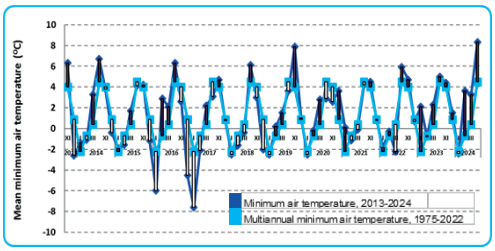


Figure 3. Mean minimum air temperature (°C), 2013-2024 (November-April) comparative with multiannual mean minimum air temperature (°C), 1975-2022 (November-April)

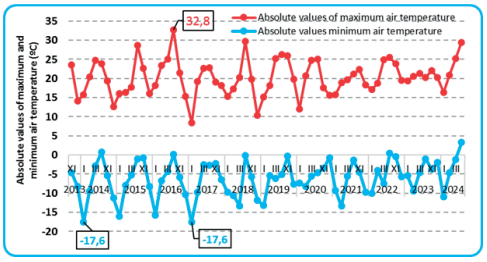


Figure 4. Absolute values of maximum and minimum air temperature (°C) in 2013-2024 (November-April) period

Characterization of the studied cultivars

Elmar is apricot cultivar that was patented in 2013. The tree vigor is medium, with a large habit. The blossoming time is late, very abundance. The cultivar is auto fertile. The ripening time is early. The fruit shape is oval with the average weight is between 45-55 g. The flesh is medium orange with red on the sunny part, good aroma and fine texture, non adherent to the stone. The recorded average yield is 18.7 t/ha. It can be used for fresh consumption or can industry.





Tudor is apricot cultivar breeded in RSFG Constanta; the tree vigor is big and recommended planting distance 5/5 m or 5/4 m; blossoming time: early, very abundance; the bearing is mainly on short fruiting; ripening time is early, 20 June to 10 July; the fruit shape is round, with orange and red skin, medium to big, firm, non-adherent at stone and the yield is high (18.7 t/ha); the stone has big size, round shape and bitter core. It is used for fresh consumption or for can industry (jam, compote, nectare).

Goldrich is an apricot cultivar of American origin. The ripening period is early, in the first half of July. It is medium to small tree and recommended planting distance 4/4m or 4/3.5 m. Blossoming is early and very abundant. The pulp is orange, with medium texture, juicy, non-adherent to the stone. The productive potential is high. Resistant to temperature oscillations during spring time.









Olimp is apricot cultivar breeded in RSFG Baneasa. The tree is of medium vigor, the inverse-conical crown, bears fruit predominantly on bunch branches (may bunches), with late flowering. The variety is partially self-fertile. The mass of a fruit is 65-75 g. The shape of the fruit is spherical-ovoid; the skin is orange with a little red on the sunny side, and the flesh is light orange with a firm texture. The ripening period is August 10-12. The cultivar is very productive. It is disease and frost resistant. Pollinators: Commander, Favorit, Selena, Sirena.





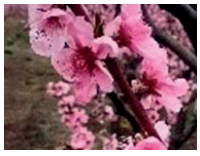
Raluca it is a standard peach that has medium vigor; the flower is a campanulate type and the blooming is abundant. Fruit shape is round, slightly asymmetrical with a little mucrone. The

fruit is Redhaven and Cardinal type; mean weight is 165 g, with yellow flesh, very juicy. The fruit will begin to ripen first week in July.



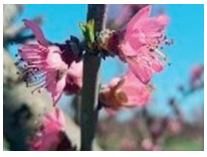


Filip it is one of the best peach cultivar with flat fruit obtained in Romania; it is not require pollinators. It is precocious and economically bearing since the 3rd year. The production is very high (23-25 t/ha for a density of 625 trees/ha, or 35-38 t/ha for 833 trees/ha) and constant. The fruit is flat, very atractive, red, with white flesh, superior taste and flavor which remember the fig and honey. Fruits destination: fresh consumption and processing.





Mimi it is a very good clingstone (pavia) cultivar, precocious with a high and constant yield (21-25 t/ha). The fruits have medium size (150-180 g), are very attractive, with orange and firm flesh, sweet (12.5-13.0% dry matter), flavoured. It has a middle ripening time. It is frost resistant and tolerant to *Taphrina deformans* and *Sphaerotheca pannossa*. It is resistent to transportation and storage better than the proper peaches. Destination: processing (jam and compote- sliced fruit) and fresh consumption.





Redhaven. This peach cultivar is a self-pollinating fruit tree, making it great for gardens and orchards. Since this tree is so vigorous, we recommend regular pruning and thinning. It is disease-resistant. The trees produce luscious, top-quality fruit, with medium-sized peaches. The flowers are pink and fragrant. The fruits ripen in July, its are sweet peaches with almost fuzzless skin over firm, creamy-textured yellow flesh. These peaches are great as a fresh snack or for canning and freezing.





In apricot, the beginning of blossoming took place at the earliest in 2020 for the Tudor cultivar (13 March) and at the latest in 2021 for the Olimp cultivar (10 April). The end of blossoming occurred at the earliest in 2014 for the Tudor cultivar (24 March) and at the latest in 2021 for the Olimp cultivar (23 April). The shortest duration of flowering was recorded in 2020 for the Tudor cultivar (5 days), and the longest duration of flowering was in 2019 for the Olimp cultivar (16 days) (Table 1).

Table 1. Phenophase of apricot blossoming in the period 2014-2023

Year	Cultivar	Blossoming phenophase		
		Beginning (BBCH 61)	End (BBCH 69)	Duration (days)
2014	Elmar	23 March	31 March	9
	Tudor	15 March	24 March	10
	Goldrich	19 March	28 March	10
	Olimp	26 March	02 April	8
2015	Elmar	26 March	05 April	11
	Tudor	17 March	28 March	12
	Goldrich	21 March	01 April	12
	Olimp	26 March	05 April	11
2016	Elmar	17 March	29 March	13
	Tudor	14 March	28 March	15
	Goldrich	14 March	28 March	15
	Olimp	19 March	02 April	15
2017	Elmar	25 March	31 March	7
	Tudor	18 March	28 March	11
	Goldrich	15 March	28 March	14
	Olimp	27 March	06 April	11
2018	Elmar	7 April	15 April	9
	Tudor	4 April	13 April	10
	Goldrich	4 April	15 April	12
	Olimp	6 April	13 April	8
2019	Elmar	19 March	01 April	14
	Tudor	19 March	02 April	15
	Goldrich	22 March	02 April	12
	Olimp	25 March	09 April	16
2020	Elmar	17 March	28 March	12
	Tudor	13 March	17 March	5
	Goldrich	16 March	29 March	14
	Olimp	25 March	06 April	13

Year	Cultivar	Blossoming phenophase		
		Beginning (BBCH 61)	End (BBCH 69)	Duration (days)
2021	Elmar	5 April	14 April	10
	Tudor	5 April	14 April	10
	Goldrich	5 April	13 April	9
	Olimp	10 April	23 April	14
2022	Elmar	1 April	09 April	9
	Tudor	1 April	06 April	6
	Goldrich	1 April	15 April	15
	Olimp	3 April	14 April	12
2023	Elmar	25 March	02 April	9
	Tudor	24 March	03 April	11
	Goldrich	20 March	30 March	11
	Olimp	27 March	08 April	13

A group of values is plotted in increasing order from left to right in Figure 5, indicating a directly proportional relationship between the beginning of the blossoming date (DOY) and the calendar year. The results indicate a positive, moderate and statistically significant correlation between Year of Calendar and DOY (r = 0.404**, p = 0.010). This correlation suggests a trend of delayed flowering over the years during the period studied (2014-2023).

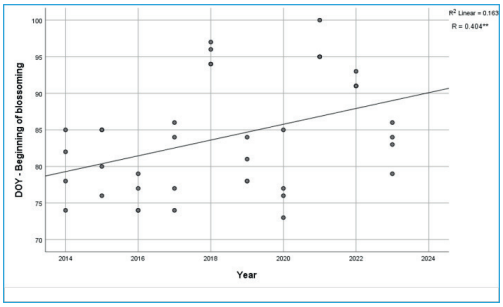


Figure 5. Directly proportional relationship between beginning of blossoming date (DOY) and calendar year of apricot

In peach, the beginning of blossoming took place at the earliest in 2020 for the Raluca cultivar (20 March) and the latest in 2015 for the Mimi cultivar (13 April).

The end of blossoming occurred the earliest in 2014 for the Raluca cultivar (03 April) and the latest in 2015, 2018, 2021 and 2022 for the Mimi cultivar (21 April).

The shortest flowering period was recorded in 2019 for the Mimi cultivar (6 days), and the longest flowering period was in 2020 for the Raluca cultivar (22 days) (Table 2).

Table 2. Phenophase of peach blossoming in the period 2014-2023

Year	Cultivar	Blossoming phenophase		
		Beginning (BBCH 61)	End (BBCH 69)	Duration (days)
2014	Raluca	23 March	03 April	12
	Filip	25 March	05 April	12
	Redhaven	27 March	07 April	12
	Mimi	28 March	09 April	13
2015	Raluca	08 April	18 April	11
	Filip	09 April	18 April	10
	Redhaven	12 April	20 April	9
	Mimi	13 April	21 April	9
2016	Raluca	24 March	05 April	13
	Filip	25 March	05 April	12
	Redhaven	27 March	07 April	12
	Mimi	29 March	09 April	12
2017	Raluca	10 April	17 April	8
	Filip	05 April	19 April	15
	Redhaven	05 April	17 April	13
	Mimi	07 April	20 April	14
2018	Raluca	10 April	17 April	8
	Filip	11 April	19 April	9
	Redhaven	07 April	19 April	13
	Mimi	10 April	21 April	12
2019	Raluca	01 April	08 April	8
	Filip	01 April	08 April	8
	Redhaven	03 April	09 April	7
	Mimi	06 April	11 April	6
2020	Raluca	20 March	10 April	22
	Filip	22 March	10 April	20
	Redhaven	25 March	11 April	18
	Mimi	29 March	11 April	14
2021	Raluca	07 April	15 April	9
	Filip	07 April	16 April	10
	Redhaven	10 April	19 April	10
	Mimi	12 April	21 April	10
2022	Raluca	05 April	12 April	8
	Filip	07 April	15 April	9
	Redhaven	10 April	18 April	9
	Mimi	07 April	21 April	15
2023	Raluca	25 March	07 April	14
	Filip	29 March	08 April	11
	Redhaven	02 April	12 April	11
	Mimi	04 April	14 April	11

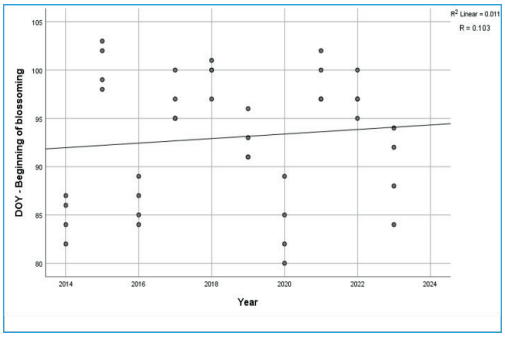


Figure 6. Directly proportional relationship between beginning of blossoming date (DOY) and calendar year of peach

There is a very weak and positive linear correlation (r = 0.103) between the calendar year and the peach blooming date (Figure 6). This suggests that, as the years pass, there is a very slight tendency for the peach tree to bloom later, but this trend is extremely weak.

CONCLUSIONS

The apricot and peach tree, thermophile plants, has found favorable conditions for growth and development in south-eastern area, where the mean annual temperatures meet the special requirements.

The moderate and significant correlation (r = 0.404**, p = 0.010) suggests that external factors, likely climatic changes, may influence apricot blooming patterns.

The variation in flowering duration among cultivars indicates genetic differences in response to environmental conditions.

The observed trend could impact pollination synchronization and fruit set, potentially requiring adjustments in orchard management.

The very weak positive correlation (r = 0.103) suggests that peach flowering remains relatively stable over time, indicating a lower sensitivity to annual climatic variations.

The variability between cultivars highlights the importance of genetic traits in determining blooming time.

ACKNOWLEDGEMENTS

The authors thank financial support from Romanian Ministry of Agriculture and Rural Development (Project: ADER 6.3.3. *Updating the zonation of fruit tree species in relation to climate change*) and Romanian Academy of Agricultural and Forestry Sciences "Gheorghe Ionescu-Şişeşti" (Project no. 7940, *The adaptation of some technological solutions to reduce the negative impact of climate change on some stone species from the south-east of the country in order to increase profitability and ensure environmental protection*).

REFERENCES

Abrahma M.G., M.J. Savage (2008). Comparison of estimates of daily solar radiation from air temperatue range for application in crop simulation. *Agr. Forest Meteorol*., 148, 401–416

Bellini, E. (2007). The Fruit Woody Species, *ARSIA*: Firenze, Italy, Volume 1–2, 1069 p

Campoy, J.A., Ruiz, D., Egea, J. (2011). Dormancy in temperate fruit trees in a global warming context: A review. *Sci. Hortic*., 130, 357–372

Challinor, A.J., T.R. Wheelar, P.Q. Craufurd, C.A.T. Fero and D.B. Stephenson (2007). Adapttion of crops to climate change through genotypic responces to mean and extreme temperatures. *Agr. Ecosys. Environ*., 119, 190–204

Challinor, A.J., T.R. Wheelar, P.Q. Craufurd and J.M. Slingo (2005). Simulation of the impact of hight temperature stress on annual crop yields. *Agr. Forest Meteorol*, 135, 180–189

Cosmulescu, S., Baciu, A., Gruia, M. (2015). Influence of climatic factors on the phenology spring in Southern Oltenia (Romania). *Journal of Horticulture, Forestry and Biotechnology*, 19(1), 147- 157

Falloon, P. and R. Betts (2010). Climate impact in European agriculture and water management in the context af adaptation and mitigation–The importance of an integrated approach. *Sci. Total Environ*. 408(23), 5667–5687

Kalvane, G., Romanovskaja, D., Briede, A., Bak$iene, E. (2009). Influence of climate change on phenological phases in Latvia and Lithuania. *Climate Research*, Vol. 39, 209–219

Lakatos, L.; Nyéki, J.; Soltész, M.; Szabó, Z.; Racskó, J. Effect of meteorological variables on the blooming time. In Morphology, Biology and Fertility of Flowers in Temperate Zone Fruits; Nyéki, J., Soltész, M., Szabó, Z., Eds.; *Akadémiai Kiadó*: Budapest, Hungary, 2008; pp. 117–140

Ledbetter, C.A. (2008). Apricots. In Temperate Fruit Crop Breeding; Hancock, J.H., Ed.; *Springer Science and Business Media B.V.*: Dordrecht, The Nederlands, 39–82

Moale, C. and Asanica, A. (2017). The effect of certain climatic parameters on the apricot tree. *Scientific Papers. Series B, Horticulture.* Vol. LXI, 69-79

Rodrigo, J.; Herrero, M. (2002). Effects of pre-blossom temperatures on flower development and fruit set in apricot. *Sci. Hortic*. 92, 125–135

Thuiller, W., Lavorel S., Araújo, M.B., Sykes, M.T., Prentice, I.C. (2005). Climate change threats to plant diversity in Europe. *Proceedings of the National Academy of Sciences of the United States of America* 102(23), 8245–8250.

Uwe Meier (2001). Growth stages of mono-and dicotyledonous plants. BBCH Monograph. *Federal Biological Research Centre for Agriculture and Forestry*, 158 p.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

NITROGEN CONTENT IN APPLE LEAVES UNDER VARIOUS FERTIGATION TREATMENTS IN YOUNG, SEMI-YOUNG AND OLD LEAVES

Antun ŠOKEC[1], Goran FRUK[2], Tomislav KARAŽIJA[1], Martina SKENDROVIĆ BABOJELIĆ[2], Mihaela ŠATVAR VRBANČIĆ[1], Kristijan KONOPKA[1], Boris LAZAREVIĆ[1], Marko PETEK[1]

[1]University of Zagreb, Faculty of Agriculture, Department of Plant Nutrition, 25 Svetošimunska cesta, Zagreb, Croatia
[2]University of Zagreb, Faculty of Agriculture, Department of Pomology, 25 Svetošimunska cesta, Zagreb, Croatia

Corresponding author email: gfruk@agr.hr

Abstract

Nitrogen (N) deficiency in apple (Malus domestica Borkh.) leads to typical physiological changes (discoloration of leaves and fruits). Fertigation can significantly increase apple growth, quality and optimize nutrient uptake. The aim of this study was to evaluate how various fertigation treatments affect the N content in apple leaves. The hydroponic cultivation of apple, variety 'Braeburn' was carried out on low vigorous rootstock (M9) in the substrate perlite. The experiment included four fertigation treatments: 1) Hoagland's solution (HS) full solution-control; 2) HS nitrogen excluded; 3) HS iron excluded; 4) HS magnesium excluded. Plant sampling was carried out in June 2024 on three positions of the shoots, based on the leaves age (young, semi-young and old leaves) followed by chemical analyses. Statistically the lowest leaves N content was detected in the young, semi-young and old leaves (1.69, 1.81 and 1.80% N, respectively) in the treatment without N, while the highest N content was detected in the treatments without iron and magnesium (2.07-2.49% N). The N content in the apple leaves varies depending on the type of fertigation treatment.

Key words*: fruit, hydroponic system, macroelement, mineral, rootstock.*

INTRODUCTION

The apple (*Malus domestica* Borkh.) is a woody perennial plant that has been cultivated for hundreds of years. Its fruit is one of the most widely cultivated and consumed fruits in the world (Cornille et al., 2012; Spengler, 2019).
Grown primarily for its fruit, the apple is known for its refreshing flavor and crisp texture, making it a popular choice for both fresh consumption and processed forms such as juice, cider, and dried snacks (Hyson, 2011).
Apples come in many varieties, each with their own balance of sweetness and tartness (Harker et al., 2002). Aside from their flavor, apples are highly nutritious and offer numerous health benefits (Boyer & Liu, 2004; USDA, 2023).
The origin of the apple can be traced back to the wild apple *Malus sieversii*, which is believed to have originated in the Caucasus region, which includes present day Turkey, Georgia, Armenia and Iran. These wild ancestors are considered to be the ancestors of today's apple varieties (Peil et al., 2011; Volk et al., 2021).
In 2023, apples were grown worldwide on an area of 46,182,85 hectares (ha) and a yield of over 973,393,38 tons (t) was achieved (FAO, 2023).
Nutrient deficiencies in apple trees can have a negative impact on yield, quality and resistance to pests and diseases, reducing the overall productivity and profitability of orchards. Apple trees often suffer from deficiencies in key macronutrients such as nitrogen (N), phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg) (Licina et al., 2021). These deficiencies often manifest themselves through visible symptoms such as leaf discoloration, stunted growth, reduced fruit size and increased susceptibility to pathogens. Identifying and correcting nutrient deficiencies in apple orchards is critical to maintaining optimal tree health, maximizing fruit yield and ensuring the

production of high-quality apples (Kowalczyk et al., 2022).

Nitrogen is a crucial element in plant metabolism, involved in the formation of amino acids, nucleic acids, proteins, biochemistry of coenzymes, photosynthetic pigments and polyamines (Lešić et al., 2016; Javornik et al., 2023). Although it makes 78.1% of the Earth's atmosphere in the form of a molecule (N_2), most plants cannot utilize N directly due to the stability of the triple bond between two N atoms. The conversion of molecular N into forms of ammonium and nitrate ions (NH_4^+ and NO_3^-, respectively) which are accessible to plants in the soil, requires a considerable amount of energy (Vukadinović & Vukadinović, 2011; Kabange et al., 2022).

The dry matter of plants usually contains between 2 and 5% N. Plants are important stores of N and continuously convert it from mineral to organic forms during vegetation and incorporate it into their tissues. However, due to limitations in mobilization from organic reserves, rapid mobility of NO_3^- and N losses, availability of N often becomes a key factor limiting plant growth and yield (Vukadinović & Vukadinović, 2011; Grzyb et al., 2021; Postma et al., 2021).

It is noteworthy that about 70% of all cations and anions taken up by the roots are in the form of NH_4^+ or NO_3^- ions. This fact has a strong influence on the ratio of uptake of cations and anions of other elements in plant nutrition (Vukadinović & Vukadinović, 2011; Zhu et al., 2021).

Nitrogen deficiency leads to slower overall plant growth and a smaller assimilation area, resulting in shorter, narrower and pale green leaves. Chlorophyll deficiency reduces the intensity of photosynthesis, accelerates plant aging and reduces yield. Nitrogen deficiency often limits plant growth as it easily returns to its molecular state and is thus rapidly lost from the soil (Bergmann, 1992; Vukadinović & Vukadinović, 2011).

An excess of N leads to the formation of a large leaf mass of dark green color. The plants become susceptible to diseases, pests, low temperatures and drought. The structure of the plants becomes brittle, which can lead to encrustation of the vegetation due to the increased formation of parenchyma tissue. The quality and taste of the vegetables are low due to reduced sugar synthesis, and it becomes more difficult to maintain freshness (Lešić et al., 2016; Fathi, 2022). On light and permeable soils, excess of N can lead to leaching of nitrates and pollution of groundwater (Vukadinović & Vukadinović, 2011).

According to Resh (2022), fertigation is a system used in hydroponics that maximizes nutrient uptake by applying vital nutrients such as N, P and K directly into the root zone of plants. Fertigation reduces the risk of nutrient deficiencies, making it an environmentally friendly method for growing apples. To avoid imbalances and ensure healthy growth of apple trees, fertigation schedules and nutrient solutions must be properly managed (Dominguez & Robinson, 2024).

The aim of this work is to evaluate the effects of different fertigation treatments on the N content in apple leaves depending on their age.

MATERIALS AND METHODS

The plant material for this study was collected from the apple orchard located at the University of Zagreb Faculty of Agriculture, in Zagreb, Croatia (longitude 45°49'36" S and latitude 16°01'47" E).

The apple variety 'Braeburn' was grown in hydroponics on a low vigorous rootstock – M9 in a substrate perlite. The cultivation of apples was carried out under four fertigation treatments: 1. Hoagland solution, full solution (control); 2. Hoagland solution, nitrogen excluded; 3. Hoagland solution, iron excluded; 4. Hoagland solution, magnesium excluded.

Hoagland solution is a nutrient-rich liquid used for the hydroponics of plants. It is prepared by mixing concentrated stock solutions of essential macro (such as KNO_3, $Ca(NO_3)_2$, $MgSO_4$, and KH_2PO_4) and micronutrients (including boron, manganese, zinc, copper and molybdenum) and a source of iron (Fe-EDTA). For the preparation, small amounts of the individual components (stock) are added to distilled water and the pH is adjusted to around 5.8-6.2. The solution provides all the nutrients that plants need for healthy growth in soil-free systems (Trejo et al. 2012).

The apple leaves were sampled in three replicates, during the 2024 growing season and depending on the different leaf position on the shoot (young, semi-young and old leaves).

The samples were submitted to the Analytical Laboratory of the Department of Plant Nutrition at the University of Zagreb Faculty of Agriculture. Homogenized samples were dried at 105 °C and grounded. Nitrogen (N) in the apple leaves was determined using the Modified Kjeldahl method (HRN ISO 11261:2004) and expressed as % N in dry weight.

The statistical software SPSS (version 22.0; SPSS Inc., Chicago, IL) was used for the statistical analysis. One-way ANOVA was used to determine the effect of the different fertigation treatments on N content in young, semi-young and old leaves. When significant differences between means, were observed, additional post hoc assessment was performed using Tukey's Test ($p < 0.05$).

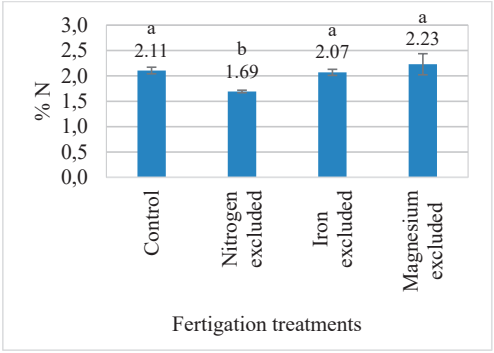
RESULTS AND DISCUSSIONS

Figure 1 shows the N content in young leaves under different fertigation treatments, determined in a range from 1.69 to 2.23 % N. The statistically significant highest N contents were found in the treatments: iron excluded, control and magnesium excluded (2.07, 2.11 and 2.23% N, respectively). Overall, the statistically lowest N content was found in treatment where N was excluded (1.69% N).

The N content in semi-young leaves under various fertigation treatments is displayed in Figure 2 and ranges from 1.81 to 2.30% N. Iron excluded and magnesium excluded treatments had the statistically significant highest N contents (2.27 and 2.30% N, respectively). Furthermore, the treatment where N was excluded had the statistically lowest N content (1.81% N).

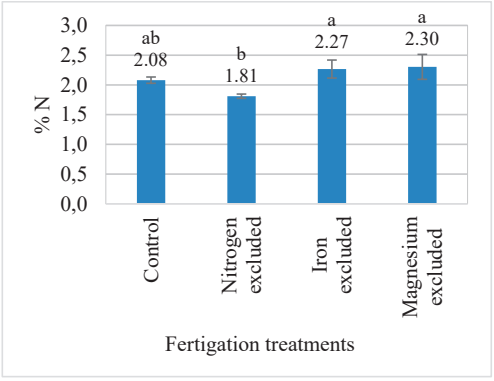
The N content of old leaves under various fertigation treatments ranges from 1.80 to 2.49 % N, as shown in Figure 3. The treatment with iron excluded had the statistically highest N content (2.49% N), while the treatment with N excluded had the statistically lowest N content (1.80% N).

Figure 4 shows dynamics of N content in young, semi-young and old leaves under the fertigation treatment in which N was excluded. The N content was determined in a range from 1.69 to 1.81% N. The statistically significant highest N contents were determined in the semi-young and old leaves (1.81 and 1.80% N, respectively), while the statistically lowest was found in the young leaves (1.69% N).



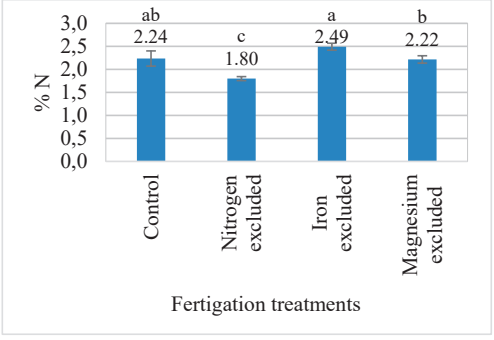
Different letters represent significantly different values according to Tukey's test, p≤0.05. The non-letter values are not significantly different

Figure 1. Nitrogen content (% N) in young leaves under different fertigation treatments



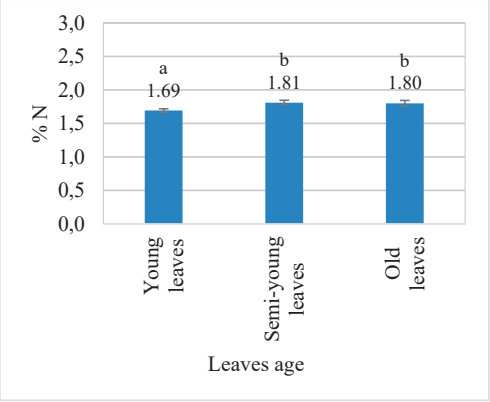
Different letters represent significantly different values according to Tukey's test, p≤0.05. The non-letter values are not significantly different.

Figure 2. Nitrogen content (% N) in semi-young leaves under different fertigation treatments



Different letters represent significantly different values according to Tukey's test, p≤0.05. The non-letter values are not significantly different.

Figure 3. Nitrogen content (% N) in old leaves under different fertigation treatments



Different letters represent significantly different values according to Tukey's test, p≤0.05. The non-letter values are not significantly different.

Figure 4. Nitrogen content (% N) in young, semi-young and old leaves in treatment with nitrogen excluded

Apple production worldwide can be limited by a number of causes, but water and N deficiency are particularly important (Haotian et al., 2023). The N content in plant leaves depends on the interaction of different macro and micronutrients which are crucial for the assimilation and utilization of N as they are contained in fertilizers. Nutrients influence N uptake (Marschner, 2012) and metabolism (Song et ač., 2025) depending on their concentration and type of application Therefore, balanced fertilization is crucial for optimizing N assimilation and establishing normal concentration in plants (Anas et al., 2020; Kowalczyk et al., 2022; Song et al., 2025). The distribution of N in apple leaves changes with age, with N gradually being transported to younger leaves, fruit or storage tissue. Transport leads to a gradual decrease in N content in older leaves (Marschner, 2012). Additionally, changes in N concentration in leaves can be influenced by season, vegetation stage, stress conditions, correct/incorrect fertilization, irrigation and the influence of other micro and macroelements (Marschner, 2012; Treder, 2022).

According to Kowalczyk et al. (2017) and MSU (2025) over-fertilization can lead to an excess of N in young leaves, while under-fertilization can lead to a deficiency in older leaves. Therefore, proper fertilization is very important for maintaining optimal levels across all leaf ages.

Old leaves, from which samples are often taken for nutrient analysis, usually contain N concentration between 1.80 and 2.50% N, which is a reliable indicator of the overall N status of the tree (Tagliavini & Marangoni, 2002; Marschner, 2012).

In this study, the N content in old leaves was determined in a range from 1.80 to 2.49% N under different fertigation treatments. The data obtained in the literature on the N content in old leaves are consistent with the data obtained in this study.

Additionally, as leaves age, their N content can even drop below 1.50% N, especially during the fruit development period. In addition, older leaves may show visible signs of deficiency by yellowing (chlorosis) as N is remobilized to support new growth or fruit production (Zheng Tan et al., 2021). On the other hand, semi-young leaves that are in the middle of the current year's shoots typically contain higher N concentrations than old leaves, ranging from 1.80 to 3.0 % N (Tagliavini & Marangoni, 2002; Marschner, 2012). In this study, the N content in semi-young leaves was determined in a range from 1.81 to 2.30% N under different fertigation treatments. The results of this study corroborate the existing literature about the nitrogen concentration of semi-young leaves.

Younger leaves, which are at the tip of the current year's shoots, typically contain higher N concentrations than older leaves, which are between 1.80 to 3.50% N as well as stated by MSU. This is due to the plant's physiological processes in which N is mobilized to support the growth of new leaves. Cheng and Fuchigami (2002) conducted a study in which they found that about 50% of the tree's N content was remobilized to support the growth of new shoots and leaves, highlighting the importance of N in the development of young leaves.

One of the studies investigated how the timing of N application affects the uptake, distribution and remobilization of N in 'Gala' apples. Zheng Tan et al., (2021) found that a pre-harvest N application resulted in significantly higher N content in the leaves, especially between 7 and 20 weeks after full bloom, than a post-harvest application. This suggests that young leaves respond more actively to early fertilization, making the timing of nutrient application a key factor in optimizing N use.

In this study, the N content in young leaves was determined in a range from 1.69 to 2.23% N

under different fertigation treatments. The data obtained in this study for the N content in the young leaves are within the range reported in the literature, except for the treatment in which no N fertilization was applied. In this treatment, a lower N content (1.69% N) was determined in the young leaves than reported in the literature.

Several explanations for the lower N content in young apple leaves compared to semi-young and old leaves can be found in the literature. Environmental factors such as drought (Xu & Zhou, 2006), excessive rainfall (Cameron et al., 2013) or extreme temperatures (Lawlor & Cornic, 2002) can disrupt N uptake and translocation which can lead to a decreased concentration of N in young leaves. These stress factors impair root function, limit nutrient uptake and alter the plants ability to distribute N efficiently, highlighting the complex interplay between environmental conditions and N dynamics in plants (Tagliavini & Marangoni, 2002; Fallahi et al., 2010).

Furthermore, Masclaux-Daubresse et al. (2010) discuss how N is remobilized from leaves into developing tissues, including young leaves and reproductive organs, during N deficiency. This process can lead to reduced N content in leaves as the plant redistributes resources to support critical growth areas.

In addition, imbalances in other nutrients, such as high levels of P or K, can interfere with N uptake and utilization, especially in young leaves, further affecting plant health and growth. These results emphasize the importance of maintaining a balanced nutrient content in plants to ensure optimal N supply (Tagliavini & Marangoni, 2002).

CONCLUSIONS

An analysis of apple leaves revealed that the lowest N content was observed in the young, semi-young, and old leaves (1.69, 1.81, and 1.80% N, respectively) in the fertigation treatment with Hoagland solution where N was excluded. In contrast, the highest concentration of N was found in treatments where iron and magnesium excluded were excluded, with values ranging from 2.07 to 2.49% N. The N content in apple leaves varies according to the type of fertigation treatment applied. It was found that the treatment where N was excluded had the statistically lowest concentration of N in the young leaves. Future research should examine how the dynamics of N distribution vary on leaf age over several
growing seasons and under different fertigation treatments.

REFERENCES

Anas, M., Liao, F., Verma, K. K., Sarwar, M. A., Mahmood, A., Chen, Z.-L., Li, Q., Zeng, X.-P., Liu, Y., Li, Y.-R. (2020). Fate of nitrogen in agriculture and environment: agronomic, eco-physiological and molecular approaches to improve nitrogen use efficiency. *Biological Research, volume 53*, Article number: 47.

Bergmann, W. (1992). *Nutritional disorders of plants: development, visual and analytical diagnosis*. Gustav Fischer.

Boyer, J., & Liu, R. H. (2004). Apple phytochemicals and their health benefits. *Nutrition Journal, 3*(1), 5.

Cameron, K. C., Di, H. J., & Moir, J. L. (2013). Nitrogen losses from the soil/plant system: A review. *Journal of Environmental Quality, 42*(2), 407 417. https://doi.org/10.2134/jeq2012.0420

Cheng, L. & Fuchigami, L.H. (2002). Growth of young apple trees in relation to reserve nitrogen and carbohydrates. *Tree Physiol. 22*(18):1297-303. doi: 10.1093/treephys/22.18.1297.

Cornille A, Gladieux P, Smulders MJ, Roldán-Ruiz I, Laurens F, Le Cam B, Nersesyan A, Clavel J, Olonova M, Feugey L, Gabrielyan I, Zhang XG, Tenaillon MI, & Giraud T. (2012). New insight into the history of domesticated apple: secondary contribution of the European wild apple to the genome of cultivated varieties. *PLoS Genet, 8(*5). doi: 10.1371/journal.pgen.1002703.

Dominguez, L. I., & Robinson, T. L. (2024). Benefits of Irrigation or Fertigation on Early Growth and Yield of a High-density Apple Planting in a Humid Climate. *HortTechnology, 34*(6), 747-760. Retrieved Mar 2, 2025, https://doi.org/10.21273/HORTTECH05497-24.

Fallahi, E., Neilsen, D., & Neilsen, G. H. (2010). Fertilizer management in apple orchards: Implications for fruit quality and yield. *HortTechnology, 20*(1), 148-154.

FAO. (2023). World Food and Agriculture – Statistical Yearbook 2023. Rome. https://doi.org/10.4060/cc8166en

Fathi, A. (2022). Role of nitrogen (N) in plant growth, photosynthesis pigments, and N use efficiency: a. *Agrisost, 28*, pp.1-8.

Grzyb, A., Wolna-Maruwka, A., & Niewiadomska, A., (2021). The significance of microbial transformation of nitrogen compounds in the light of integrated crop management. *Agronomy, 11*(7), p.1415.

Haotian, S., Liyao, H., Xinpeng, X., Yuanjun, Z., Bingnian Z., & Zhanjun L. (2023). Effects of different rates of nitrogen fertilizer on apple yield, fruit quality, and dynamics of soil moisture and nitrate in soil of rainfed apple orchards on the Loess Plateau, China,

European Journal of Agronomy, 150, 126950. https://doi.org/10.1016/j.eja.2023.126950.

Harker, F. R., Marsh, K. B., Young, H., Murray, S. H., Gunson, F. A., & Walker, S. B. (2002). Sensory interpretation of instrumental measurements 2: Sweet and acid taste of apple fruit. *Postharvest Biology and Technology, 24*(3), 241-250.

Hyson D. A. (2011). A comprehensive review of apples and apple components and their relationship to human health. *Adv Nutr., 2*(5), 408-20. doi: 10.3945/an.111.000513.

Javornik, T., Carović-Stanko, K., Gunjaca, J., Vidak, M., & Lazarević, B. (2023). Monitoring Drought Stress in Common Bean Using Chlorophyll Fluorescence and Multispectral Imaging. *Plants. 12*. 1386. 10.3390/plants12061386.

Kabange, N. R., Lee, S. M., Shin, D., Lee, J. Y., Kwon, Y., Kang, J. W., Cha, J. K., Park, H., Alibu, S. & Lee, J. H. (2022). Multiple Facets of Nitrogen: From Atmospheric Gas to Indispensable Agricultural Input. *Life, 12*(8), p.1272.

Kowalczyk, W., Wrona, D., & Przybyłko, S. (2022). Effect of Nitrogen Fertilization of Apple Orchard on Soil Mineral Nitrogen Content, Yielding of the Apple Trees and Nutritional Status of Leaves and Fruits. *Agriculture, 12*(12), 2169. https://doi.org/10.3390/agriculture12122169.

Kowalczyk, W., Wrona, D., Przybyłko, S. (2017). Content of minerals in soil, apple tree leaves and fruits depending on nitrogen fertilization. *J. Elem., 22*(1): 67-77. DOI: 10.5601/jelem.2016.21.1.1011

Lawlor, D. W., & Cornic, G. (2002). Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. *Photosynthesis Research, 73*(1-3), 121-136. https://doi.org/10.1023/A:1020356403476.

Lešić, R., Borošić, J., Butorac, I., Herak Ćustić, M., Poljak, M., & Romić, D. (2016). *Povrćarstvo,* III. dopunjeno izdanje. Zrinski, Čakovec.

Licina, V., Krogstad, T., Simić, A., Akšić, M. F. & Meland, M. (2021). Nutrition and fertilizer application to apple trees-a review. *NIBIO Rapport*.

Marschner, H. (2012). *Mineral Nutrition of Higher Plants* (3rd ed.). Academic Press.

Masclaux-Daubresse, C. Daniel-Vedele, F., Dechorgnat, J., Chardon, F., Gaufichon, L., Suzuki, A. (2010). Nitrogen uptake, assimilation and remobilization in plants: challenges for sustainable and productive agriculture. *Annals of Botany, 105* (7), pp. 1141–1157, https://doi.org/10.1093/aob/mcq028

MSU - Michigan State Universit; MSU Extension, Apples. by Hanson, e. *Apple nutrition*. Available online: https://www.canr.msu.edu/uploads/files/Applenutrition-EricHanson.pdf (Accessed on 20 May 2025).

Peil, A., Kellerhals, M., Höfer, M., & Flachowsky, H., (2011). Apple breeding - from the origin to genetic engineering. *Fruit Veg Cereal Sci Biotechnol, 5*, pp.118-138.

Postma, J. A., Hecht, V. L., Hikosaka, K., Nord, E. A., Pons, T. L., & Poorter, H., (2021). Dividing the pie: A quantitative review on plant density responses. *Plant, Cell & Environment, 44*(4), pp.1072-1094.

Resh, H. M. (2022). *Hydroponic Food Production: A Definitive Guidebook for the Advanced Home Gardener and the Commercial Hydroponic Grower*. CRC Press.

Song, X-L., Wang, Z-J., Yin, X-W., Sun, Y-L., Jang, D-J., Hong, S-K. (2025). The impact of nitrogen deposition on nitrogen metabolism in ryegrass lawn with different soil nutrient levels. *Scientific Reports , 15,* Article number: 16755

Spengler, R. N. (2019). Origins of the apple: The role of megafaunal mutualism in the domestication of Malus and rosaceous trees. *Frontiers in Plant Science, 10*. Frontiers Media S.A. https://doi.org/10.3389/fpls.2019.00617.

Tagliavini, M., & Marangoni, B. (2002). Major nutritional issues in deciduous fruit orchards in Northern Italy. *HortTechnology, 12*(1), 26-31.

Treder, W., Klamkowski, K., Wójcik, K., Tryngiel-Gać, A., Sas-Paszt, L., Mika, A. & Kowalczyk, W. (2022). Apple leaf macro-and micronutrient content as affected by soil treatments with fertilizers and microorganisms. *Scientia Horticulturae, 297*, p.110975.

Trejo, T., Libia, I. & Gómez-Merino, F. (2012). *Nutrient Solutions for Hydroponic Systems*. 10.5772/37578.

USDA (United States Department of Agriculture) (2023). National Nutrient Database for Standard Reference.

Volk, G., Cornille, A., & Durel, C. E. (2021). *Botany, Taxonomy, and Origins of the Apple*. 10.1007/978-3-030-74682-7_2.

Vukadinović, V., & Vukadinović, V. (2011). *Ishrana bilja* (Vol. 3). Poljoprivredni fakultet u Osijeku.

Xu, Z., & Zhou, G. (2006). Combined effects of water stress and high temperature on photosynthesis, nitrogen metabolism, and lipid peroxidation of a perennial grass Leymus chinensis. *Plant and Soil, 284*(1-2), 255-264.

Zheng Tan, B., Close, D. C., Quin, P.R., Swarts, N.D. (2021). Nitrogen use efficiency, allocation, and remobilization in apple trees: uptake is optimized with pre-harvest N supply. *Front. Plant Sci. Sec. Plant Nutrition. 12,* https://doi.org/10.3389/fpls.2021.657070

Zhu, Y., Qi, B., Hao, Y., Liu, H., Sun, G., Chen, R. & Song, S., (2021). Appropriate NH4+/NO3–ratio triggers plant growth and nutrient uptake of flowering Chinese cabbage by optimizing the pH value of nutrient solution. *Frontiers in Plant Science, 12*, p.656144.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

FLORAL BIOLOGY OF SOME AUTOCHTHONOUS SOUR CHERRY GENOTYPES FROM THE GERMPLASM FUND

Ioana Adelina STAN (ZOICAN)[1, 2], Mădălina BUTAC[1], Dorel HOZA[2]

[1]Research Institute for Fruit Growing Pitești - Mărăcineni, 402 Mărului Street, Mărăcineni, Piteşti, Argeş, Romania
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăști Blvd, District 1, Bucharest, Romania

Corresponding author email: adelinazoican@yahoo.com

Abstract

Knowledge of flower biology has a significant importance, particularly in sour cherry which shows different degrees of incompatibility. The main objective of this paper is to study some aspects regarding floral biology of 42 autochthonous sour cherry genotypes: beginning, ending and intensity of flowering, self-fertility, as well as the viability and germination capacity of pollen. The phenological observations were made noting the date on 1-5% of flowers opened (beginning of flowering) and when 95-100% of flowers fell (end of flowering). The degree of self-fertility was determined by isolating 100 flowers and counting the fruits set. The pollen viability was determined in acetic carmine. The pollen germination capacity was determined in vitro on solid medium (15% sucrose, 1.5 g agars, 0.01 g boric acid in 100 ml distilled water), under laboratory conditions (temperature 18-20ºC and relative humidity of 70-90%). In 2024, the average date of beginning of flowering for the 42 sour cherry genotypes was April 6, varying from April 3 at 'Drobeta', 'Turcești', 'Mari timpurii', 'Mocănești 16', 'Timpurii de Pitești' cvs. and April 9 at the 'Bucovina', 'Crișana 2' and clones, 'Dropia', 'Ilva', 'Nana', 'Stelar', 'Vrâncean', 'Mărculești' and his clones. Average flowering duration was 9 days and average flowering intensity was medium to high (notes 3.5-4). The average value of pollen viability was 78.47%, varying from 91.85% at 'Selecție M' genotype and 37.89% at 'Mari timpurii cl. 11' genotype. The average value of pollen germination was 18.01%, varying from 38.55% at 'Rival' cv. and 4.87% at 'Topologu Tulcea' cv. Regarding self-fertility, the sour cherry genotypes studied were classified into 3 groups: self-fertile (e.g. 'Bucovina', 'Ilva', 'Nana', 'Rival', 'Suraia Vrancea' cvs. - over 10% fruits set); partially self-fertile (e.g. 'Mocănești 16', 'Scuturător', 'Stelar', 'Timpurii de Osoi', 'Brezniţa' cvs. - 5-10% fruits set) and self-sterile (e.g. 'Dropia', 'Ţarina', 'Vrâncean', 'Crișana 2', 'Locale de Bistriţa' cvs. – below 5% fruits set). The results regarding floral biology in sour cherry provide information regarding the use of a variety as a pollinator in commercial orchards, as well as a parent in breeding works.

Key words*: phenology, pollen viability, pollen germination, self-fertility, sour cherry.*

INTRODUCTION

Sour cherry (*Prunus cerasus* L.) is an important fruit species in worldwide, and Romania is among the top ten producing countries. The area cultivated with sour cherry in Romania was 2,760 ha, which ensured a production of 29,000 tones (at the level of the year 2023) (FAOSTAT, 2025).

Sour cherry belongs to the group medium to early blooming species, but is the latest among stone fruits (Nyéki et al., 2002; Fotirić Akšić et al., 2013).

It is known that flowering is the most important process in the development of fruit trees, fruit production and their quality depending on the flowering course conditions and especially on the pollination and fecundation of flowers (Lech, 1990; Szpadzik et al., 2010).

Realization of the pollination process in good conditions positively influences the fruits setting.

Pollination and fecundation of flowers at a high level depends on the pollen quality, the degree of self-fertility of the varieties, the climatic conditions during the flowering, but also on the presence of bees in the sour cherry orchards during the flowering period.

Some authors believe that the viability and germination of pollen are positively correlated with fruit setting (Wocior, 1975; Milutinovic et al., 1997; Szpadzik et al., 2008, 2010).

The authors note that pollen germination is even more important, depending on the pollen viability and the climatic conditions during the flowering. Sometimes the viable pollen grains have not a good germination.

From the point of view of the behavior in the pollination and fecundation process the sour cherry varieties are grouped into 3 classes: self-fertile, partially self-fertile and self-sterile (Cimpoieş, 2002).
Lech (1984) believes that when it sets 30% of fruits after pollination the yield is satisfying. Nyeki et al. (1997) think that even if 20% of flowers set fruits, the production is economical. Budan and Gradinariu (2000), Butac (2004) consider that when the percentage of fruit set is between 10 and 15% a normal fruit harvest is obtained.
Most scientists consider that the cultivars which have a high degree of self-fertility give a good yield. Also, self-fertile cultivars give a good production regardless of the evolution of the climatic conditions (low temperature or rain) (Wocior, 1977; Szpadzik et al., 2008; 2010).
The main objective of this paper is to study some aspects regarding floral biology of 42 autochthonous sour cherry genotypes from national germplasm fund: beginning, ending and intensity of flowering, self-fertility, as well as the viability and germination capacity of pollen.

MATERIALS AND METHODS

Field trial and plant material

The research was carried out in sour cherry germplasm collection from Genetic and Breeding Department of Research Institute for Fruit Growing located in Mărăcineni - Argeş area.
The sour cherry collection was planted in 2010 and comprises 144 genotypes of which 54 Romanian and 90 of foreign origin.
Of these, 42 bred and local Romanian genotypes were evaluated from the point of view of the flowering phenophases, degree of self-fertility, viability and germination capacity of pollen.

Measurements

The observations regarding blooming time were carried out, as follows (Nyeki et al., 2014):
- Beginning of flowering – noting the date when 1-5% of flowers are opened;
- Ending of flowering - noting the date when 95-100% of flowers fell;
- Duration of flowering - was calculated the number of days from the beginning until the end of flowering;
- Intensity of flowering -was appreciated by notes from 1 (very low intensity) to 5 (very high intensity.
The viability of pollen was determined by the method of coloring with acetic carmine by which the fertile pollen is colored in red, and the sterile pollen remains colorless (Cociu and Oprea, 1989; Botu and Botu, 1997).
The germination capacity of pollen was determined *in vitro* on solid medium (15% sucrose, 1.5 g agars, 0.01 g boric acid in 100 ml distilled water), under laboratory conditions (temperature 18-20ºC and relative humidity of 70-90%).
The reading of the preparations was performed at the MC-5 electron microscope, with the objective x40.
The degree of self-fertility was determined by isolating 100 flowers and counting the fruits set.

Statistical analysis

The data were included in an Excel database and statistically interpreted with some statistic indices: average, standard deviations and variability coefficient (%).

RESULTS AND DISCUSSIONS

Blooming time

The observations regarding flowering phenophases was carried out in 2024 year which was the hottest in the last 54 years, with 2.6ºC over normal (12.7ºC compared to 10.1ºC as normal), but also much poorer in precipitation, with 146.2 mm below normal (527.0 mm compared to 673.2 mm as it represents the normal period 1969-2023). Also, the average temperatures of the first four months of the year were much higher than the normal area (with 0.8ºC in January, with 5.8ºC in February, with 2.9ºC in March and with 3.0ºC in April).
All these high temperatures have had the effect of earlier flowering than the normal flowering time in Mărăcineni - Argeş area for sour cherry, respectively April 18 for beginning of flowering and April 28 for ending of flowering.

It is observed that in 2024 the average date regarding the beginning of flowering for all autochthonous genotypes studied was April 6, being with 12 days earlier than the average flowering date in the Mărăcineni - Argeş area (Tables 1 and 2).

The average date of the end of flowering for all Romanian genotypes was April 15, being with 13 days earlier than the normal average date of this phenophase (Tables 1 and 2).

From the point of view of the blooming time, they were noted for the late flowering: 'Bucovina', 'Crişana 2', 'Dropia', 'Ilva', 'Nana', 'Stelar', 'Vrâncean' – bred cultivars; 'Crişana Cluj', 'Crişana Nazarcea', 'Crişana 4/6', 'Mărculeşti 1/7', 'Mărculeşti 15/2', 'Mărculeşti 32/20', 'Mărculeşti P2 Vie', 'Băneasa 6/26', 'Tg. Jiu 200', 'Selecţie Vrancea', 'Topoloveni' and 'Selecţie M' – local genotypes.

The same period of flowering and earliness of blooming was also reported in Serbia for 'Oblačinska' cv., in Hungary for 'Ujfehértói fürtös' cv., as well as in Poland for some cultivars such as 'Schattenmorelle', 'Debreceni Botermo' and 'Ujfehértói fürtös' cvs. (Szpadzik et al., 2010; Mika et al., 2011; Fotirić Akšić et al., 2013; Nyéki et al., 2014).

Table 1. Flowering phenophases of sour cherry bred cultivars from genetic resources fund - 2024, RIFG Piteşti, Romania

Cultivars	Beginning (day/month)	Ending (day/month)	Duration (days)	Intensity (note)
Bucovina	9.04	17.04	8	4
Crişana 2	9.04	17.04	8	4
De Botoşani	4.04	13.04	9	4
Dropia	9.04	16.04	7	4
Ilva	9.04	18.04	9	4
Mocăneşti 16	3.04	15.04	12	4
Nana	9.04	18.04	9	4
Rival	4.04	12.04	8	4
Scuturător	4.04	13.04	9	4
Stelar	9.04	19.04	10	4
Timpurii de Osoi	3.04	12.04	9	4
Timpurii de Piteşti	3.04	12.04	9	4
Ţarina	4.04	13.04	9	4
Vrâncean	9.04	18.04	9	4
Average	**6.04**	**15.04**	**9**	**4**

The average flowering duration was short, only 9 days due to the very high temperatures from blooming time. However, there were some genotypes where the flowering period was a little longer, 10-13 days: 'Bizigheşti', 'Mocăneşti 16', 'Stelar', 'Mărculeşti P2 Vie', 'Mărculeşti P4 Vie', 'Suraia Vrancea', 'Focşani 3' (Tables 1 and 2).

In 2024 the intensity of flowering was high (note 4), being higher at bred cultivars than at local ones (Tables 1 and 2). All the bred cultivars had a high intensity of the flowering (note 4), while in the case of the local genotypes, 12 of them had a medium flowering intensity (note 3).

Table 2. Flowering phenophases of sour cherry genotypes (local cultivars and selections) from genetic resources fund - 2024, RIFG Piteşti, Romania

Cultivars	Beginning (day/month)	Ending (day/ month)	Duration (days)	Intensity (note)
Crişana Cluj	9.04	18.04	9	4
Crişana Nazarcea	9.04	18.04	9	3
Crişana 4/6	9.04	17.04	8	3
Mărculeşti 1/7	9.04	17.04	8	3
Mărculeşti 15/2	9.04	18.04	9	3
Mărculeşti 32/20	9.04	18.04	9	3
Mărculeşti 33/13	4.04	12.04	8	3
Mărculeşti P2 Vie	9.04	20.04	11	4
Mărculeşti P4 Vie	4.04	17.04	13	3
Drobeta	3.04	12.04	9	3
Băneasa 4/7	9.04	18.04	9	3
Băneasa 6/26	9.04	18.04	9	3
Breznița	4.04	11.04	7	3
Bizigheşti	4.04	16.04	12	4
Mari timpurii	3.04	11.04	8	4
Mari timpurii cl. 11	4.04	12.04	8	3
Tg. Jiu 200	9.04	17.04	8	4
Tg. Jiu 505	3.04	14.04	11	4
Tg. Jiu 404	4.04	12.04	8	4
Focşani 3	4.04	14.04	10	4
Locale de Bistrița	3.04	11.04	8	4
Suraia Vrancea	3.04	15.04	12	4
Selecţie Vrancea	9.04	17.04	8	4
Turceşti	3.04	12.04	9	4
Topologu Tulcea	4.04	12.04	8	4
Topoloveni	9.04	17.04	8	4
Selecţie Leordeni	3.04	12.04	9	4
Selecţie M	9.04	14.04	5	4
Average	**6.04**	**15.04**	**9**	**3.5**

Quality of pollen (viability and germination capacity)

Research regarding the viability and germination capacity of pollen, in addition to the practical importance related to the quantity and quality of the yield to obtain under optimal pollination conditions, also have a theoretical valence correlated with their potential use as

genitors in the breeding works (Zoican and Maresi, 2022).

According to Davarynejad et al. (2008) high percentage of pollen viability and germination capacity are important to decide the best pollinator. Also, it is known that not all pollen grains germinated on stigma can reach to the ovule.

On bred cultivars, the average value of pollen viability was 78.24% ranging from 91.27 at 'Bucovina' cv. and 54.62 at 'Vrâncean' cv. (Table 3).

Table 3. Pollen viability and germination capacity of sour cherry bred cultivars from genetic resources fund - 2024, RIFG Piteşti, Romania

Cultivars	Pollen viability (%)	Pollen germination (%)	Self-fertility (% fruit set)
Bucovina	91.27	12.97	15.62
Crişana 2	71.32	15.26	3.51
De Botoşani	81.73	19.25	3.69
Dropia	75.55	19.92	2.36
Ilva	82.95	11.34	16.48
Mocăneşti 16	79.06	10.87	8.94
Nana	76.21	29.10	11.34
Rival	86.78	38.55	10.25
Scuturător	83.96	23.52	5.38
Stelar	71.66	17.11	9.49
Timpurii de Osoi	86.49	13.60	5.81
Timpurii de Piteşti	89.78	20.94	6.47
Ţarina	63.95	16.25	3.74
Vrâncean	54.62	9.44	3.63
Average	**78.24**	**18.44**	**7.62**
Standard deviation	**10.29**	**7.90**	**4.55**
Coefficient of variability (%)	**13.15**	**42.86**	**59.69**

The results regarding the pollen viability are in accordance with the results reported by Zoican and Maresi in 2022 for 'Ţarina' cv.

On local genotypes, the average value of pollen viability was 78.70%. The highest viability was obtained from 'Selecţie M', while the lowest was from 'Mari timpurii cl. 11' (Table 4).

The coefficient of variability was 13.15% for bred cultivars and 17.87% for local genotypes, which indicates medium variability for this trait.

The pollen germination was 18.44% for bred cultivars and 17.58% for local genotypes.

The highest germination was obtained from 'Nana', 'Scuturător', 'Timpurii de Piteşti' (bred cultivars) and 'Crişana 4/6', 'Mărculeşti 15/2', 'Mărculeşti P2 Vie', 'Drobeta', 'Băneasa 6/26', 'Breznița', 'Bizigheşti', 'Tg. Jiu 505', 'Turceşti', 'Vişin Leordeni', 'Selecţie M' (local genotypes) (over 20%) (Tables 3 and 4).

Table 4. Pollen viability and germination capacity of local cultivars and selections from genetic resources fund - 2024, RIFG Piteşti, Romania

Cultivars	Pollen viability (%)	Pollen germination (%)	Self-fertility (% fruit set)
Crişana Cluj	44.44	13.46	8.49
Crişana Nazarcea	70.21	16.66	9.44
Crişana 4/6	83.70	23.70	3.51
Mărculeşti 1/7	82.09	6.22	1.41
Mărculeşti 15/2	87.70	28.98	2.35
Mărculeşti 32/20	80.08	19.90	4.78
Mărculeşti 33/13	65.64	11.38	6.53
Mărculeşti P2 Vie	87.16	20.77	4.23
Mărculeşti P4 Vie	63.65	15.34	5.46
Drobeta	91.36	31.59	10.58
Băneasa 4/7	77.83	7.40	1.25
Băneasa 6/26	91.18	30.09	8.45
Breznița	84.61	22.34	8.76
Bizigheşti	88.33	21.40	11.26
Mari timpurii	53.70	9.04	3.28
Mari timpurii cl. 11	37.89	6.07	2.89
Tg. Jiu 200	74.02	14.61	5.18
Tg. Jiu 505	81.71	26.70	11.64
Tg. Jiu 404	86.48	14.19	6.57
Focşani 3	78.43	10.74	3.25
Locale de Bistriţa	90.27	8.04	3.56
Suraia Vrancea	87.37	19.52	15.97
Selecţie Vrancea	78.07	16.04	6.72
Turceşti	87.33	23.00	10.86
Topologu Tulcea	88.88	4.87	1.15
Topoloveni	80.23	12.40	7.51
Vişin Leordeni	89.50	27.23	10.36
Selecţie M	91.85	30.58	8.45
Average	**78.70**	**17.58**	**6.57**
Standard deviation	**14.06**	**8.16**	**3.74**
Coefficient of variability (%)	**17.87**	**46.44**	**56.93**

There is no correlation between the viability of pollen and its germination capacity. For example, on 'Ţarina' cv. the viability of the pollen is low (63.95%), but the germination is good (16.25%). On 'Topologu Tulcea' variety, the viability of the pollen is high (88.88%), but the germination is very low (4.87%). On 'Rival' cv., both the viability and the germination of the pollen have high values (86.78%, respectively 38.55%). On 'Turceşti' variety the germination capacity of the pollen was good (23%), but the pollen tubes were very short, being the possibility that they do not reach the ovule (Figure 1).

The values of the coefficient of variability for this character are very high (over 40%) which indicates an extremely high variability and the possibility to selecting potential genitors for breeding works.

Tosun and Koyuncu (2007) studied sour cherry pollens quality and reported that pollen viability rate reached 80-93% and pollen germination rate was 57-67%.

Our results were similar to those reported by Tosun and Koyuncu (2007) for pollen viability, but much smaller regarding pollen germination.

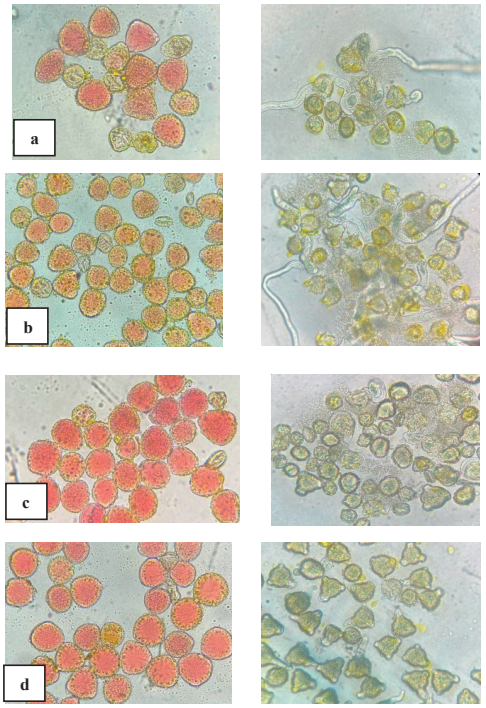


Figure 1. Pollen viability and germination capacity on some genotypes studied: a - 'Ţarina'; b - 'Rival'; c - 'Topologu Tulcea'; d - 'Turceşti'

Self-fertility

The percentage of fruits set after self-pollination was 7.62% for bred cultivars and 6.57% for local genotypes (Tables 3 and 4). According to data reported by Szabo (2007) and Davarynejad (2008), a percentage of fruits set to over 5% can be considered acceptable. After the percentage of fruits set after self-pollination, Nyeki et al. (2000) classified the sour cherry cultivars in three groups: adequate (above 10%), acceptable (5-10%) and weak (below 5%).

In our study 23.80% of the studied genotypes had a percentage of fruits set over 10% (Tables 3 and 4).

According to Nyeki's classification, the genotypes studied were grouped as follows:

- adequate – self fertile (over 10% fruits set) – 10 genotypes: 'Bucovina', 'Ilva', 'Nana', 'Rival', 'Drobeta', 'Bizigheşti', 'Tg. Jiu 505', 'Suraia Vrancea', 'Turceşti', 'Vişin Leordeni';
- acceptable – partially self fertile (5-10% fruits set) – 16 genotypes: 'Mocăneşti 16', 'Scuturător', 'Stelar', 'Timpurii de Osoi', 'Timpurii de Piteşti', 'Crişana Cluj', 'Crişana Nazarcea', 'Breznița', 'Topoloveni', etc.;
- weak – self sterile (below 5% fruits set) – 16 genotypes: 'Crişana 2', 'De Botoşani', 'Dropia', 'Ţarina', 'Vrâncean', 'Mari timpurii', 'Locale de Bistriţa', 'Topologu Tulcea', etc.

It can be observed that, compared to the 'Crişana 2' cv. (with a small degree of self - fertility), two clones ('Crişana Cluj' and 'Crişana Nazarcea') have a higher degree of self -fertility.

It can also be seen that there is no always a correlation between the pollen germination capacity and the degree of self-fertility. For example, there are genotypes that have acceptable the pollen germination and the percentage of fruits set after self pollination ('Bucovina', 'Ilva', 'Nana', 'Rival', 'Stelar', 'Crişana Cluj', 'Crişana Nazarcea', 'Drobeta', 'Bizigheşti', 'Tg. Jiu 505', 'Suraia Vrancea', 'Turceşti'), but there are genotypes that have a large percentage of pollen germination but a small percentage of fruits set ('Crişana 2', 'De Botoşani', 'Dropia', 'Timpurii de Piteşti', 'Ţarina', 'Vrâncean', 'Crişana 4/6', 'Băneasa 6/26', etc.) (Tables 3 and 4).

The values of the coefficient of variability for this character are very high (56.93%) which indicates an extremely high variability.

CONCLUSIONS

The results regarding floral biology in sour cherry provide information regarding the use of a variety as a pollinator in commercial orchards, as well as a parent in breeding works.

Regarding the flowering period, it can be said that in 2024 due to the very high temperatures from the beginning of the year, the genotypes studied have blossomed 12 days earlier than the normal flowering period of the Mărăcineni - Argeş area, thus being exposed to the late spring frosts. However, in the germplasm fund there are also genotypes with late flowering that can be used as gene sources for this trait.

The tests regarding the pollen viability and germination have highlighted the fact that in the collection of genetic resources exit genotypes with high germination and therefore

with the possibility of being used as pollinators in commercial orchards but also as genitors in breeding works (e.g. 'Nana', 'Scuturător', 'Timpurii de Piteşti', 'Drobeta', 'Bizigheşti', 'Brezniţa', 'Turceşti', etc.).

Regarding self-fertility, the sour cherry genotypes studied were classified into 3 groups: self-fertile (e.g. 'Bucovina', 'Ilva', 'Nana', 'Rival', 'Suraia Vrancea' cvs. – over 10% fruits set); partially self-fertile (e.g. 'Mocănești 16', 'Scuturător', 'Stelar', 'Timpurii de Osoi', 'Brezniţa' cvs. - 5-10% fruits set) and self-sterile (e.g. 'Dropia', 'Ţarina', 'Vrâncean', 'Crişana 2', 'Locale de Bistriţa' cvs. – below 5% fruits set).

REFERENCES

Botu, I. & Botu, M. (1997). *Metode şi tehnici de cercetare în pomicultură*. Ed. Conphys, pp. 100 – 104 (in Romanian).

Budan, S. & Grădinariu, G. (2000). *Cireşul*. Ed. Ion Ionescu de la Brad, Iaşi, pp. 42-44 (in Romanian).

Butac, M. (2004). Biologia înfloritului şi fructificării la soiurile de prun din sortimentul naţional în scopul stabilirii celor mai buni polenizatori. *Teză de doctorat*. Editura INVEL-Multimedia, Bucureşti (in Romanian).

Cimpoieş, Gh. (2002). *Pomicultură specială*. Ed. Colograf-Com, Chişinău, 220-235 (in Romanian).

Cociu, V. & Oprea, St. (1989). *Metode de cercetare în ameliorarea plantelor pomicole*. Ed. Dacia, Cluj (in Romanian).

Davarynejad, G.H., Szabo, Z., Nzeki, J., Szabo, T. (2008). Phenological stages, pollen production level, pollen viability and *in vitro* germination capability of some sour cherry cultivars. *Asian Journal of Plant Sciences 7*(7): 672-676.

Fotirić, Akšić M., Rakonjac, V., Nikolić, D., Zec, G. (2013). Reproductive biology traits affecting productivity of sour cherry. *Pesq. Agropec. Bras., Brasilia*, *48*, n. 1: 33-41.

Lech, W. (1984). Kwitnienie: Fizjologia roslin sadowniczych. Praca zbiorowa pod red. *L.S. Jankiewicza. PWN, Warszawa*: 420-462.

Lech, W. (1990). Badania wybranych ezynnikow wplywajacych na owocowanie wisni. *Zesz. Nauk. AR w Krakowie, 144*. Rozprawa habilitacyjna.

Milutinovic, M., Rakonjac, V., Nikolic, d. (1997). Functionaly of pollen and fruit set in sour cherry cultivars. *Acta Hort. 468*: 591-594.

Mika, A., Buler, Z., Michalska, B. (2011). Biology of flowering and fruiting of sour cherry trees grown at high density. *Journal of Fruit and Ornamental Plant Research, 19*(1): 123-126.

Nyéki, J., Szabó, Z., Andrasfalvy, A. (1997). Morphological and phenological properties of sour cherry varieties grwon in Hungary and their inter-incompatibility relations. *Acta Hort. 468*: 595-602.

Nyéki, J., Szabó, Z., Szabó, T., Soltesz, M. (2000). Morphological and phenological properties of sour cherry varieties grown in Hungary and their inter-incompatibility relations. *Int. J. Hortic. Sci., 6*:114-117.

Nyéki, J., Soltész, M. and Szabó, Z. (eds.) (2008). Morphology, Biology and Fertility of Flowers in Temperate Zone Fruits. *Akadémiai Kiadó*, Budapest.

Nyéki, J., Papp, N., Lakatos, L., Soltesz, M., Szabo, Z. (2014). Bloom, phenology and fertility of sour cherry cultivars of Hungarian origin at two different growing sites. *Acta Hort. 1020*: 197-201.

Szabo, T. (2007). Resultand economic importance of the North-Eastern Hungarian sour cherry landrance cultivar selection. *PhD tesis* Corvinus University of Budapest.

Szpadzik, E., Jadczuk-Tobjasz, E., Lotocka, B. (2008). Preliminary evaluation of pollen quality, fertility relations and fruit set of selected sour cherry cultivars in Polish conditions. *Acta Agrobotanica*, *2*(1): 307-309.

Szpadzik, E., Jadczuk-Tobjasz, E., Lotocka, B. (2010). Floral bioliogy of some sour cherry cultivars and their suitability for cultivation. *Annals of Warsaw University of Life Sciences, Horticulture and Landscape Architecture, 31*: 43-51.

Tosun, F. & Koyuncu, F. (2007). Investigation of suitable pollinator for 0900 Ziraat sweet cherry cv.: pollen performance tests, germination tests, germination procedures, *in vitro* and *in vivo* pollinations. *Hort. Sci. (Prague), 34*(2): 47-53.

Wocior, S. (1975). Badania nad biologia pylku 6 odmian wisni. *Rocz. Nauk Rol*., *ser. A 100*(4): 7-15.

Wocior, S. (1977). Badania nad ezynnikami wplywajacymi na przebieg i efektywnosc zapylenia wisni. *Rozprawy naukowe 49*. Habilitation Thesis.

Zoican, A. & Mareşi, E. (2022). Studii privind fertilitatea şi capacitatea de germinare a polenului la soiuri folosite ca genitori în lucrările de ameliorare la speciile măr, păr, prun, cireş şi vişin. *Buletin ştiinţific ICDP Piteşti-Mărăcineni, vol. 5*: 2-9 (in Romanian).

*** (2025). FAOSTAT data base.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

REPRODUCTIVE MANIFESTATIONS OF APPLES BY CONVENTIONAL FARMING

Vera STEFANOVA, Galya DOBREVSKA, Manol DALLEV

Agricultural University Plovdiv, 12 Mendeleev Blvd, Plovdiv, Bulgaria

Corresponding author email: vera.v.stefanova@abv.bg

Abstract

There are a number of innovative projects looking for techniques that can improve production while reducing resource input and impact on the environment. A study is made of growth and reproductive manifestations of apples (variety Florina) on the territory of the, Plovdiv region, Bulgaria country. Conventional production is applied in two variants of area - on cultivated area and on grassplot area. The following parameters regarding the growth parameters of Florina apples are monitored: cross-sectional area of the stem, average weight of fruit, number of fruits per tree, productivity coefficient. The different indicators give a different reflection in the two variants of area, but in the same conventional production. The average weight of fruit, number of fruits per tree and the coefficient of productivity give better results on growing apples in a grassplot area. The indicator `cross-sectional area of the stem` presents better values when growing apples on a cultivated area. The results are visualized by presentation in a digital technology, using Geographic Information Systems (GIS), with the aim of faster and rational use by modern farmers.

Key words*: apple reproductive manifestations, conventional production, cultivated area, grassplot area, GIS.*

INTRODUCTION

Due to the harmful effects of excessive pesticide use, increasing attention is being paid to maintaining a balanced nutrient composition in soils. One key factor in addressing this issue is the proper selection of a soil surface management system in orchards. The evolution of these systems is closely linked to the intensification of fruit production and the increasing adoption of environmentally friendly production methods.

Historically, two main soil management systems have been predominant - sod cover (grass swarding) and bare black fallow (Rubin, 1967; Spivakovsky, 1963; Trocme & Gras, 1964; Todorov, 1966). However, in recent decades, with scientific and technical advancements in countries with developed horticultural sectors, these traditional methods have been replaced by more modern systems. These include turf-mulch systems, herbicide fallows, and various hybrid approaches (Kotov, 1973; Otto & Winkler, 1975; Stamatov et al., 1982).

With the introduction of biological farming practices in orchards, the effects of chemical and organic herbicides have also been explored. Studies have shown that prolonged use of chemical herbicides leads to soil erosion, a significant reduction in organic matter content, and an increase in nitrate concentrations in groundwater (Himmelsbach & Kleisinger, 1995).

In Bulgaria, Rusaliev and Rankova (2004) also emphasized the importance of minimizing soil surface tillage in orchards as a means to preserve soil structure and fertility.

Research has also focused on how different soil maintenance systems affect the physiology, productivity, and fruit quality of apple orchards (Hogue & Nielsen, 1987; Bugg & Waddington, 1994; Mika et al., 1998; Sanches et al., 2007; Zhang, 2008).

While many findings highlight the positive impacts of various systems, some negative effects - especially in the inter-row zones - have also been reported (Szewczuk, 2000).

In addition to plant response, the influence of soil maintenance systems on the physical and biological properties of soil has also been investigated. Grass coverage and swarding are particularly effective in reducing erosion and nutrient leaching, improving soil structure, water retention, microbial activity, and organic matter content, and in suppressing weed development (Mattern, 1989; Tzvetkova et al., 1995; Kabourakis, 1995; Glen, 1999; Higgins,

2001; Lang et al., 2001; Jordan & Jones, 2002; Loyd et al., 2002; Tasseva, 2003; Tanimu et al., 2007).

Nevertheless, the data collected in any experimental work is only valuable to producers if presented in an accessible and practical format. One of the most effective ways to achieve this is through the use of Geographic Information Systems (GIS), which offer a powerful tool for spatial analysis and visualization in precision agriculture.

Geographical Information Systems (GIS) have become indispensable tools in modern apple production, enabling the creation of thematic maps that facilitate various aspects of orchard management, site selection, and crop monitoring. By integrating spatial data with analytical techniques, GIS supports informed decision-making to enhance productivity and sustainability in apple cultivation.

GIS facilitates the monitoring of apple orchards by enabling the analysis of temporal and spatial data. A study in China developed a method to accurately identify the planting years of apple orchards using satellite remote sensing images. By analyzing phenological characteristics and employing spatiotemporal data fusion techniques, researchers could determine the age distribution of orchards, which is vital for predicting production and planning rejuvenation strategies (Zhang et al., 2020).

GIS is extensively employed to assess land suitability for apple orchards by analyzing multiple environmental factors such as climate, soil properties, topography, and water availability. For instance, a study in the central west of the southern Pyrenees utilized GIS combined with fuzzy logic and the Analytic Hierarchy Process (AHP) to identify optimal sites for apple orchards. This approach considered factors like reduced risk of late spring frosts and mild slopes, providing a modifiable tool for crop suitability assessment in mountainous regions (Casadó-Tortosa et al., 2023).

In apple orchards, GIS enables detailed spatial analysis of key factors such as soil properties, yield variability, and water distribution, which are crucial for implementing precision agriculture techniques. For instance, Aggelopoulou et al. (2011) demonstrated the effectiveness of GIS in mapping soil spatial variability to support site-specific fertilization strategies, thus improving input efficiency and sustainability.

Yield estimation is another critical area where GIS has proven valuable by integrate image processing techniques and can facilitate predictive modeling of apple production (Aggelopoulou, Bochtis, Fountas, et al., 2011).

Furthermore, GIS is used to assess the impact of varying irrigation regimes on spatial variability within orchards, allowing for targeted water management and improved fruit quality (Rud et al., 2018).

Recent advancements also include the use of unmanned aerial vehicles (UAVs) and deep learning within a GIS framework to produce high-resolution yield maps, offering a cloud-based solution for real-time decision-making in orchard management (Apolo-Apolo et al., 2020).

Collectively, these studies underscore the critical role of GIS in enhancing the precision, efficiency, and sustainability of apple orchard cultivation.

Conventional apple production remains a dominant and efficient agricultural system, but it also faces growing scrutiny due to its environmental footprint and potential health risks. As consumer awareness increases and regulations evolve, the industry may need to adopt more sustainable practices to balance productivity with ecological responsibility.

MATERIALS AND METHODS

The experiment was conducted in an apple orchard located at the Pomology Research and Training Station of the Agricultural University Plovdiv, Bulgaria, during the 2020-2021 period. The orchard consisted of trees of the Florina/MM106cultivar-rootstock combination. A conventional cultivation system was applied, including standard plant protection practices, mineral fertilization, drip irrigation, and mechanical soil management. The plant protection program included three preventive winter treatments targeting overwintering forms of economically significant pests and diseases, as well as additional applications during the growing season. Fertilization was carried out using mineral fertilizers, tailored to the specific nutritional needs of the orchard.

Irrigation was provided through a localized drip system, ensuring precise water delivery based on plant growth stages and prevailing weather conditions.

Soil cultivation was conducted using specialized equipment for both deep and shallow tillage suitable for orchard management (Stamatov et al., 1982; Todorov et al., 1974). Two different soil surface management systems were implemented in the inter-rows: (1) bare fallow (cultivated area) and (2) grass-covered (sodded area). The bare fallow method involved several shallow inter-row tillage operations to enhance nutrient availability, improve air and water exchange, and reduce weed infestation. Additionally, deep ploughing was performed in autumn at a depth of 18-20 cm.

The grass-covered system involved maintaining inter-rows with species such as *Poa pratensis* and *Trifolium repens*, which were periodically mowed. The root systems of these grasses contributed to natural soil loosening, enhancing aeration and water dynamics in the root zone. This system also stimulated microbial activity by increasing the organic matter content in the soil (Lichev et al., 2020; Stamatov et al., 1982). These two technological approaches represent distinct strategies for managing soil and plant protection resources under conventional apple production, each offering specific advantages and challenges related to sustainability, soil fertility, and long-term orchard productivity.

The experimental trees - Florina grafted onto MM106 - were at full fruit-bearing stage under both management systems and of equal age. Their crowns were trained to a free spindle form, planted in a rectangular pattern at 4.00 m × 1.80 m spacing.

Two experimental variants treatments were established:

- Treatment I: Maintenance of cultivated inter-rows (bare fallow);
- Treatment II: Maintenance of grassed inter-rows.

Each variant included four replications with three trees per replication.

The following parameters were monitored:

- Cross-sectional area of the trunk (cm^2): Determined using the formula $S = \pi r^2$;
- Average fruit weight (kg): Based on 10 randomly sampled fruits from each replication;
- Number of fruits per tree;
- Productivity coefficient (kg/cm^2): Calculated as the ratio of total fruit weight to trunk cross-sectional area;
- Instantaneous soil moisture (%): Measured at a soil depth of 0-60 cm during the key phenological phase of vigorous growth using a soil moisture meter. Three measurements were taken per replication.

All results were statistically processed using analysis of variance (ANOVA).

One of the most effective methods for presenting the collected data from the study area was through the implementation of Geographic Information Systems (GIS). GIS facilitates visualization and interpretation of how different soil surface management systems influence productivity, orchard health, and soil condition. It provides producers with highly useful visual and analytical insights through thematic maps, thus enabling data-driven decision-making and enhancing orchard management efficiency.

The GIS platform used for data visualization was QGIS 3.40.4, incorporating open-access datasets. Land boundaries were derived from the digital cadastral map and database of the Republic of Bulgaria. An interpolation method was applied for data visualization and thematic map generation.

RESULTS AND DISCUSSIONS

GIS offers a wide range of capabilities for data representation and analysis, with its most significant function being the ability to visually present research findings on maps. This approach facilitates easier interpretation of results, enables rapid and efficient analysis of large datasets, and allows for straightforward editing and modification. The system generates multilayered information that can be easily transformed into thematic maps, effectively illustrating the achieved outcomes.

In the study of various growth and reproduction indicators of the Florina apple variety under conventional observation on both cultivated and grassed areas, we used GIS to represent the studied area and its specific localization.

The studied area is located in the village of Brestnik, Burgas district, Bulgaria. The following Figure 1 is a thematic map.

The studied area is located in Brestnik village, Plovdiv region, Bulgaria country. The next Figure 1 presents it on the thematic map.

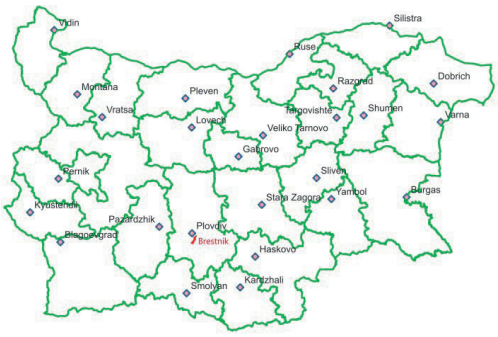


Figure 1. Localization of the studied area in Plovdiv region in border of Republic of Bulgaria

Brestnik village, located in Plovdiv Region, lies approximately 4 km south of the city of Plovdiv, at the beginning of the Rhodope ridge "Chernatitsa," also known as the "Rhodope collar."



Figure 2. Localization of Village Brestnik in Plovdiv region

This geographical position determines the specific relief, soil, and climatic characteristics of the area (Figure 2).

The village is situated on a slope with a distinct elevation difference between its northern and southern ends. The terrain has an inclination of 1% to 5%, typical of accumulative plains, covered with alluvial-deluvial deposits of relatively homogeneous composition.

The geological base of the area includes both carbonate and non-carbonate redeposited materials. The soils are represented by deluvial and proluvial deposits, typical for the Rhodope collar. The climate in the region is transitional-continental, characteristic of the Plovdiv plain in the Upper Thracian Lowland. The temperature regime is marked by hot summers and mild winters.

These natural conditions create a favorable environment for the development of agriculture and orchards in the territory of Brestnik village (Figure 3).

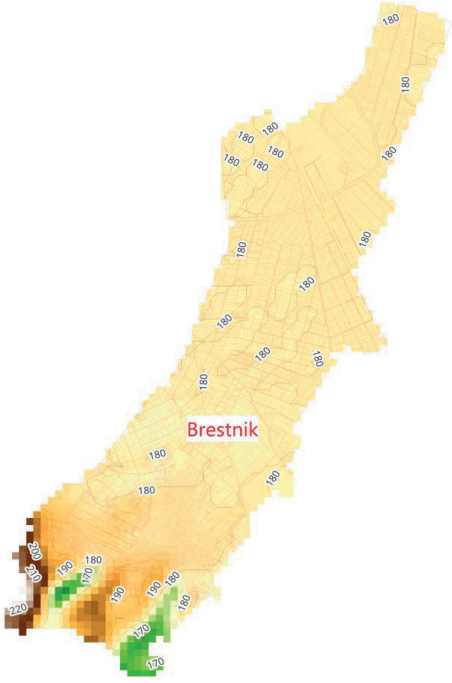


Figure 3. Topography map of studied area



Figure 4. Area of Florina apples orchard

The research includes two versions in conventional apple production - cultivated and grassed alleyways in orchard, located in Brestnik village.

A comparative analysis was conducted between cultivated and grassed inter-row management systems under conventional production of Florina apples (Figure 4). The results are presented in Table 1.

Table 1. Growth and reproductive manifestations of apples variety Florina in conventional production

Types of area / Parameters	Florina apple conventional production		GD at		
	Cultivated	Grassed	5%	1%	0.1%
Cross-sectional area of the stem (cm^2)	162.58	157.08	23.08	57.63	179.69
Average weight of fruit (kg)	0.182	0.194	0.15	0.39	1.42
Number of fruits per tree	142.91	145.27	119.05	271.01	781.03
Productivity coefficient (kg/cm^2)	0.16	0.18	0.35	0.89	4.01

Regarding the cross-sectional area of the trunk in the experimental trees under the two inter-row soil surface management systems - cultivated (162.58 cm^2) and grassed (157.08 cm^2) - no statistically significant differences were observed. This parameter is one of several that contribute to determining tree productivity. However, in this experiment, the trees in both treatments were not affected by the soil surface management system applied.

Examining the fruit mass in both variants (0.182 kg for the cultivated area and 0.194 kg for the grassed area), a similar trend was observed. Although a slightly higher value was recorded for the fruits grown under the grassed system, the difference was too small to be statistically significant. Thus, fruit mass also appears to be unaffected by the type of inter-row soil surface management.

The difference in the number of fruits per tree between the two soil surface management systems was also minimal, and again, no statistically significant differences were detected between the treatments.

The most important indicator of fruit tree productivity is the productivity coefficient (kg/cm^2), calculated as the ratio of fruit yield to the trunk cross-sectional area. As a natural outcome of the previously discussed results, this parameter also demonstrated minimal and statistically insignificant differences between the two treatments.

Table 2. Instantaneous soil moisture at 0-60 cm depth depending on inter-row soil surface management

Soil Surface Management	Soil Moisture (%)
Cultivated inter-rows	7.1
Grassed inter-rows	8.3

Table 2 presents the measured instantaneous soil moisture at a depth of 0-60 cm under two inter-row management systems in a conventional apple orchard. The results show that grassed inter-rows (sodded areas) maintained a higher soil moisture content (8.3%) compared to cultivated inter-rows (bare soil), which recorded 7.1%.

This difference, although numerically modest, highlights the positive effect of grass cover on soil water retention. The root system of grass species such as *Poa pratensis* and *Trifolium repens* contributes to improved soil structure and organic matter content, both of which enhance water-holding capacity.

Interpolate the results from apples study into GIS is presented by next thematic maps.

The baseline data include the boundaries of the area, markers of the studied plot, soil characteristics, and others.



Figure 5. Spatial distribution of different soil maintenance systems

Interpolate the results from apples study into GIS is presented by next thematic maps.
The baseline data include the boundaries of the area, markers of the studied plot, soil characteristics, and others.
The application of GIS in our study, through the creation of maps presents the spatial distribution of different soil management systems; a comparison between areas with varying soil surface conditions in terms of fruit yield; and zones with different current soil moisture levels depending on the implemented management system.
The two developed variants are presented in Figures 5 and 6. The cultivated and grassed areas are presented, including the corresponding repetitions.



Figure 6. Comparison between zones with different soil surface systems in terms of fruit production

The results of the conducted study on Florina apple variety regarding the productive parameters under the two surface soil treatment variants do not show significant or substantial differences (Figure 6).
However, the analysis of the samples taken for current soil moisture reveals notable differences between the treatments. In the grass-covered inter-rows, the soil moisture content is the higher compared to the tilled ones (Figure 7).



Figure 7. Zones with different soil moisture levels depending on the applied system

CONCLUSIONS

Based on the obtained results regarding the influence of two soil surface management systems in the inter-row space (cultivation and grassing) on the reproductive indicators of the apple cultivar-rootstock combination Florina/MM106, on the instantaneous soil moisture in the root zone, and on the visualization through the GIS system, the following conclusions can be drawn:

1. The type of soil surface management system did not have a statistically significant effect on the productivity of the Florina cultivar grafted onto MM106 rootstock. The absence of significant differences does not imply that further studies should be disregarded, particularly with other cultivar-rootstock combinations. This experiment may serve as a model system for such investigations.
2. Higher instantaneous soil moisture was recorded in the root zone when grassed inter-row management was applied.

3. GIS-based maps are developed to provide producers with spatial information regarding the distribution of different soil surface management systems and their associated effects - namely, the productivity of the Florina/MM106 apple cultivar-rootstock combination and the levels of instantaneous soil moisture in the orchard.

ACKNOWLEDGEMENTS

This research work was carried out with the support of Agricultural University Plovdiv, Bulgaria.

REFERENCES

Aggelopoulou, A.D., Bochtis, D., Fountas, S., et al. (2011). Yield prediction in apple orchards based on image processing. *Precision Agriculture, 12*(4), 448–456. https://doi.org/10.1007/s11119-010-9187-0

Aggelopoulou, K.D., Pateras, D., Fountas, S., et al. (2011). Soil spatial variability and site-specific fertilization maps in an apple orchard. *Precision Agriculture, 12*(2), 118–129. https://doi.org/10.1007/s11119-010-9161-x

Apolo-Apolo, O.E., Pérez-Ruiz, M., Martínez-Guanter, J., et al. (2020). A cloud-based environment for generating yield estimation maps from apple orchards using UAV imagery and a deep learning technique. *Frontiers in Plant Science, 11*, 1086. https://doi.org/10.3389/fpls.2020.01086

Bugg R. E. and C. Waddington (1994). Using cover crops to manage arthropod pests of orchards. Review, Agric. *Ecosyst. Environ*., 50, 11-28.

Casadó-Tortosa, A., de Herralde, F., Peris, M., et al. (2023). GIS-Based Approach for Modeling Vineyard and Apple Orchard Suitability in Mountainous Regions. *SSRN Electronic Journal*.

Glenn J. (1999). Producers expand line of mulch products. *BioCycle*, 40: 45-47.

Higgins A. (2001). Mulch starting to border on too much. *The Washington Post*. http://homes.mainetoday.com/gardening/010518mulch.shtml.

Himmelsbach J., S. Kleisinger (1995). Bodenpflegemasnahmen im obstbau. Erfahrungen und wirtschaftlichkeit. *Erwerbsobstb*, 37, 66-72.

Hogue E. J., G. H. Nielsen (1987). Orchard floor vegetation managment. *Hort. Rev*, 9, 377-430.

Jordan K. K., Jones S. C. (2002). Invertebrate fauna associated with mulch in urban environments. *Proceedings of the 4th International Conference on Urban Pests*, p. 87-94.

Kabourakis E. (1995). Biologikal olive cultivation. *Agricultural Tehnology*.

Kotov V. (1973). Sadovodstvo [Fruit Growing], No. 4.

Lang A., M. H. Behboudian, J. Kidd, H. Brown (2001). Mulch enhances apple fruit storage quality. *Acta Horticulturae*, *v. 1*, 557-561.

Loyd J. E., D. A. Herms, B. R. Stinner, H. A. J. Hoitink (2002). Mulch effects on soil microbial activity, nutriens cycling, and plant growth in ornamental landscapes. *Annual reports and Research Reviews*, pp. 83-92.

Mattern V. (1989). Mulch made easy. Organic Gardening, p. 38-42.

Mika A., D. Krzewińska, T. Olszewski (1998). Effects of mulches, herbicidesand cultivation as orchard groundcover management systems in young apple orchard. *J. Fruit Ornam. Plant Res*., 6: 1-13.

Otto G., H. Winkler (1975). Archiv fur Gardenbau, 7.

Rubin S. (1967). Soil Management in Orchards. Moscow.

Rud, R., Käthner, J., Giesser, J., et al. (2018). Monitoring spatial variability in an apple orchard under different water regimes. *Acta Horticulturae, 1197*, 19-26. https://doi.org/10.17660/ActaHortic.2018.1197.2

Rusaliev Zh., Rankova Z. (2004). Minimization of Soil Surface Tillage in Orchards. *Journal of Crop Science*, 41, pp. 14-17.

Sanches E. E, A. Giayetto, L. Chichon, D. Fernandes, M. Aruani, M. Curetti (2007). Cover crops influence soil properties and tree performance in an organic apple orchard in northern Patagonia. *Plant Soil*, 292, 193-203.

Spivakovsky N. (1963). Soil Management in Orchards. Kyiv.

Stamatov I., Todorov V., Gogova K., Makariev Z. (1982). *Soil Maintenance Systems in Fruit Orchards*. Hr. G. Danov Publishing, Plovdiv.

Szewczuk A. (2000). Influence of irrigation and mulching with pine bark and nonwoven polypropylene in tree rows on yielding in apple-tree Elstar. *J. Zeszyty Naukowe Akademii Rolniczej we Wroclawiu. Rolnictwo*, no. 396(77), p. 163-174.

Tanimu J., E. N. O. Iwuafor, A. C. Odunze, G. Tian. (2007). Effect of incorporation of leguminous cover crops on yield and yield components of maize. *World Journal of Agricultural Sciences*, 3(2): 243-249

Tasseva V. (2003). Influence of the Apple Growing Technology over the Soil Microflora in: *Proceedings of the International Scientific Conference "50 Years University of Forestry", Session - Agriculture*, 74-76

Todorov V. (1966). Use and Maintenance of Soil Surface in Orchards: A Review.

Trocme S., R. Gras (1964). *Sol et fertilisation en arboriculture fruitiere*, Ed. Perrin.

Tzvetkova E., T. Mitova, D. Hristova (1995). Possibilities for soil erosion protection and for improving the soil productivity by incorporation the straw and green manure. *Proceeding. Scientific Papers. Second scientific practikal conference "Ecological problems of agriculture', AGRO-ECO'95*, Plovdiv, 119-122.

Zhang J. (2008). Application of reflective mulch in pistachio production. *Acta Horticulturae*, *v. 1*, 912-919.

Zhang, Y., et al. (2020). Identification of Apple Orchard Planting Year Based on Remote Sensing and GIS. *Remote Sensing*, *12*(7), 1199.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

PRELIMINARY ASSESSMENT OF SOME MORPHOLOGICAL TRAITS OF JUJUBE FRUITS PRODUCED IN SOUTHERN OLTENIA

Ana-Maria STOENESCU[1, 2], Florin STĂNICĂ[3]

[1]University of Craiova, Faculty of Horticulture, Department of Horticulture and Food Science, 13 A.I. Cuza Street, 200585, Craiova, Romania
[2]Research and Development Station for Plant Culture on Sands, 217 Petre Baniță Street, 207220, Călărași, Romania
[3]University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Horticulture, Department of Bioengineering of Horti-Viticultural Systems, 59 Mărăști Blvd, 011464, District 1, Bucharest, Romania

Corresponding author emails: anamaria_stoenescu@yahoo.com, flstanica@yahoo.co.uk

Abstract

This work aimed to analyse the main morphological characteristics of jujube fruits (Ziziphus jujuba Mill.) produced in a small orchard in Southern Oltenia, Romania. Ten genotypes were evaluated for fruit key morphological traits (height, diameter, weight, and shape index). Fruit height varied from 22.37 mm (R5) to 41.79 mm ('Lang' cv.), while fruit diameter ranged from 17.77 mm (S1) to 35.74 mm ('Lizao' cv.). Total fruit weight ranged from 4.13 g (Jurilovca) to 22.52 g ('Lizao' cv.), and the shape index varied between 0.96 ('Fengmiguan' cv.) and 1.82 (S1). The analysis revealed a high coefficient of variability in total fruit weight, ranging from 15.73% (Fellini selection) to 35.25% (R5), indicating significant morphological diversity among the genotypes. The observed variability can be attributed mainly to the genetic characteristics of the genotypes, with additional influences from pedoclimatic conditions and applied orchard management practices. These results highlight the morphological diversity of jujube fruits in Southern Romania and underscore the role of genetic and environmental factors in fruit development.

Key words*: jujube, biometric analysis, cultivar, genotype.*

INTRODUCTION

Jujube, also known as Chinese date (*Ziziphus jujuba* Mill.), belongs to the Rhamnaceae family and the *Ziziphus* genus. It is one of the oldest cultivated fruit tree species in the world, with a history spanning over 7,000 years, and holds significant economic, ecological, and social importance (Liu et al., 2020; Xu et al., 2016; Zhang et al., 2024), with over 900 cultivars worldwide (Liu, 2018). Native to China, specifically the Central and Southern regions of the Yellow River basin (Li et al., 2024; Zhang et al., 2024; Poșta et al., 2021), the species is highly adaptable to climatic conditions, thriving in arid and semi-arid areas of temperate and subtropical climates (Liu, 2006; Khadivi et al., 2021). The nutritional content of jujube fruits, their use in traditional medicine, and application in the food industry further contribute to their economic importance (Shahrajabian et al., 2020; Ma et al., 2021). Jujubes are highly precocious, often setting fruit the second year after planting and sometimes even during the year of planting or grafting (Yao, 2013; Liu, 2006). The fruit is a drupe, with a crisp, fleshy, juicy, and sweet mesocarp (which varies by cultivar), a thin epicarp, and a hard, sharp endocarp containing none to two seeds (Yao, 2013; Liu, 2006). Jujube fruits differ in shape, colour, size, and organoleptic characteristics, influenced by genetic traits, location, and the pedoclimatic attributes of the cultivation area. Jujubes are classified into five groups: for fresh consumption (table cultivars), drying, candied, multipurpose, and ornamental cultivars (Liu, 2006). Current global trends focus on producing large fruits that meet consumer demands, are resilient to climate change, and can thrive on marginal, less-productive land. Additionally, resistance to pests and diseases, preserve their qualities for extended periods (whether fresh or processed), and ultimately generate profit. Therefore, understanding the primary morphological characteristics of fruits

is crucial for selecting the most suitable cultivars based on their intended use. Gaius Plinius Secundus (AD 23-79), commonly known as Pliny the Elder, documented in his *Historia Naturalis* that the Roman Emperor Octavian Augustus's counselor introduced the Chinese jujube from Syria to Italy, from where it subsequently spread to other Mediterranean countries (Stănică et al., 2020; Stănică, 2019; Stănică, 1997). In Romania, the Chinese jujube exists in a semi-spontaneous state solely in the Dobrogea region, situated between the Danube River and the Black Sea (Stan et al., 2021). Notably, the populations of Chinese jujube found in Romania are located near ancient sites with Greek, Roman, and Byzantine ruins, suggesting that these ancient civilizations may have played a significant role in the introduction of the Chinese jujube to the Mediterranean basin (Stănică, 2009). Since 1996, research focused on the propagation, cultivation practices, and genetic improvement of Chinese jujube genotypes has been conducted at the Faculty of Horticulture, University of Agronomic Sciences and Veterinary Medicine (USAMV) of Bucharest (Stoli & Stănică, 2021). Currently, jujube is not extensively cultivated in Romania, except in a limited number of experimental research stations, institutes, and educational fields. However, more people are beginning to appreciate and cultivate jujube in private gardens. Currently, the most common cultivars in Romania include 'Xuancheng Jian' ('Xuanchengjianzao'/XCJ), 'Lizao' ('Linyilizao'/'Li'), 'Lang', 'Dongzao', and 'Hupingzao'. Khadivi et al. (2021) mention that the main goals of jujube breeding are increasing yield, fruit size, and flesh/stone ratio. Luo et al. (2024) highlight the key quality traits of the jujube fruit as crucial in jujube breeding. This study aimed to identify the main morphological characteristics of jujubes produced in Southern Oltenia and provide additional insights into the fruit characteristics of various jujube genotypes in Romania.

MATERIALS AND METHODS

Materials

Jujube fruits (100 fruits per genotype) were harvested at the full red stage (crisp and ripe) from an orchard in Bratovoești Commune, Dolj County, Oltenia region, Romania (44°07'N 23°54'E). Ten jujube fruit trees were selected for the study: cultivars 'Fengmiguan', 'Lang', 'Xuancheng Jian', 'Dabaizao', and 'Lizao', Fellini selection, and K1, Jurilovca, R5, and S1 genotypes. The jujube trees are 4 years old, with a slender spindle canopy (cone shape), a support system, and planting distances of 1.5 m between plants within a row. Grass coverage with a mixture of species, respectively *Festuca pratensis*, *Phleum pratense*, *Festuca rubra*, *Dactylis glomerata, Agrostis gigantea, Festulolium, Bromus inermis, Lolium hybridum, Lolium multiflorum, and Lolium perenne*, is used to improve soil quality. The orchard is irrigated using a sprinkler system, with no fertilizers or phytosanitary treatments applied.

Literature review regarding some of the studied genotypes

- 'Xuancheng Jian' is a cultivar suitable for candied fruit but can also be eaten fresh. Originating from Anhui Province, China, it has a history of over 200 years (Liu & Wang, 2009). This late-ripening cultivar (harvested in late September to early October) exhibits high resistance to fruit cracking. The fruit is medium to large, oblong, and column-shaped with a pointed tip, averaging 22.5 g in weight. The cultivar is known for its early bearing, high yield, and high fruit set (Li et al., 2013);
- 'Fengmiguan' (known as 'Honey Jar' in the US) originates from Shaanxi Province and reaches the crisp maturity stage in mid-September. It is an early to mid-ripening cultivar that is highly resistant to diseases and exhibits light fruit cracking. The fruits are small, nearly round, averaging 9.2 g in weight, and are irregular in size. It is considered one of the sweetest jujube cultivars (Wang et al., 2017);
- 'Lizao' originates from Shanxi Province and has a cultivation history of over 3,000 years. According to Yao S. in Karp & Gasic (2024), it may be synonymous with 'Linyi Li', 'Shanxi Li', and 'Redlands 4' cultivars, as they share identical DNA sequences with 'Li' based on genotyping with SNP markers (Sapkota et al., 2024). The tree has moderate vigour, bears early, and ripens in late September. The fruits are exceedingly large, obovate, and globose to

ovoid, with an average weight of 31.6 g (up to 40 g) and irregular sizes. The fruit is crisp, juicy, and sweet. It is resistant to cracking, making it suitable for intensive planting due to its moderate vigour. 'Lizao' has the largest cultivation area and widest distribution among middle-late ripening table cultivars, though it has recently been surpassed by the 'Dongzao' cultivar in China (Zhang et al., 2024);

- 'Lang' cultivar, according to Yao S. in Karp & Gasic (2024), may be synonymous with the Chinese 'Junzao' cultivar, as both share identical DNA sequences based on genotyping with SNP markers (also mentioned by Sapkota et al., 2024). Originating from Shanxi Province, it has a cultivation history of over 1,000 years. The tree is vigorous, characterized by a strong central trunk and a conical-shaped crown, bearing fruit early. The fruits are pear-shaped, late-ripening, and range from medium to large (15.1–18.2 g, up to 26.3 g). They have a mild flavour, distinguishing them from the Chinese 'Langzao' cultivar, which is column or oval-shaped and averages 12.1 g. This cultivar is suitable for both drying and multipurpose use, although it is highly susceptible to fruit cracking;
- 'Dabaizao', according to Cossio & Bassi (2013), is a vigorous cultivar with medium productivity and low fruit uniformity, ripening toward the end of September or early October. According to Li et al. (2013), it is native to Hubei Province and is primarily used as a table cultivar for fresh consumption. The fruits are irregular in shape and size, weighing between 20 g and 40 g;
- Jurilovca is a wild type of *Ziziphus jujuba*, or maybe *Ziziphus acido-jujuba* from the Dobrogea region in South-East Romania. This biotype has been described by several researchers (Stănică, 2009; 2016; 2019; Stoli & Stănică, 2023; Stoli, 2023) in terms of its morphological and nutritional characteristics. In Romania, it is primarily used as a rootstock (Stănică, 2009; Stoli et al., 2022);
- Fellini is a selection of unknown origin (probably Chinese) propagated and cultivated by an Italian fruit grower from Gambetolla, Italy;
- R5 genotype may represent a selection from Russia, potentially of Chinese origin, identified as Ruski 5 (Russian 5/L5).

Climatic data

The experimental location is characterized by a continental climate with an annual precipitation of 500 mm, with most of this occurring in May and June, and smaller amounts in August and September. The temperature range exceeds 25°C, with hot summer days exceeding 35°C. Based on hardiness zones, the location is classified as zone 7b. Climate factors, including temperature, humidity, light intensity, and precipitation, influence fruit development. For example, higher temperatures have been observed to decrease fruit size and hardness (Gómez-Devia & Nevo, 2024), while heavy rainfall can cause fruit cracking, a common occurrence in jujube fruits.

Methods

Fruit measurements, including height, diameter, and total weight, were recorded. Fruit height and diameter were determined using a high-precision electronic calliper (precision of 0.01 mm), while total fruit weight was measured with a KERN EMB 200-2 analytical balance (precision of 0.01 mg). The fruit shape index (FSI) was calculated as the ratio between fruit height and diameter. According to Liu & Wang's (2009) classification, jujube fruit shapes are categorized as follows: oblate for index values <0.90, globose or nearly globose for index values between 0.90 and 1.10, oblong globose for index values between 1.10 and 1.30, cylindrical for values between 1.30 and 1.60, and long cylindrical for index values >1.60.

Statistical analysis

Statistical analysis was conducted using IBM SPSS Statistics 26 software. One-way ANOVA and Duncan's multiple range tests ($p < 0.05$) were applied. Pearson correlation analysis was used to identify relationships among the measured morphological traits, while cluster analysis helped determine possible similarities between genotypes. The coefficient of variation (CV) was also calculated using the formula CV = (SD/X) *100.

RESULTS AND DISCUSSIONS

The main jujube analysed morphological characteristics are shown in Table 1.

Statistical analysis of the obtained data (one-way ANOVA) revealed significant differences

between the jujube genotypes ($p < 0.05$), as indicated by the Duncan multiple range tests. The size, shape, and pattern of jujube fruits are shown in Figure 1.

Table 1. Morphological characteristics of jujube fruits

Genotype	Descriptive statistics*	Height (mm)	Width (mm)	Total weight (g)	Shape index (FSI)
Fellini selection	Mean±SD	29.93±1.71^{e**}	30.00±2.02^{c}	13.29±2.09^{c}	1.00±0.06^{fg}
	Variation limits	26.85-34.74	26.26-35.13	9.90-18.20	0.86-1.11
	CV%	5.71	6.73	15.73	6.00
'Fengmiguan'	Mean±SD	25.77±1.71^{f}	26.92±1.61^{d}	9.66±1.60^{d}	0.96±0.05^{g}
	Variation limits	22.57-29.94	23.90-30.15	7.20-12.40	0.87-1.10
	CV%	6.63	5.98	16.56	5.20
K1	Mean±SD	22.37±1.17^{g}	20.98±1.87^{f}	4.88±1.01^{e}	1.07±0.08^{e}
	Variation limits	20.41-24.85	16.13-24.60	2.60-7.50	0.94-1.29
	CV%	5.23	8.91	20.69	7.47
'Lang'	Mean±SD	41.79±3.34^{a}	29.98±2.20^{c}	17.15±2.97^{b}	1.40±0.08^{c}
	Variation limits	34.14-46.55	24.68-33.84	10.30-22.20	1.24-1.60
	CV%	7.99	7.33	17.31	5.71
S1	Mean±SD	32.28±3.01^{d}	17.77±1.38^{h}	4.71±0.96^{e}	1.82±0.16^{a}
	Variation limits	25.53-37.38	15.81-20.11	2.70-6.60	1.53-2.20
	CV%	9.32	7.76	20.38	8.79
Jurilovca	Mean±SD	23.14±1.58^{g}	19.46±1.36^{g}	4.13±0.78^{e}	1.19±0.06^{d}
	Variation limits	20.33-27.25	16.70-22.84	2.80-6.10	1.09-1.37
	CV%	6.82	6.98	18.88	5.04
'Xuancheng Jian'	Mean±SD	40.36±2.18^{a}	25.47±2.82^{e}	12.32±2.67^{c}	1.60±0.17^{b}
	Variation limits	36.23-44.68	21.07-33.50	7.90-19.40	1.14-1.94
	CV%	5.40	11.07	21.67	10.62
R5	Mean±SD	35.60±5.37^{c}	28.93±3.87^{c}	13.90±4.90^{c}	1.23±0.08^{d}
	Variation limits	26.12-43.45	22.15-34.38	7.40-21.90	1.11-1.35
	CV%	15.08	13.37	35.25	6.50
'Dabaizao'	Mean±SD	33.70±1.96^{d}	33.15±2.45^{b}	16.55±3.24^{b}	1.02±0.02^{efg}
	Variation limits	31.84-36.27	30.87-36.46	12.80-20.60	0.99-1.03
	CV%	5.81	7.39	19.57	1.96
'Lizao'	Mean±SD	37.64±2.99^{b}	35.74±4.17^{a}	22.52±7.08^{a}	1.06±0.07^{ef}
	Variation limits	32.17-41.37	29.28-42.31	12.40-35.70	0.94-1.17
	CV%	7.94	11.66	31.43	6.60

*Mean ± Standard Deviation; Variation limits: Minimum-Maximum; CV%-Coefficient of variation; **Different letters indicate statistically significant differences between the analysed parameters ($p<0.05$).

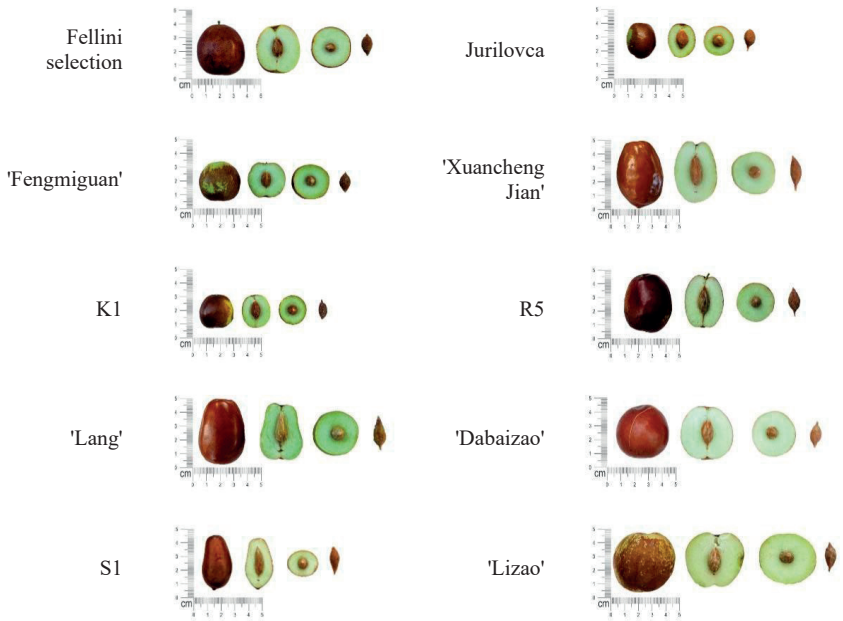


Figure 1. Fruit morphological traits of the studied jujube genotypes (single fruit, longitudinal section, cross-section, and kernel)

The highest fruit height was observed in the 'Lang' cultivar (46.55 mm), while the lowest was found in Jurilovca (20.33 mm). The coefficient of variation for fruit height ranged from 5.23% (K1) to 15.08% (R5). Fruit diameter varied from 15.81 mm (S1) to 42.31 mm ('Lizao'), with coefficients of variation ranging from 5.98% ('Fengmiguan') to 13.37% (R5). The total fruit weight ranged from 2.60 g (K1) to 35.70 g ('Lizao'), with coefficients of variation between 15.73% (Fellini selection) and 35.25% (R5).

Șumălan et al. (2025) report an average fruit height of 39.30 mm, an average diameter of 26.10 mm, and a weight of 10.32 g for the 'Junzao' cultivar. In contrast, Stoli (2023) recorded a fruit height of 43.81 mm, a diameter of 37.03 mm, and a weight of 18.67 g for the same cultivar, which are lower values than those found for the 'Lang' cultivar in the present study. Regarding the Jurilovca selection, Șumălan et al. (2025) reported an average fruit height of 20.20 mm, an average diameter of 16.30 mm, and an average weight of 3.43 g, which are lower results compared to those obtained in this study, likely due to differing pedoclimatic conditions.

Stoli (2023), in a study on jujube genotypes cultivated in Dobrogea, Romania, reported three Jurilovca selections with fruit height ranging from 18.34 to 23.76 mm, fruit diameter from 16.75 to 20.33 mm, and fruit weight between 2.18 and 3.30 g. Stănică (2009) also noted fruit weights for two Jurilovca selections, ranging from 1.30 g to 1.35 g, which differ from the results found in this study. Poșta et al. (2021) observed two genotypes from Timișoara, Romania, with fruit heights ranging from 19.24 to 22.68 mm, diameters from 18.13 to 21.82 mm, and fruit weights between 5.47 and 6.13 g. Stănică and Vasile (2008) reported for the 'Xuancheng Jian' cultivar a fruit height of 36.9 mm, a diameter of 20/21 mm, and a fruit weight of 8.55 g, while Stoli (2023) found a fruit height of 41.00 mm, a diameter of 33.85 mm, and a fruit weight of 13.98 g - values differing from those identified in the present study under different cultivation conditions. For the 'Fengmiguan' cultivar, Stănică and Vasile (2008) recorded a fruit height of 27.00 mm, a diameter of 23/25 mm, and a fruit weight of 9.17 g, consistent with this study's results. Grygorieva et al. (2014) reported 28 jujube genotypes from Ukraine with fruit heights ranging from 20.73 to 44.84 mm, diameters from 16.64 to 38.87 mm, and fruit weights between 2.90 g and 20.99 g. Čizmović and Prenkić (2018) found that the 'Lang' and 'Li' cultivars had the highest fruit weights (24.32 g and 23.97 g, respectively). Esmail (2006) for the same cultivars in Giza, Egypt, found fruit heights between 40.70 and 43.30 mm, widths between 32.10 and 33.50 mm, and fruit weights between 16.51 and 19.42 g for 'Lang' cv. and fruit heights between 39.70 and 38.40 mm, widths between 33.10 and 33.70 mm, and fruit weights between 18.79 and 16.73 g for 'Li' cv., measured over two consecutive years. The highest coefficient of variation in this study was observed in the total fruit weight, while the lowest was found in the shape index, as confirmed by Șumălan et al. (2025). Regarding the shape index, according to Liu & Wang (2009), the Fellini selection, 'Fengmiguan', K1, 'Dabaizao', and 'Lizao' fall into the category of globose or nearly globose fruits. Jurilovca and R5 are categorized as oblong-globose fruits; 'Lang' and 'Xuancheng Jian' have cylindrical fruits, and S1 has an elongated cylindrical shape. Stoli (2023) reported a fruit shape index of 1.1 for Jurilovca selections and 1.2 for 'Xuancheng Jian' and 'Junzao'.

The correlation coefficients (Table 2) for all analysed fruits were $r = 0.933$ ($R^2 = 0.8913$) for the relationship between fruit diameter and total weight, $r = 0.728$ ($R^2 = 0.5299$) for the relationship between fruit height and total weight, $r = 0.509$ ($R^2 = 0.2586$) for the correlation between fruit height and diameter, and $r = 0.574$ ($R^2 = 0.3294$) for the correlation between height and shape index. Some genotypes also showed positive correlations between shape index and fruit height and width, respectively (Table 2). The Pearson correlation coefficients (r) quantify the degree of correlation between the analysed morphological parameters for each genotype and all jujube fruits by assessing the strength and direction of the linear relationship between the two variables. In contrast, R^2 represents the proportion of variance in the dependent variable that is explained by the independent variables.

Table 2. Pearson correlation coefficients between the analysed morphological traits of jujube fruits

Cultivar	Fruit parameter	Height	Width	Weight	Shape index
Fellini selection	Height	1	.502**	.687**	.365*
	Width	.502**	1	.956**	-.619**
	Weight	.687**	.956**	1	-.403**
	Shape index	.365*	-.619**	-.403**	1
'Fengmiguan'	Height	1	.654**	.790**	.516**
	Width	.654**	1	.903**	-.309*
	Weight	.790**	.903**	1	-.024
	Shape index	.516**	-.309*	-.024	1
K1	Height	1	.677**	.780**	-.142
	Width	.677**	1	.960**	-.819**
	Weight	.780**	.960**	1	-.677**
	Shape index	-.142	-.819**	-.677**	1
'Lang'	Height	1	.741**	.842**	.487**
	Width	.741**	1	.903**	-.225
	Weight	.842**	.903**	1	.045
	Shape index	.487**	-.225	.045	1
S1	Height	1	.494**	.881**	.639**
	Width	.494**	1	.672**	-.351
	Weight	.881**	.672**	1	.353
	Shape index	.639**	-.351	.353	1
Jurilovca	Height	1	.734**	.825**	.340*
	Width	.734**	1	.934**	-.387**
	Weight	.825**	.934**	1	-.169
	Shape index	.340*	-.387**	-.169	1
'Xuancheng Jian'	Height	1	.277	.431**	.232
	Width	.277	1	.903**	-.862**
	Weight	.431**	.903**	1	-.685**
	Shape index	.232	-.862**	-.685**	1
R5	Height	1	.894**	.933**	.483
	Width	.894**	1	.939**	.042
	Weight	.933**	.939**	1	.231
	Shape index	.483	.042	.231	1
'Dabaizao'	Height	1	.989**	.991**	-.812*
	Width	.989**	1	.978**	-.888*
	Weight	.991**	.978**	1	-.801
	Shape index	-.812*	-.888*	-.801	1
'Lizao'	Height	1	.803**	.837**	-.186
	Width	.803**	1	.973**	-.732*
	Weight	.837**	.973**	1	-.643*
	Shape index	-.186	-.732*	-.643*	1
All jujube fruits	Height	1	.509**	.728**	.574**
	Width	.509**	1	.933**	-.390**
	Weight	.728**	.933**	1	-.095
	Shape index	.574**	-.390**	-.095	1

**. Correlation is significant at the 0.01 level (2-tailed).

Ivanišová et al. (2017) reported correlation coefficients for jujubes from Ukraine of r = 0.97 (between fruit diameter and weight), r = 0.77 (between fruit height and weight), and r = 0.65 (between fruit height and diameter). Ma et al. (2021) observed an r = 0.95 for the relationship between fruit weight and diameter in the 'Lingwu Changzao' cultivar. Khadivi et al. (2021) found positive correlations between fruit weight and width (r = 0.75), fruit length and width (r = 0.71), and fruit weight and length (r = 0.70) in several jujube accessions from Iran. Luo et al. (2024) also found positive correlations between the same parameters in some jujube F1 hybrids. Fruit diameter has a strong influence on total fruit weight, as evidenced by the results obtained and supported by findings in the literature.

Cluster analysis (Figure 2), conducted using the Ward method, divided the studied genotypes into two main groups. Analysing the dendrogram based on morphological characteristics, it can be deduced that the genotypes Jurilovca and K1; 'Lang' and 'Xuancheng Jian'; and 'Dabaizao', R5, and the Fellini selection share similarities in their analysed morphometric traits.

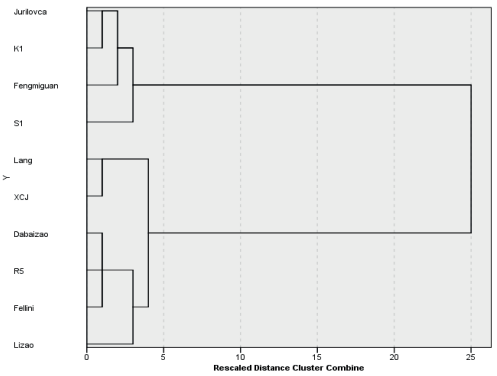


Figure 2. Ward cluster analysis of the studied *Z. jujuba* genotypes based on morphological traits

The morphological characteristics of jujube fruits vary according to genotype, age, and location, and are influenced by pedoclimatic factors and applied cultivation practices (Khadivi et al., 2021). An important factor in evaluating the potential of jujube cultivars for Romania is their resistance to fruit-cracking disease. This common issue affects jujube yield and quality, leading to significant economic losses (Wang et al., 2021; Liu et al., 2025). Based on morphological observations and comparisons with scientific literature, all the studied genotypes are highly resistant to fruit cracking, except for the 'Lang' cultivar. According to Li & Gao (1990), the elasticity and plasticity of the fruit flesh are the main factors influencing cultivar susceptibility. Fruit cracking is a response to unfavourable environmental conditions and the growth of fruit flesh, creating favourable conditions for pathogens. Hua et al. (2015) highlight both internal physiological factors (such as mineral nutrition, sugar accumulation, and fruit firmness) and external factors (including

climate, soil, fertilizers, and crop management) as key contributors to fruit cracking disease. Li et al. (2019) mention that jujube fruits are prone to cracking during the colouring period, with the fruit surface serving as the primary point of water entry, which ultimately contributes to the cracking. Stomata and surface microcracks act as sites for water absorption, making them the key factors responsible for fruit cracking. Wang et al. (2021) also emphasize that a combination of physiological, genetic, and environmental factors influences fruit cracking. Jujube is a species that thrives in poor soil with minimal water, is highly adaptable to varying climate conditions, and produces early with high yields. Due to these traits, it has the potential to be intensively cultivated in areas prone to desertification. Genetic diversity is fundamental for agricultural programs and future development. Morphological characterization is a crucial initial step in identifying plant genetic resources. Therefore, it is imperative to comprehend the characteristics and potential of these valuable resources, as their collection and analysis enable researchers and specialists to effectively utilize them in enhancing traits within breeding programs (Khadivi & Beigi, 2022). The analysed jujube genotypes confirm the species' suitability for Southern Romania. Both genetic variation and environmental conditions contribute to the morphometric traits of jujube fruits.

CONCLUSIONS

The results of this study highlight the main morphometric characteristics of jujube fruits produced in the Southern Oltenia region of Romania. This data can be valuable for selecting suitable cultivars for orchard establishment. Further research will focus on determining the biochemical composition of the fruits and examining the plants' behaviour under the influence of environmental factors.

REFERENCES

Čizmović, M., & Prenkić, R. (2018). Morphological characteristics of fruits and pits of some jujube (*Ziziphus jujuba* Mill.) genotypes. *Book of Proceedings*, 227-232.

Cossio, F., & Bassi, G. (2013). Field performance of six Chinese jujube cultivars introduced and tested in Northern Italy. *Acta Horticulturae*, 993, 21-28. doi: 10.17660/actahortic.2013.993.2

Esmail, S. E. (2006). Morphological and physiological studies on some *Ziziphus jujuba* Mill varieties under Giza conditions. *Journal of Plant Production*, *31*(8), 5213-5226.

Gómez-Devia, L., & Nevo, O. (2024). Effects of temperature gradient on functional fruit traits: an elevation-for-temperature approach. *BMC Ecology and Evolution*, *24*(1), 94. https://doi.org/10.1186/s12862-024-02271-w

Grygorieva, O., Abrahamová, V., Karnatovská, M., Bleha, R., & Brindza, J. (2014). Morphological characteristics of fruits, drupes, and seeds in genotypes of *Ziziphus jujuba* Mill. *Potravinarstvo*, *8*(1), 306-314. doi:10.5219/414

Hua, Y., Song, Y., Li, J., Tian, C., Zhou, X. G., & Li, L. (2015). A proposed systemic modelling software for jujube fruit cracking. *American Journal of Plant Sciences*, *6*(5), 565-573. http://dx.doi.org/10.4236/ajps.2015.65061

Ivanišová, E., Grygorieva, O., Abrahamova, V., Schubertova, Z., Terentjeva, M., & Brindza, J. (2017). Characterization of morphological parameters and biological activity of jujube fruit (*Ziziphus jujuba* Mill.). *Journal of Berry Research*, *7*(4), 249-260. https://doi.org/10.3233/JBR-170162

Karp, D., & Gasic, K. (2024). Register of new fruit and nut cultivars list 52. *HortScience*, *59*(8), 1220-1292. https://doi.org/10.21273/HORTSCI.57.9.1174

Khadivi, A., Mirheidari, F., Moradi, Y., & Paryan, S. (2021). Identification of superior jujube (*Ziziphus jujuba* Mill.) genotypes based on morphological and fruit characterizations. *Food Science & Nutrition*, *9*(6), 3165-3176. https://doi.org/10.1002/fsn3.2276

Khadivi, A., & Beigi, F. (2022). Morphological and chemical characterizations of jujube (*Ziziphus jujuba* Mill.) to select superior accessions. *Food Science & Nutrition*, *10*(7), 2213-2223. https://doi.org/10.1002/fsn3.2831

Li, D., Niu, X., & Tian, J. (2013). *The illustrated germplasm resources of Chinese jujube*. Beijing: China Agriculture Press. ISBN 978-7-109-14752-2

Li, K. Z., & Gao, Z. S. (1990). Preliminary study on the mechanism of fruit cracking in Chinese jujube. *Journal of Fruit Science*, *7* (4), 221-226.

Li, N., Fu, L., Song, Y., Li, J., Xue, X., Li, S., & Li, L. (2019). Water entry in jujube fruit and its relationship with cracking. *Acta Physiologiae Plantarum*, 41, 162. https://doi.org/10.1007/s11738-019-2954-2

Li, Y., Zhou, X., Zhao, K., Liu, J., Chen, G., Zhang, Y., Ma, J., Sun, N., & Li, X. (2024). Cultivation and morphology of jujube (*Ziziphus jujuba* Mill.) in the Qi River Basin of Northern China during the Neolithic Period. *Scientific Reports*, *14*(1), 2305. https://doi.org/10.1038/s41598-024-52260-8

Liu, J., Hou, L., Liu, N., Zhang, C., Zheng, Y., Pang, X., & Li, Y. (2025). Comparative analyses for determining fruit cracking in jujube (*Ziziphus jujuba* Mill.). *Euphytica*, *221*(2), 11. https://doi.org/10.1007/s10681-024-03459-3

Liu, M. (2006). Chinese jujube: botany and horticulture. *Horticultural reviews*, *32*, 229-298.

Liu, M. (2018). The challenges and countermeasures of jujube industry during transition period. *China Fruits*, 1, 1-4.

Liu, M. J., & Wang, M. (2009). *Germplasm resources of Chinese jujube*. China Forestry Publishing House.

Liu, M., Wang, J., Wang, L., Liu, P., Zhao, J., Zhao, Z., Yao, S., Stănică, F., Liu, Z., Wang, L., Ao, C., Dai, L., Li, X., Zhao, X., & Jia, C. (2020). The historical and current research progress on jujube–a superfruit for the future. *Horticulture research*, *7*, 119. https://doi.org/10.1038/s41438-020-00346-5

Luo, Y., Chen, W., Pan, Y., Ge, L., Wu, C., Wang, J., Liu, M., & Yan, F. (2024). Comparison and genetic variation analysis of important fruit traits in jujube F1 hybrids by different male parents. *Agronomy*, *14*(3), 459. https://doi.org/10.3390/agronomy14030459

Ma, Y., Zhang, D., Wang, Z., Song, L., & Cao, B. (2021). Fruit morphology measurements of jujube cultivar 'Lingwu Changzao' (*Ziziphus jujuba* Mill. cv. Lingwuchangzao) during fruit development. *Horticulturae*, *7*(2), 26. https://doi.org/10.3390/horticulturae7020026

Poșta, D. S., Rózsa, S., & Gocan, T. M. (2021). Morphological and chemical analysis of *Ziziphus jujuba* Mill. fruits from Timișoara Botanical Park. *Current Trends in Natural Sciences*, *10*(19), 328-332. https://doi.org/10.47068/ctns.2021.v10i19.042

Sapkota, D., Zhang, D., Park, S., Meinhardt, L. W., Lozada, D. N., Steiner, R., & Yao, S. (2024). Genetic diversity and population structure of jujube cultivars in the United States revealed by single nucleotide polymorphism markers. *Journal of the American Society for Horticultural Science*, *149*(2), 107-120. https://doi.org/10.21273/JASHS05370-23

Shahrajabian, M. H., Sun, W., & Cheng, Q. (2020). Chinese jujube (*Ziziphus jujuba* Mill.)–a promising fruit from Traditional Chinese Medicine. *Annales Universitatis Paedagogicae Cracoviensis Studia Naturae*, 194-219. https://doi.org/10.24917/25438832.5.13

Stan, E. G., Iliescu, L. M., & Stănică, F. (2021). Jujube processing: methods, products and nutraceutical value. *Scientific Papers. Series B. Horticulture*, *65*(2), 83-92.

Stănică, F. (1997). Rezultate preliminare privind microînmulțirea *in vitro* a curmalului chinezesc (*Ziziphus jujuba* Mill.). *Scientific Debates Cluj-Horticulture XX*. USAMV Cluj-Napoca. 236-238.

Stănică, F. (2009). Characterization of two Romanian local biotypes of *Ziziphus jujuba. Acta Horticulturae,* 840, 259–262. doi:10.17660/actahortic.2009.840

Stănică, F. (2016). Chinese dates (jujubes): a new promising fruit plant for Romania. *In* Liu, D., Ye, X., & Jiang, Y. (Eds.). *Chinese Dates: A Traditional Functional Food*, 363-373. ISBN 9780429160509

Stănică, F. (2019). Twenty years of jujube (*Ziziphus jujuba* Mill.) research in Romania. *Scientific Papers. Series B, Horticulture*, *63*(2), 17-23.

Stănică, F., & Vasile, S.A. (2008). Chinese date - a new promising fruit plant for Romania Southern areas. *Acta Horticulturae*, 769, 265-269. DOI: 10.17660/ActaHortic.2008.769.38

Stănică, F., Dicianu, D. E., Butcaru, A. C. & Liu, M. J. (2020). Jujube fruits behavior at controlled atmosphere storage. *Acta Horticulturae*, 1287, 317-320. DOI: 10.17660/ActaHortic.2020.1287.40

Stoli, I. (2023). Studiul comportării unor genotipuri de curmal chinezesc (*Ziziphus jujuba* Mill.) în condițiile din zona Dobrogea. (PhD Thesis, Universitatea de Științe Agronomice și Medicină Veterinară din București, Școala doctorală Ingineria și managementul resurselor vegetale și animale). rei.gov.ro

Stoli, I., & Stănică, F. (2021). Review on some features of the Chinese jujube (*Ziziphus jujuba* Mill.). *Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Horticulture*. *78*(1), 10-16. https://doi.org/10.15835/buasvmcn-hort:2020.0033

Stoli, I., & Stănică, F. (2023). Chemical analysis of some local and cultivated jujube genotypes (*Ziziphus jujuba* Mill.) from Dobrogea region. *Scientific Papers. Series B. Horticulture*, *67*(1), 186-192.

Stoli, I., Stănică, F., Liu, M.J., Asănică, A.C. & Peticilă, G.A. (2022). Preliminary jujube grafting results on different Romanian rootstocks. *Acta Horticulturae*. 1350, 263-268. DOI: 10.17660/ActaHortic.2022.1350.31

Șumălan, R. L., Copolovici, D. M., Crișan, M., Stănică, F., Șumălan, R. M., Lupitu, A., Vicas, S. I., Mot, S., Copolovici, L., & Ciulcă, S. (2025). Assessment of fruit traits and antioxidant capacity in wild and cultivated genotypes of *Ziziphus* sp. *Plants*, *14*(1), 134. https://doi.org/10.3390/plants14010134

Wang, Y., Guo, L., Zhao, X., Zhao, Y., Hao, Z., Luo, H., & Yuan, Z. (2021). Advances in mechanisms and omics pertaining to fruit cracking in horticultural plants. *Agronomy*, *11*(6), 1045. https://doi.org/10.3390/agronomy11061045

Wang, Y., Sui, C., Li, D., Zhao, A., Ren, H., Xue, X., and Du, X. (2017). Breeding of a new very early ripening Chinese jujube cultivar 'Zaohongmi'. *Journal of Fruit Sci*ence, *34* (10):1361-1364. DOI: 10.13925/j.cnki.gsxb.20170213

Xu, C., Gao, J., Du, Z., Li, D., Wang, Z., Li, Y., & Pang, X. (2016). Identifying the genetic diversity, genetic structure and a core collection of *Ziziphus jujuba* Mill. var. jujuba accessions using microsatellite markers. *Scientific reports*, 6(1), 31503. https://doi.org/10.1038/srep31503

Yao, S. (2013). Past, present, and future of jujubes—Chinese dates in the United States. *HortScience*, *48*(6), 672-680. https://doi.org/10.21273/HORTSCI.48.6.672

Zhang, Y., Cao, D., Yao, J., Li, M., Wang, M., Li, J., Feng, J., & Li, J. (2024). Jujube, nutritious fruit on Chinese traditional diet. *Scientific Bulletin. Series F. Biotechnologies*, *28*(1), 79-86.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

DAMAGES ON *FRAGARIA ANANASSA* VAR. 'SNOW WHITE' CAUSED BY *ERWINIA AMYLOVORA* AND POTENTIAL OF *CHAENOMELES* EXTRACT FOR DISEASE CONTROL

Mariya STOYANOVA[1], Elena GRANCHAROVA[1], Miroslava VALKOVA[1], Teodora MIHOVA[2]

[1]Institute of Soil Science, Agrotechnologies and Plant Protection "Nikola Pushkarov", Agricultural Academy - Sofia, 7 Shosse Bankya Str., Sofia, Bulgaria
[2]Research Institute of Mountain Stockbreeding and Agriculture, Agricultural Academy, 281 Vasil Levski Str., Troyan, Bulgaria

Corresponding author email: mistoyanova@abv.bg

Abstract

Plant pathogens present serious challenges before producers. Erwinia amylovora is a polyphagous pathogen that also affects strawberries. However, data on bacterial pathogens on this crop is scarce worldwide and lacks in Bulgaria. The present study aimed to investigate the damages that E. amylovora induces in greenhouse-grown strawberries (Fragaria ananassa) from the variety "Snow white" in Bulgaria, with respect to different irrigation and fertilization levels, and the potential of a plant-derived extract from fruits of Chaenomeles sp. to be used for disease control. The extract has previously shown to have good antibacterial activity and it exhibited no phytotoxicity on the strawberry plants. The results showed raised leaf losses for the inoculated with pathogen vs the non-inoculated plants and vs the inoculated and treated with extract plants. The flower losses due to infection are significant and the inoculated plants lose about 2.3 times more flowers compared to the non-inoculated plants and about 2 times more flowers compared to the inoculated and treated plants, thus revealing a clear tendency towards flower damaging caused by the pathogen and a protective effect of the used extract.

Key words*: Erwinia amylovora, Fragaria ananassa, disease control, Chaenomeles sp., plant extract.*

INTRODUCTION

Plant pathogens present serious challenges before producers. *Erwinia amylovora* is a polyphagous pathogen that poses a major threat worldwide, infecting, spreading and surviving on an extremely large number of plant species. These factors make this devastating disease difficult to control. Copper preparations and antibiotics are still the most effective solutions to combat the pathogen, although their use contributes to environmental pollution and the development of resistant populations. In Europe, the use of antibiotics for agricultural purposes is prohibited, due to considerations of increasing antibiotic resistance among medical strains of pathogens and the protection of human health. Therefore, the need of new plant protection products is still urgent and pressing.

Plants synthesize secondary metabolites, some of which are highly active against certain pathogens, and offer promising prospects for alternative plant disease control and bioproduction. The scientific interest in these natural sources of potential control agents is not waning. Plant extracts have been investigated for antimicrobial properties against pathogens, but still from a very limited number of plant species (Stangarlin et al., 1999; Schwan-Estrada et al., 2005; Mohamed, 2010; Stoyanova & Valkova, 2014; 2015a; 2015b). The limited data is mainly due to several elements: a large number of pathogens, local strain differences, a huge number of plant species that could be tested, different extraction methods with corresponding differences in the composition of the finished extracts and potential differences in the methods of application and/or the concentrations used. Most investigations are *in vitro* studies and chemical analyses, but experiments with living plants are extremely insufficient. The reasons are different, and in some cases the main problem is the host crop of a certain pathogen. The nature of fruit tree species, which suffer the most from *E. amylovora*, is a major obstacle to experimental

work. Experiments with orchards require large areas, specific isolation requirements, years for trial preparation, industrial quantities of the tested candidate drug and potential eradication of the orchard at the end of the study. A potential solution to the problem is the discovery of plant-based products with activity against the causal agent, development and optimization of their application on more convenient for cultivation, treatment and monitoring host crops, such as strawberries. This approach can be compared to the practice in pharmacy and medicine, where research on potential drugs is tested on mice before moving on to testing on volunteers. On the other hand, the discovery of a biological agent for controlling fire blight of strawberries would be beneficial itself given the way of consumption of the fruits - after simply washing with water without the possibility of "scrubbing" the surface or peeling. Data on bacterial pathogens on strawberries is scarce worldwide.

Some plant extracts have shown activity against *E. amylovora in vitro* (Stoyanova & Valkova, 2014; Stoyanova et al., 2024). A promising option for alternative biocontrol of the pathogen presents the extract from *Chaenomeles* sp. fruits. The plant species is unpretentious, native for Asia and introduced in many European countries. Extracts from several genotypes have been tested, all of which possessed antibacterial activity against *E. amylovora* (Stoyanova et al., 2024). The experiment in this study was based on the hypothesis that preventive spraying of host plants with the plant extract may reduce the pathogen population on the leaf surface and thus, to prevent its entry into plant tissues and to reduce the development of disease symptoms on plants.

The study aimed to investigate the damages that *E. amylovora* induces in greenhouse-grown strawberry plants (*Fragaria ananassa*) from the variety “Snow white”, with respect to different irrigation and fertilization levels, and the potential of a plant-derived extract from fruits of *Chaenomeles* sp. to be used for disease control.

MATERIALS AND METHODS

Plant material: Fruits from *Chaenomeles* sp. plants genotype 6’ and genotype 29 originating from the field of the Research Institute of Mountain Stockbreeding and Agriculture, Agricultural Academy, Troyan, were used in this study. The fruits were collected in the autumn of the year prior to the experiment and stored freezed at -20°C.

Extractions: Extracts were prepared from smashed fruits in methanol (HPLC grade) using Soxhlet at 80°C for 5 hours. The solvent was separated in vacuum vaporizer (model Hei VAP Precision, Heidolph Inst.) at 55°C, 30 000 Pa. The extracts were concentrated at decreasing pressure up to 7 200 Pa and increasing temperature up to 70°C. Storage of the extracts was in brown bottles, at 18°C, in the dark. Working solutions of the extracts was 2% (w/v, in water), freshly prepared 1 h before the treatments. The extracts were applied by spraying. Choice of dilution was based on a previous study for *in vitro* antibacterial activity and minimal inhibitory concentration of extracts from the two genotypes (Stoyanova et al., 2024).

Bacterial strains: *Erwinia amylovora* NBIMCC 2331 (=ATCC 15580, type strain, isolated from pear) and NBIMCC 8473 (Bulgarian strain from *Fragaria ananassa*) were used for inoculations of the plants. Bacteria were applied as water suspensions with transparency 65% (NBIMCC 2331) and 75% NBIMCC 8473 (measured with turbidimeter Biolog Inc.) by spraying.

Test plants: A total of 153 strawberry plants *Fragaria ananassa* variety 'Snow White' grown in a non-heated polyethylene tunnel greenhouse, located at Experimental base “Chelopechene” of the Institute of Soil Science, Agrotechnologies and Plant Protection “Nikola Pushkarov”, Agricultural Academy, Sofia, Bulgaria (latitude 42°44′22.8″N, longitude 23°28′3.7″E and altitude 550 m above sea level), were subjects of this study. The plants were drip irrigated and mulched with silver-black UV polyethylene film with a thickness of 30 μm. Covering of the greenhouse was with a five-layer UV+EVA+IR+AD+dif -150 μm polyethylene film.

Phytotoxicity tests: During the growing season, prior to the main experiment, a preliminary investigation to establish possible phytotoxicity of the used extracts on white strawberry plants in 2 replicates was carried out. In the first replicate, strawberries were grown in pots with a diameter of 30 cm and 10 L of soil at outdoor temperatures, outdoor humidity and artificial

irrigation, and in the second - in pots with a diameter of 30 cm and soil 10 L of soil under outdoor conditions. The test plants were divided into four lots with five plants per lot for each of the two extracts: 1) non-treated (unsprayed) plants; 2) non-treated plants sprayed with water; 3) treated plants, sprayed with 2% extract; 4) treated plants, sprayed with 5% extract. Treatments were applied 3 times every 7 days.

Treatments: The experiment included two consecutive rounds, each including: a) treatment, letting dry for 1 h, inoculation; b) treatment every 7 days for the next 3 weeks. The first round of the experiment was performed with genotype 6' extract and the type strain of the pathogen. The second round was performed with genotype 29 extract and the strawberry strain of the pathogen. The observations were carried out every week before the treatments. The numbers of withered flowers and leaves of each plant were recorded and the same were removed from the plants and the greenhouse to avoid recounting.

Variants of irrigation and fertilization (VIF): We used 5 VIF: 100% irrigation (I0F0), 50% irrigation and 100% fertilization (I2F1), 75% irrigation and 100% fertilization (I1F1), 75% irrigation and 75% fertilization (I1F2), 50% irrigation and 75% fertilization (I2F2).

Setting of the experiment: For each VIF, plants were divided into three groups: treated non-inoculated (negative control, healthy), treated and inoculated with pathogen (experimental) and inoculated only (positive control, infected). 10±3 plants were used for each option. The study was conducted in the period May-June of 2024.

Statistics: The collected data were statistically analyzed by Duncan's Multiple Range tests at significance levels $p<0.05$ and $p<0.01$ using STATISTICA 8.0.

RESULTS AND DISCUSSIONS

In the preliminary phytotoxicity tests, no negative changes were observed. The leaves of the treated plants did not exhibit any changes in appearance and there were only single withered flowers in all pots with similar count. The overall development of the plants was good and similar in all pots, so it could be concluded that the extracts from *Chaenomeles* were not phytotoxic to strawberry plants.

The symptoms of infection with *E. amylovora* on the leaves of white strawberries in the greenhouse were mainly in the form of marginal necrosis of the young leaves, although diffuse necrosis and chlorosis could also be observed. Necrosis on flower stalks and sepals was very common (Figure 1).



Figure 1. Symptoms of fire blight on leaves and flowers of *F. ananassa* var. 'Snow White'

When considering the total number of dead leaves and flowers (for all VIFs), the highest number of dead leaves was observed in the inoculated non-treated (infected) plants in both rounds of the experiment (Figure 2). In the treated plants (both inoculated - experimental, and non-inoculated - healthy), no dependence was observed in the loss of leaves when comparing the two consecutive experiments - in the second round the experimental plants lost 39% less leaves and the number was also lower compared to the healthy plants. The infected plants also lost less leaves (Figure 1). For the total period of 8 weeks, the results show an increased leaf loss by ~47% of the diseased plants compared to the healthy ones and by ~21% compared to the experimental ones. Much greater differences were observed in the loss of flowers: the diseased plants lose ~2.3 times more flowers compared to the healthy plants and by ~2 times more compared to the experimental plants (Figure 2).

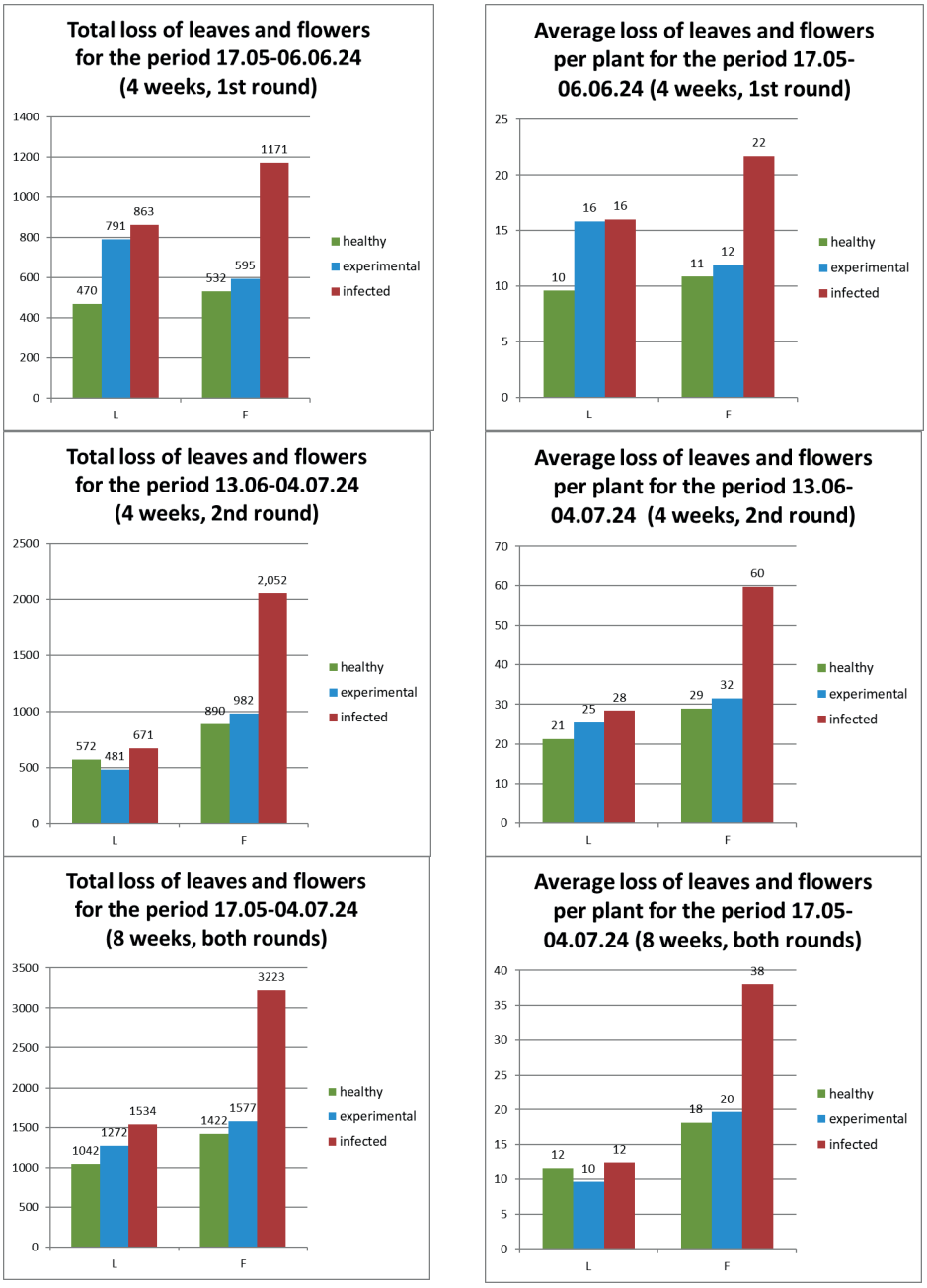


Figure 2. Loss of leaves (L) and flowers (F) of treated, experimental and diseased plants

Flower loss followed the same trend in the both rounds of the experiment - the ratios among the three groups are very similar (Table 1).

Table 1. Approximate ratios of leaf and flower loss between the two rounds of the experiment (values are rounded)

	H: E: I	
	leaves	flowers
1 round	2.5: 4: 4	1.1: 1.2: 2.2
2 round	2.6: 3: 3	1: 1.1: 2

Distribution among VIFs of leaf loss did not follow a clear trend (Figure 3). Greatest leaf loss was observed for infected plants from variants I1F1 and I1F2, which are the richest combinations between irrigation and fertilization. These are also the only two VIFs, at which the plants lost less leaves if treated compared to the infected plants. At the variants with the most restricting conditions I2F1 and I2F2 leaf loss was intricate. At I0F0 leaf loss of experimental plants is similar to the infected ones (Figure 3).

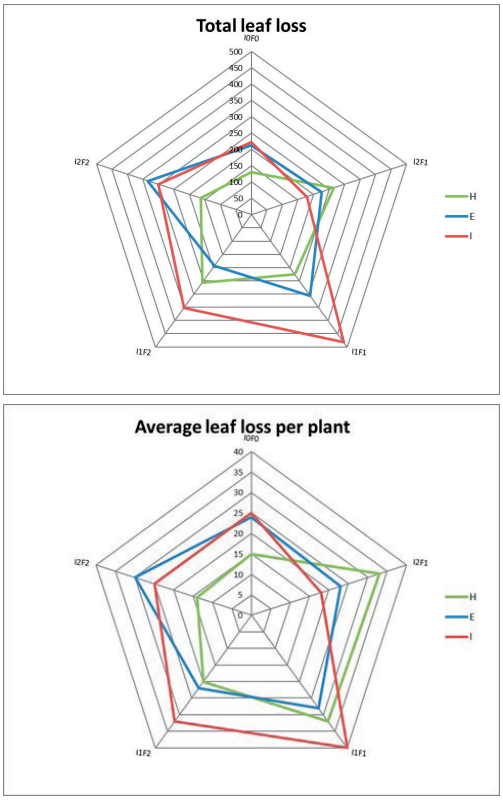


Figure 3. Leaf loss by variants (VIFs)

The loss of flowers was much greater and followed a certain trend - the counts were always the highest for the infected only plants (Figure 4). Greatest leaf loss was observed for infected plants from variants I1F1 and I2F2, which are the richest and the most restricting combinations, respectively, between irrigation and fertilization. At all variants leaf loss was greatly reduced for the experimental plants (Figure 4).

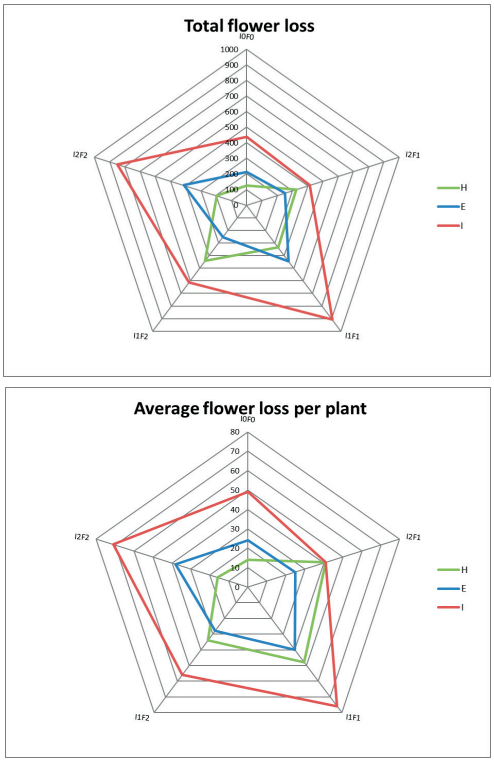


Figure 4. Flower loss by variants (VIFs)

At this point, the richest combination of irrigation and fertilization I1F1 seems to favor the development of fire blight in means of leaf and flower loss and the treatments with chaenomeles extract was able to lower these losses.

Distribution among VIFs of flower loss (with the exception of I2F1) showed a repetitive tendency between the two rounds of the experiment: I0F0 and I2F2 plants (grown at the most water favorable and the most unfavorable conditions) lost the most flowers if infected and the least - if healthy, with the experimental ones positioning in the middle. In the other variants, the experimental plants lost less flowers compared to the negative control (Figure 5).

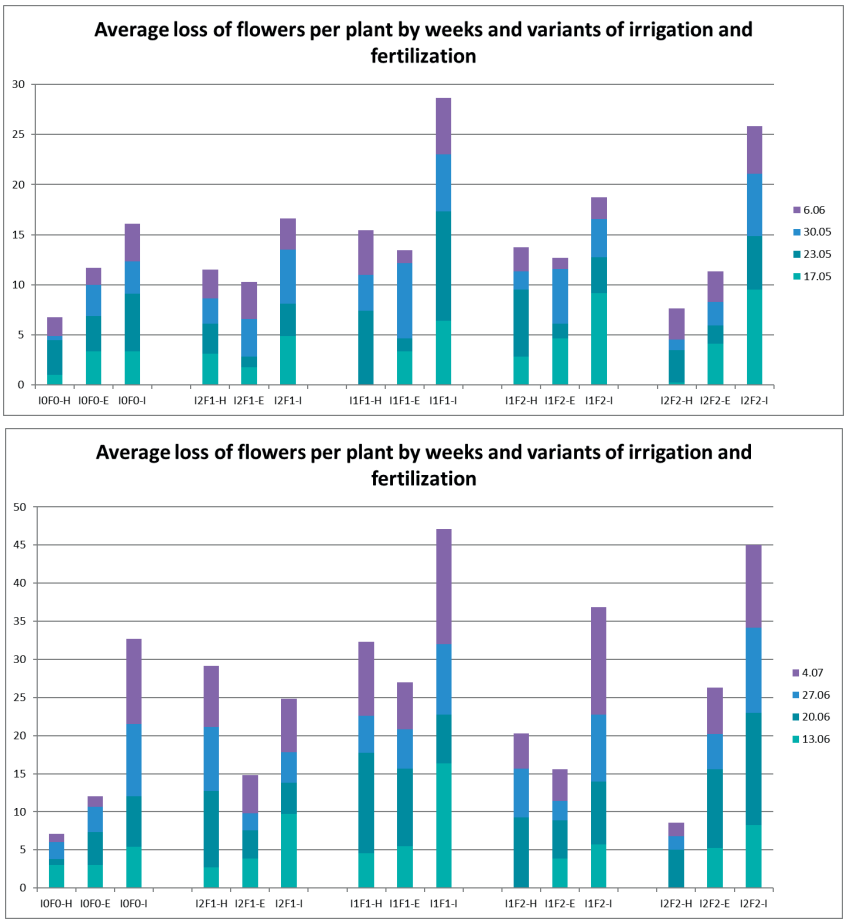


Figure 5. Average flower loss per plant by variants (VIFs)

Statistical analysis of all collected data for leaf loss showed significant differences ($p<0.05$) among all 15 variant groups (experimental groups with regards to the VIF), although only I0F0-H, I2F2-H (the healthy plants, grown at the most water favorable and the most restricting conditions) and I1F1-I are clearly separated from all others (Table 2).

Table 2. Average loss of leaves of treated, experimental and infected plants with regard to VIFs

Leaves	Healthy	Experiment	Infected
I0F0	7.28a	11.78ab	12.33abc
I2F1	16.56bc	11.30ab	8.90ab
I1F1	16.07bc	13.91abc	20.04c
I1F2	9.88ab	10.78ab	16.05bc
I2F2	6.83a	15.12bc	12.50abc

Values with same lowercase letter for the same parameter were not statistically different.

There were no statistically significant differences in leaf loss among the three groups (healthy-treated, experimental-treated inoculated and infected-inoculated) in each round of the experiment as well as among the VIFs.

Statistical analysis of all collected data for flower loss showed significant differences ($p<0.01$) among all 15 variant groups (experimental groups with regards to the VIFs), although only I0F0-H (the healthy plants, grown at the most water favorable conditions) and I1F1-I (the infected plants from the richest combination of VIF) are more clearly separated from all others (Table 3).

Very high statistical significance $p<0.001$ was observed among the groups in flower loss: the infected plants differentiate from the healthy and experimental ones (Table 4).

Table 3. Average loss of flowers of treated, experimental and infected plants with regard to VIFs

Flowers	Healthy	Experiment	Infected
I0F0	6.94a	11.83abc	24.39cde
I2F1	20.31bcd	12.55abc	20.7bcd
I1F1	23.86cde	20.23bcd	37.875f
I1F2	17.04abcd	14.11abc	27.77def
I2F2	8.13ab	18.82abcd	35.42ef

Values with same lowercase letter for the same parameter were not statistically different.

Table 4. Average loss of flowers of the groups

Experiment	Flowers
healthy	15.26a
experiment	15.51a
infected	29.23b

Values with same lowercase letter for the same parameter were not statistically different.

There was also a significant (p<0.01) difference between the two rounds of the experiment due to the much greater flower loss during the second round. However, the ratio of the values between the three groups is similar (Table 1). Three factors can have an effect on the experimental results – the pathogen strain, the genotype used for extraction and the temperature range during the experimentation period. Since the trend of losses between the two rounds is the same (losses increased in all the three groups with a similar ratio) it is very unlikely that the strain or the genotype exerted great effects, since both factors influenced only two of all three groups. At the same time, temperatures during the second period are much higher (Table 5) and they influence all the plants. The maximum temperature of the second period is 4°C higher compared to the first period and a gradual rising trend of the maximum and the average temperatures during the weeks of the first period can be observed. Higher temperatures can have an additive effect to the physiological leaf and flower loss.

Table 5. Basic temperature data during the experimental period (average data, °C)

Week	09.05-17.05	18.05-23.05	24.05-30.05	31.05-06.06	Average ±SD
Min.	4.82	9.46	7.80	11.03	8.28±2.66
Max.	38.06	43.13	44.94	47.71	43.46±4.06
Average	16.28	20.36	21.93	27.29	21.46±4.55

Week	07.06-13.06	14.06-20.06	21.06-27.06	28.06-04.07	Average ±SD
Min.	13.93	9.24	16.94	11.10	12.80±3.37
Max.	49.51	50.89	51.70	48.91	50.25±1.27
Average	29.21	28.67	29.90	28.23	29.01±0.72

Round	09.05-06.06	06.06-05.07	variance
Min.	4.82	9.24	4.42
Max.	47.71	51.70	3.99
Average	21.14	29.02	7.87

The flower losses in the two rounds of the experiment reveal the real effect which the pathogen *E. amylovora* exerts on strawberry plants. Though the loss of leaves is not so obvious, the infection progresses in a "sneaking" way causing significant flower loss, which can be missed below the surfaces of the leaves. At the same time, treatments with *Chaenomeles* extract lower these losses to levels that are comparable to the healthy plants

CONCLUSIONS

E. amylovora can cause a "sneaking" infection on strawberry plants *F. ananassa* var. 'Snow White', grown in greenhouse, causing significant loss of flowers. Water solution of concentrated *Chaenomeles* extract can exert a protective effect against the infection with the pathogen, lowering the flower loss to levels comparable with these of the healthy plants.

ACKNOWLEDGEMENTS

This research work was supported by the Bulgarian National Science Fund under grant No. КП-06-Н66/4.

REFERENCES

Mohamed, E. (2010). Antiviral properties of garlic cloves juice compared with onion bulbs juice against potato virus Y (PVY). *Journal of American Science*, *6*(8), 302–310.

Schwan-Estrada, K.R.F., Stangarlin, J.R. (2005). Extracts and essential oils of medicinal plants in the resistance induction against plant pathogens. In: Cavalcanti, L.S., Di Piero, R.M., Cia, P., Pscholati, S.F., Resende, M.L.V., Romeiro, R.S., eds. *Resistance induction in plants against pathogens and insects*. Piracicaba: FEALQ, p. 125–138.

Stangarlin J.R., Schwan-Estrada, K.R.F., Cruz, M.E.S., Nozaki, M.H. (1999). Medicinal plants and alternative control of phytopathogens, *Biotecnologia Ciência & Desenvolvimento,* 11, 16–21.

Stoyanova, M., & Valkova, M. (2014). Plant extracts with potential to control *Clavibacter michiganensis* subsp. *michiganensis* - causal agent of bacterial canker of tomato. In: *Proceedings of Seminar of Ecology - 2014 with International Participation*, 24-25 April 2014, Sofia, Bulgaria, p. 104–110. ISBN: 979-853-476-132-4

Stoyanova, M., &Valkova, M. (2015a). Antibacterial activity of extracts from medicinal and weed plants against phytopathogens of tomato and pepper. *Proceedings of the Union of Scientists – Ruse*, *7*(3), 246–251. ISSN 1311-1078

Stoyanova, M., & Valkova, M. (2015b). Antibacterial activity of extracts from *Chelidonium majus* against *Xanthomonas euvesicatoria* causing bacterial spot of pepper. *Science and Technologies*, *5*(6), 8-12. ISSN 1314-4111

Stoyanova, M., Valkova, M., Mihova T. (2024). Antibacterial properties of plant extracts against the pathogen of *Rosaceae* plants *Erwinia amylovora*. *Acta Microbiologica Bulgarica, 40*(3), 358–365. https://doi.org/10.59393/amb24400310.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

TOWARDS *IN VITRO* CONSERVATION OF SEVERAL PLUM CULTIVARS (*PRUNUS DOMESTICA* L.)

Larisa-Daniela STRÎMBU (VLAȘIN)[1, 2], Luminița-Antonela ZAGRAI[1], Zsolt JAKAB-ILYEFALVI[1], Ioan ZAGRAI[1], Georgeta Maria GUZU[1, 2], Mirela Irina CORDEA[2]

[1]Fruit Research and Development Station Bistrița, 3 Drumul Dumitrei Nou Street, 420127, Bistrița, Romania
[2]Faculty of Horticulture and Business in Rural Development, University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, 3-5 Calea Mănăștur Street, 400372, Cluj-Napoca, Romania

Corresponding authors emails: luminita.zagrai@scdp-bistrita.ro, mcordea@usamvcluj.ro

Abstract

Prunus domestica L. is one of the most economically important fruit species in the global plum industry, serving as a key species for both fresh consumption and processed products. In vitro micropropagation is one of the most suitable methods to preserve the valuable cultivars of species. Twenty nine plum cultivars certified at Prebasic category were initiated on Woody Plant Medium (WPM) with benzylaminopurine (BAP) or meta-topolin (mT), to assess their effect on initiation rate. Although the average of both variants showed a similar initiation rate, there were significant differences between some cultivars. During the multiplication stage, the explants from 19 plum cultivars were cultivated on two medium (WPM or Murashige & Skoog) with the same hormones, and the multiplication coefficient (number of proliferated shoots) and regeneration efficiency (proportion of explants developing into healthy plants) were assessed. Results revealed that both indicators proved to be highly variable dependending mainly on cultivar and less of the four culture variants.

Key words*: cultivars, cytokinins, initiation rate, multiplication coefficient, plum preservation.*

INTRODUCTION

Prunus domestica L.*,* commonly known as European plum, is one of the two key pillars of the plum industry. Its adaptability for both fresh consumption and processing has made it valuable crop worldwide, especially in Europe and North America (Hartmann & Neumüller, 2009). Over the past two decades, Romania has ranked as the second - larger producer globally (FAOSTAT, 2025).

Micropropagation is a widely used technique in plant biotechnology for vegetative propagation and an effective tool for the conservation of plant genetic resources, providing several advantages over traditional methods (Bettoni et al., 2024; Sharma & Jain, 2024).

This technique facilitates the preservation of valuable cultivars in a controlled environment, protected from external stressors and pathogens (Nezami & Gallego, 2023). Organogenesis is an important pathway in tissue culture, extremely useful in micropropagation for plant regeneration and in germplasm conservation of horticultural crops (Canli & Tian, 2009; Yao et al., 2011; Romadanova et al., 2016).

Furthermore, it enables rapid multiplication of plant material, which is particularly advantageous for slow-growing woody species, such as plums (Vujović et al., 2022; Nacheva et al., 2023).

In vitro conservation of plum cultivars, together with *ex situ* conservation in an insect-proof house, forms an integrated strategy for genetic resources conservation (Zagrai & Zagrai, 2015; Zagrai et al., 2022). Preserving diverse plum cultivars is essential for maintaining genetic diversity and ensuring the availability of desirable traits for breeding programs and commercial production (Yuan et al., 2009). However, prolonged successive subculturing of *in vitro* plants on fresh medium, along with their maintenance under standard growth conditions, may lead to a decline or loss of the culture's morphogenetic potential (Chauhan et al., 2019).

Moreover, tissue culture technology facilitates the conservation of plant genetic resources for short-, medium-, or long-term, depending on specific requirements and the technique employed (Grout, 1990; Engelmann, 1997). Short-term conservation requires regular sub-culturing to maintain cultures (Sharma & Jain, 2024). Due to a great potential for preserving plant material via micropropagation, our study aims to evaluate the short-term *in vitro* conservation of several plum cultivars by assessing their responses to different culture media and growth regulators. Initiation rate, multiplication coefficient, and regeneration efficiency will be analyzed to identify the most effective strategies for preserving plum cultivars.

MATERIALS AND METHODS

This study was carried out at the Fruit Research and Development Station Bistrița, Romania, in the period of 2023-2025. Annual young shoots from 29 plum cultivars were collected in winter from trees grown in an insect-proof screen house. These included 23 Romanian cultivars ('Agent', 'Andreea', 'Carpatin', 'Centenar', 'Dani', 'Delia', 'Diana', 'Doina', 'Elena', 'Flora', 'Geta', 'Gras ameliorat', 'Iulia', 'Ivan', 'Jubileu 50', 'Matilda', 'Minerva', 'Pescăruș', 'Romaner', 'Silvia', 'Tita', 'Tuleu dulce' and 'Zamfira') and 6 foreign cultivars ('Anna Späth', 'Blue free', 'd`Agen', 'President', 'Reine Claude d'Althan' and 'Stanley').

Nodal segments excised and surface sterilized with a fungal solution containing copper hydroxide (50%) and metallic copper equivalent (50%). They were then washed in sterile distilled water on a plate with a magnetic stirrer for 15 minutes.

The following steps were performed in a horizontal laminar air flow hood: uninodal shoots were immersed in ethanol 70% for 1 minute, then in sodium hypochlorite 20% for 20 min with 2-3 drops of Tween 20. Subsequently, they were rinsed four times with sterile distilled water.

The cultures were incubated in a vegetative growth room at 23±1°C under artificial light conditions, providing 4000 lux and 16/8 hours photoperiod for initiation and multiplication stages.

Initiation stage

Apexes explants (1-3 mm) were excised and cultured on Woody Plant Medium (WPM) supplemented with salts and vitamins (Lloyd & McCown, 1980) in the initiation stage. Various growth hormones were added in two different variants (Table 1).

For the initiation and multiplication stages the culture medium was supplemented with PVP (polyvinylpyrrolidone) 0.05 g/L, acid ascorbic 0.05 g/L and sucrose 30 g/L, and the medium was solidified using Phytoagar 5.8 g/L. Hormones were prepared as stock solutions. The medium was adjusted to pH 5.8 with NaOH and HCl, prior to dispensing and autoclaving at 121°C, 1.1 atm for 20 min. All chemicals were from Duchefa Biochemie B. V.

Table 1. Culture variants for initiation and multiplication stages

Stage	Culture variants	Medium culture	Growth hormones (mg/L)		
			BAP	mT	GA_3
Initiation	VI-1	WPM	0.3	-	-
	VI-2		-	0.3	-
Multiplication	VM-1	WPM	0.5	-	0.5
	VM-2		-	0.5	0.5
	VM-3	MS	0.5	-	0.5
	VM-4		-	0.5	0.5

The apexes were initially cultured in test tubes containing 10 mL of medium with one explant per tube. After four weeks of incubation in the growth room, the initiation rate was assessed based on the cytokinins used. Initiation rate was defined as the percentage of explants actively growing from initial cultured explants, after four weeks on the initiation medium.

Multiplication stage

Media used for the multiplication stage was WPM or MS medium (Murashige and Skoog, 1962), both supplemented with salts and vitamins, supplemented with growth hormones in different combinations (Table 1).

Explants obtained during the initiation stage were transferred to multiplication medium in 200 ml jar containing 50 ml solid medium. In this stage, 19 cultivars were selected for regeneration, including 17 Romanian cultivars ('Andreea', 'Carpatin', 'Centenar', 'Delia', 'Diana', 'Doina', 'Elena', 'Geta', 'Gras ameliorat', 'Matilda', 'Minerva', 'Pescăruș', 'Romaner', 'Silvia', 'Tita', 'Tuleu dulce',

'Zamfira') and 2 foreign cultivars ('Anna Späth' and 'Blue free') in different culture media.

After subcultivation, at 4 weeks on each variant, the multiplication coefficient was determined. The multiplication coefficient was defined as the number of shoots that had proliferated from the initial *in vitro* plant for all replications. In this stage was determined also the regeneration efficiency, which represents the percentage of explants that successfully developed into new and healthy plants after a subcultivation cycle on each variant of culture.

The research involves a comparative evaluation of Woody Plant Medium (WPM) and Murashige & Skoog (MS) medium, along with the plant growth regulators benzylaminopurine (BAP) and meta-topolin (mT), to determine the optimal composition for each cultivar.

Statistical analysis

The data were organised as followed: three replications per each variant and cultivar in initiation phase and four replications for every variant and cultivar in multiplication stage. All data were subjected to an analysis of variance (ANOVA). In the initiation stage, the differrences between the variants were assessed using the Student's t-test ($p<0.05$), while in the multiplication stage, mean values were analysed with the Fisher post hoc test ($p<0.05$, $p<0.01$). Data are expressed as means ± standard deviation (SD). Principal component analysis (PCA) was performed using the average values of the variants in the multiplication stage. Research data was analysed with the aim of XLSTAT statistical software (Addinsoft, France) ver. 2019 1.1, embedded in MS Excel.

RESULTS AND DISCUSSIONS

Initiation stage

The average values of the initiation rate of the 29 cultivars studied on two cultures media was quite similar, 59.05% for VI-1 and 60.14% for VI-2 with no statistical differences (Table 2). This indicates that both cytokinin-type hormones incorporated into the culture media facilitated optimal regeneration and development of the explants during the initial stage for studied plum cultivars.

When take into account the two cultures media for each cultivar, the situation is a bit different. A comparative analysis using the *t-test* indicates that the differences in initiation rates between VI-1 and VI-2 are statistically significant in most cases (cultivars marked with an asterisk).

Table 2. Initiation rate depending the variants (%)

Origin of cultivars	Cultivars	Culture variants	
		VI-1 (%)	VI-2 (%)
Romanian	Agent*	18.17±0.82	**54.5±4.55**
	Andreea	70.58±4.41	66.28±1.75
	Carpatin	85.71±1.29	80.0±5.0
	Centenar*	**50.0±5.0**	27.27±1.72
	Dani*	**37.50±2.5**	8.33±7.23
	Delia*	25.0±21.73	**100.0±0.0**
	Diana	73.68±1.32	73.68±2.32
	Doina	50.0±0.0	57.13±22.86
	Elena	66.67±16.67	42.86±7.13
	Flora*	30.0±3.33	**38.75±1.25**
	Geta	64.70±5.29	64.28±1.71
	Gras ameliorat*	**55.55±2.44**	33.32±1.67
	Iulia*	**78.57±1.43**	54.54±4.55
	Ivan*	50.0±1.0	**55.56±2.43**
	Jubileu 50	72.85±12.85	71.25±8.75
	Matilda	33.49±34.66	44.58±25.35
	Minerva	77.30±7.31	68.89±8.89
	Pescăruș*	**88.89±1.11**	81.82±3.17
	Romaner	58.82±1.07	58.82±2.17
	Silvia*	**77.78±0.21**	62.5±2.5
	Tita*	63.63±3.36	**84.62±3.37**
	Tuleu dulce*	40.0±3.0	**80.0±4.0**
	Zamfira	62.22±8.37	63.07±16.92
Foreign	Anna Späth	53.12±21.87	48.21±23.21
	Blue free*	**84.61±3.38**	60.0±5.0
	d`Agen	38.46±1.54	41.76±3.32
	President	80.0±5.0 0	83.33±3.67
	Reine Claude d'Althan*	50.0±1.00	**63.63±4.26**
	Stanley	75.0±5.0	75.0±5.0
Average		**59.05±20.94**	**60.14±20.78**

*p<0.05. Cultivars followed by the * are significantly different according to Student's t-test.

The highest average initiation rate was found on medium with mT (VI-2) for 'Delia' cultivar (100%). The use of BAP (VI-1) resulted in obtaining average values of the initiation rate in the range from 18.17±0.82 ('Agent') to 88.89±1.11 ('Pescăruș'), while on medium with mT (VI-2) from 8.33±7.23 ('Dani') to 100.0±0.0 ('Delia'). The statistical difference in the means of initiation rates was observed for several cultivars, the highest values on medium with BAP (VI-1) for 'Centenar', 'Dani', 'Gras ameliorat', 'Iulia', 'Pescăruș', 'Silvia' and 'Bluefree', or the highest values on

medium with mT (VI-2) for 'Agent', 'Delia', 'Flora', 'Ivan', 'Tita', 'Tuleu dulce' and 'Reine Claude d'Althan'.

These results confirmed the importance of selecting an appropriate cytokinin type to optimize the initiation phase of micropropagation, particularly for specific cultivars.

In a previous study, similar establishment rates were obtained for 'Delia' (20.6%) and 'Tita' (46%) cultivars on MS medium supplemented with 0.2 mg/L BAP (Vescan et al., 2011). Another study revealed that the medium containing BAP in concentration of 0.5 or 1 mg/l showed significant positive response for shoot induction and shoot multiplication than kinetin hormone for 'Stanley' cultivar (Wolella, 2017).

Multiplication stage

Multiplication coefficient

The average values of the multiplication coefficient for the 19 cultivars studied on four cultures variants (Table 3) showed that plants propagated on WPM with each hormone (VM-1 and VM-2) had the highest significant values than those on medium MS medium with each hormone (VM-3 and VM-4).

The highest average value of the multiplication coefficient of plum cultivars was found on nutrient medium WPM supplemented with BAP for 'Doina' cultivar (6.10±4.14). For all cultivars on nutrient medium WPM supplemented with BAP (VM-1), relatively high values of the multiplication coefficient are observed, ranging from 2.05±0.66 ('Anna Späth') to 6.10±4.14 ('Doina') shoots per explants. On nutrient medium WPM supplemented with mT (VM-2), the range of multiplication coefficient was from 1.48±0.97 ('Gras ameliorat') to 5.22±4.26 ('Blue free') shoots per explants. In contrast, the range of the multiplication coefficient was narrower when nutrient medium MS supplemented with BAP (VM-3) was used, more specifically from 1.21±0.26 ('Blue free') to 3.09±1.39 ('Pescăruș') shoots per explants. Regarding the nutrient medium MS supplemented with mT (VM-4), the multiplication coefficient ranged from 1.45±0.15 ('Anna Späth') to 3.27±1.21 ('Geta') shoots per explants.

Table 3. Multiplication coefficient depending the culture variants

Origin of cultivars	Cultivars	Culture variants			
		VM-1	VM-2	VM-3	VM-4
Romanian	Andreea*	**3.12±1.45 a**	2.49±0.69 ab	1.63±0.42 b	1.74±0.98 ab
	Carpatin**	3.06±2.54 a	4.37±2.23 a	2.51±2.38 a	2.41±0.85 a
	Centenar**	**3.91±1.47 a**	2.42±0.58 ab	1.78±0.67 b	2.21±0.80 ab
	Delia*	2.81±1.39 ab	**4.80±1.98 a**	2.18±0.74 b	3.00±2.02 ab
	Diana**	2.32±1.30 a	3.30±0.96 a	2.06±0.63 a	1.98±0.43 a
	Doina*	**6.10±4.14 a**	3.37±1.11 ab	2.89±0.53 ab	2.15±0.16 b
	Elena**	2.43±0.87 a	3.06±1.71 a	2.65±0.75 a	2.58±0.71 a
	Geta**	2.37±0.43 a	2.25±1.89 a	3.00±1.81 a	3.27±1.21 a
	Gras ameliorat**	2.74±1.31 a	1.48±0.97 a	2.18±0.83 a	2.83±1.00 a
	Matilda**	**3.73±1.87 a**	1.39±0.49 b	2.31±0.75 ab	2.33±0.48 ab
	Minerva*	3.02±1.24 ab	**3.14±0.82 a**	2.45±1.32 ab	1.49±0.19 b
	Pescăruș*	3.81±1.59 ab	**4.91±2.00 a**	3.09±1.39 ab	2.31±1.21 b
	Romaner*	**5.12±2.63 a**	3.22±1.52 ab	2.75±0.80 ab	2.05±0.59 b
	Zamfira**	**2.21±0.54 a**	1.81±0.60 ab	1.40±0.08 ab	1.12±0.08 b
	Silvia**	**4.42±1.45 a**	2.66±0.65 ab	2.10±1.19 ab	2.01±0.84 b
	Tita*	**4.75±2.09 a**	4.04±1.29ab	2.29±1.00 b	2.23±0.29 b
	Tuleu dulce*	**5.83±2.87 a**	3.76±2.61 ab	1.45±0.48 b	3.00±2.34 ab
	Zamfira**	**2.21±0.54 a**	1.81±0.60 ab	1.40±0.08 ab	1.12±0.08 b
Foreign	Anna Späth**	2.05±0.66 a	2.81±1.31 a	2.56±1.56 a	1.45±0.15 a
	Bluefree*	2.89±1.93 ab	**5.22±4.26 a**	1.21±0.26 b	1.52±0.45 b
Average**		**3.51±2.04 A**	**3.18±1.85 A**	**2.24±1.08 B**	**2.19±1.02 B**

*p<0.05, ** p<0.01. Means followed by the different letters are significantly different according to Fisher test.

Based on the comparative analysis, significant differences of multiplication coefficients were observed between VM-1 and VM-4 for the cultivars 'Doina', 'Romaner', 'Zamfira' and 'Silvia', and between VM-1 and VM-3 for the cultivars 'Andreea', 'Centenar' and 'Tuleu dulce'. Similarly, for the 'Matilda' cultivar, VM-1 showed higher multiplication coefficients compared to VM-2 while for 'Tita' cultivar in contrast with VM-3 and VM-4.

On nutrient medium WPM supplemented with mT (VM-2), the highest significant multiplication coefficients were observed for 'Delia' cultivar in comparison to VM-3, for 'Minerva' and 'Pescăruș' cultivars in contrast with VM-4, and for 'Blue free' cultivar in comparison to VM-3 and VM-4.

These findings suggest that WPM, particularly when supplemented with BAP or mT, provides optimal conditions for micropropagation. However, cultivar-specific responses must be considered when selecting the most suitable medium for efficient shoot multiplication.

The composition of the culture medium plays an essential role in *in vitro* micropropagation, as it directly affects plant growth, development, and regeneration (Figure 1). Medium nutritive culture most used for *Prunus domestica* are MS (Alam & Barua, 2015; Gago et al., 2020), WPM (Yao et al., 2011) or another such as Quoirin & Lepoivre Medium - QL (Plopa et al., 2010), DKW/Junglans medium and Westvaco WV5 medium (Jakab et al., 2009).

Similar results were obtained for cultivars 'Anna Späth', 'Carpatin',' Dani', 'Ivan', 'Jubileu 50', 'Reine Claude d'Althan' and 'Stanley' proliferated on MS medium and BAP (Clapa et al., 2013). Another previous study showed the lowest proliferation rate on medium with mT compared to BAP, but was observed a positive effect on the growth and quality for the shoots with mT for *Prunus domestica* 'Torinel' and *P. domestica x insititia* 'Ferdor' (Gentile et al., 2014).

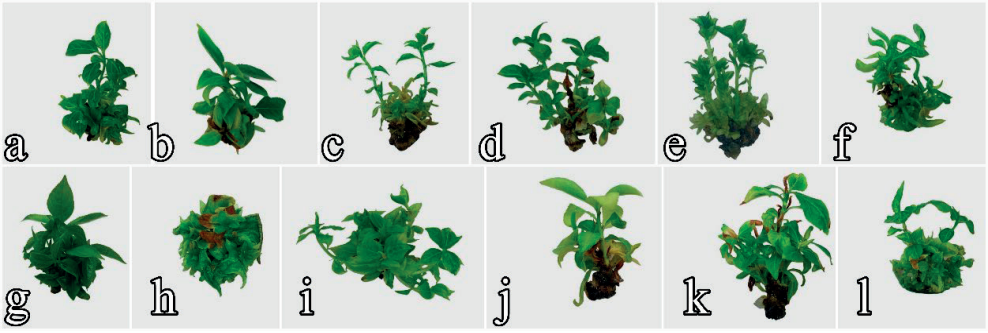


Figure 1. Micropropagation of high regenerated cultivars: 'Andreea' (a), 'Bluefree' (b), 'Centenar' (c), 'Delia' (d), 'Doina' (e), 'Matilda' (f), 'Minerva' (g), 'Pescăruș' (h), 'Romaner' (i), 'Silvia' (j), 'Tita' (k), 'Tuleu dulce' (l)

Principal Component Analysis (PCA), which was performed in order to explore the relationships between cultivars and culture multiplication variants (Figure 2), revealed a total variability of 63.13%, explained by the two components (F1 and F2) of the total variance. Regarding the culture variants, VM-1 and VM-2 are positively correlated and contribute strongly to F2, and VM-3 and VM-4 are negatively correlated with F2 and positively with F1. A cultivar high in VM-1 and VM-2 is likely to be low in VM-3 and VM-4 (and vice versa).

'Tuleu Dulce' and 'Tita' cultivars have high values for VM-2, likely have moderate values for VM-1 and lower values for VM-3 and VM-4.

'Doina', 'Pescăruș', 'Carpatin', 'Romaner' and 'Delia' cultivars have strong correlations with VM-1 and tend to have high values for this trait, moderate influence from VM-2 but lower values in VM-3 and VM-4.

'Elena' and 'Geta' cultivars show high values for VM-3 and VM-4 and conversely, they have low values for VM-1 and VM-2.

'Blue free' cultivar was strongly influenced by VM-2. However, it is far from VM-1, VM-3, and VM-4, suggesting it has lower values for these variants.
'Zamfira', 'Andreea', 'Centenar', 'Silvia' 'Anna Späth', 'Minerva', 'Diana', 'Matilda' and 'Gras ameliorat' cultivars cluster near the centre or slightly to the negative side, suggesting moderate to low values in all variants.

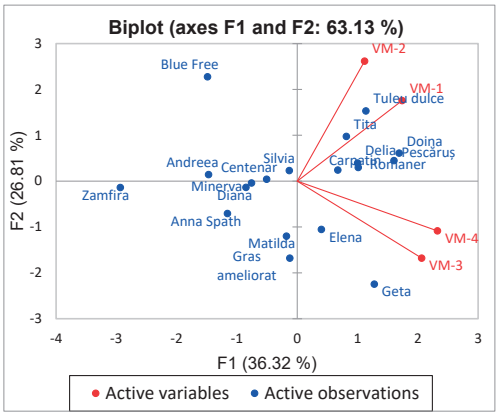
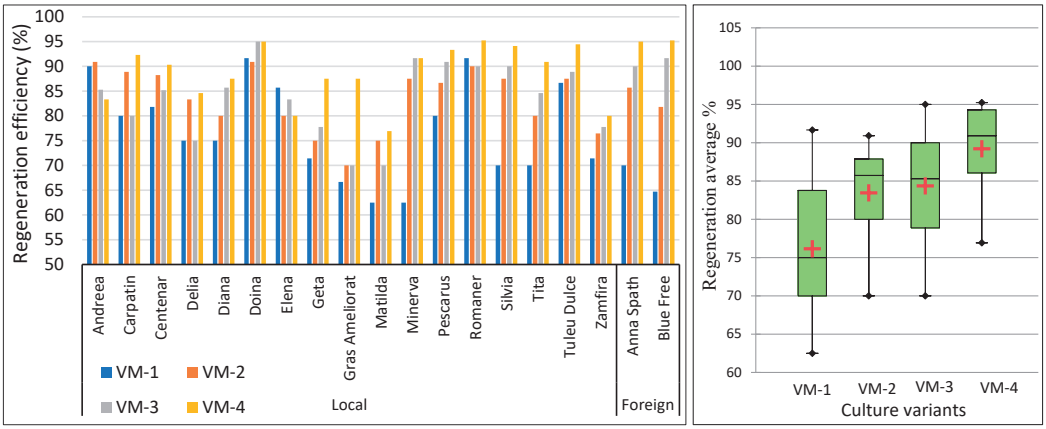


Figure 2. Principal Component Analysis (PCA) of 19 cultivars on four culture variants of multiplication medium

Regeneration efficiency

Regeneration efficiency varied from 63% ('Matilda' and 'Minerva') on variants VM-1 to 95% ('Doina', 'Romaner', 'Anna Späth' and 'Blue free') on variants VM-4 and on variants VM-3 for 'Doina' cultivar (Figure 3a). Some cultivars, with regeneration efficiency between 65-80% ('Delia', 'Geta', 'Gras ameliorat', 'Matilda' and 'Zamfira') showed a high level of necrotic plants, unable to regenerate. In this case medium composition or hormonal balance should be adjust for each genotype.
The highest average regeneration efficiency was obtained for VM-4 (Figure 3b). Regarding of culture variants, all lead to good regeneration efficiency.
Regeneration efficiency is mostly influenced by the cultivars and also by the medium composition (Long et al., 2022). Our results are in accordance with other report regarding successfully regenerated microshoots for three plum cultivars and on three different medium culture (Batukaev et al., 2018).
Short term conservation of plum cultivars was successfully achieved using micropropagation techniques through tissue culture. This approach proved effective for eleven selected cultivars that demonstrated a high multiplication coefficient and superior regeneration efficiency ('Doina', 'Blue free', 'Carpatin', 'Centenar', 'Elena', 'Minerva', 'Pescăruș', 'Romaner', 'Silvia', 'Tita', 'Tuleu dulce'). These cultivars exhibited strong *in vitro* adaptability, ensuring their rapid propagation and maintenance under controlled conditions. The high regeneration efficiency of these cultivars highlights the potential of micropropagation as a valuable tool for the preservation.



a b

Figure 3. Regeneration efficiency: a - for 19 cultivars, b - average regeneration rate for variants

The findings of this study contribute to the development of tailored *in vitro* conservation protocols for *Prunus domestica* L. cultivars, providing valuable material for the medium- and long-term conservation of plum genetic resources.

CONCLUSIONS

In the initiation stage both cytokinin-type hormones (BAP and mT) incorporated into the culture media effectively supported the initiation stage of plum cultivars. However, cultivar-specific variations were evident and revealed statistically significant differences in initiation rates for 14 cultivars, suggesting that certain genotypes respond more favorably to one cytokinin over the other.

The choice of nutrient medium and cytokinin type significantly influences the multiplication coefficient of plum cultivars, WPM medium supplemented with BAP (VM-1) highlighting its effectiveness in promoting shoot proliferation. Similarly, WPM medium with mT (VM-2) supported superior multiplication rates for specific cultivars.

Regeneration efficiency varied significantly among cultivars, with the highest success observed on VM-4. To optimize propagation, medium composition and hormonal balance should be adjusted for each genotype, particularly for those with higher necrosis rates.

All these findings showed that micropropagated plum cultivars are suitable for short-term conservation using tissue culture techniques, providing an effective method for preserving and propagating valuable cultivars.

ACKNOWLEDGEMENTS

This research work was carried out with the support of Ministry of Agriculture and Rural Development through ADER 2026 Program, financed from Project no. 6.3.4/2023, and also by Fruit Research and Development Station Bistrita, which we are grateful for the generous help and assistance.

REFERENCES

Alam, M. J., & Barua, R. (2015). *In vitro* regeneration and antibacterial activity of *Prunus domestica* L. *Journal of BioScience & Biotechnology*, *4*(1).

Batukaev, A. A., Bamatov, I. M., & Vinter, M. A. (2018). Studying tolerance of prune (*Prunus domestica*) to the plum pox virus (PPV) by criterion" Efficiency of microshoots' regeneration" in controlled *in vitro* conditions. *Journal of Pharmaceutical sciences and researc*h, *10*(1), 59-64.

Bettoni, J. C., Wang, M. R., & Wang, Q. C. (2024). *In Vitro* Regeneration, Micropropagation and Germplasm Conservation of Horticultural Plants. *Horticulturae*, *10*(1), 45.

Canli, F. A., & Tian, L. (2009). Regeneration of adventitious shoots from mature stored cotyledons of Japanese plum (*Prunus salicina* Lindl). *Scientia Horticulturae*, *120*(1), 64-69.

Chauhan, R., Singh, V., & Quraishi, A. (2019). *In vitro* conservation through slow-growth storage. *Synthetic Seeds: Germplasm Regeneration, Preservation and Prospects,* 397-416.

Clapa, D., Fira, A. L., Vescan, A., Zagrai, I., Zagrai, L., Jakab, Z. S., & Plopa, C. (2013). The micropropagation and *in vitro* maintenance of some plum cultivars. *Acta Hortic*., 981, 437-442

Engelmann, F. (1997). *In vitro* conservation methods. *Biotechnology in Agriculture Series*, 119-162.

(FAO) Food and Agricultural Organization. www.fao.org. Accesed in 25 february 2025.

Gago, D., Sánchez, C., Aldrey, A., Christie, C. B., Bernal, M. Á., & Vidal, N. (2022). Micropropagation of plum (*Prunus domestica* l.) in bioreactors using photomixotrophic and photoautotrophic conditions. *Horticulturae*, *8*(4), 286.

Gentile, A., Jàquez Gutiérrez, M., Martinez, J., Frattarelli, A., Nota, P., & Caboni, E. (2014). Effect of meta-Topolin on micropropagation and adventitious shoot regeneration in *Prunus* rootstocks. *Plant Cell, Tissue and Organ Culture (PCTOC),* 118, 373-381.

Grout, B. W. (1990). *In vitro* conservation of germplasm. *Plant Tissue Culture. Application and Limitations*, 394-411.

Hartmann, W., & Neumüller, M. (2009). Plum breeding. *Breeding plantation tree crops: temperate species*, 161-231.

Jakab, Z. I., Clapa, D., & Fira, A. (2009). Contributions concerning the *in vitro* multiplication in *Prunus domestica*. *Annals of the Romanian Society for Cell Biology*, *14*(1), 246-252.

Lloyd, G., & McCown, B. (1980). Commercially-feasible micropropagation of mountain laurel, Kalmia latifolia, by use of shoot-tip culture. *Combined Proceedings, International Plant Propagators' Society*, 30, 421-427.

Long, Y., Yang, Y., Pan, G., & Shen, Y. (2022). New insights into tissue culture plant-regeneration mechanisms. *Frontiers in plant science*, 13, 926752.

Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia plantarum*, *15*(3), 473-497.

Nacheva, L., Dimitrova, N., Koleva-Valkova, L., Stefanova, M., Ganeva, T., Nesheva, M., ... & Vassilev, A. (2023). *In vitro* multiplication and rooting of plum rootstock 'Saint Julien'(*Prunus*

domestica subsp. *insititia*) under fluorescent light and different LED spectra. *Plants*, *12*(11), 2125.

Nezami, E., & Gallego, P. P. (2023). History, phylogeny, biodiversity, and new computer-based tools for efficient micropropagation and conservation of pistachio (*Pistacia* spp.) germplasm. *Plants*, *12*(2), 323.

Plopa, C., Milusheva S., Butuc, M. M., Presa, S. A. & Isac, V. (2010). Obtaining virus free planting material of plum cultivars by *in vitro* method. *Scientific Paper of the RIFG Piteşti*, *XXVI*.

Romadanova, N. V., Mishustina, S. A., Matakova, G. N., Kushnarenko, S. V., Rakhimbaev, I. R., & Reed, B. M. (2016). *In vitro* collection methods for Malus shoot cultures used for developing a cryogenic bank in Kazakhstan. In *XXIX International Horticultural Congress on Horticulture: Sustaining Lives, Livelihoods and Landscapes (IHC2014): 1113* (pp. 271-277).

Sharma, P., & Jain, S. M. (2024). Micropropagation Applications in Conservation of Horticultural Crops. In *Sustainable Utilization and Conservation of Plant Genetic Diversity* (pp. 683-710). Singapore: Springer Nature Singapore.

Vescan, L. A., Pamfil, D., Zagrai, I., Ioana, V. B., Clapa, D., Ciuzan, O., & Iulia, F. (2011). *In vitro* techniques for Plum Pox Virus elimination from two infected Romanian plum cultivars. *Bulletin UASVM Animal Science and Biotechnologies*, *68*, 1-2.

Vujović, T., Jevremović, D., Anđelić, T., & Vasilijević, B. (2022). Optimization of the protocol for *in vitro* propagation of autochthonous plum genotype 'Metlaš'. *Book of Proceedings, XIII International Scientific Agriculture Symposium "AGROSYM 2022"*, 166-172.

Wolella, E. K. (2017). Surface sterilization and *in vitro* propagation of *Prunus domestica* L. cv. Stanley using axillary buds as explants. *Journal of Biotech Research*, *8*, 18.

Yao, Y. X., Sun, Y. W., Li, G. G., & Li, G. H. (2011). Regeneration of plants from *in vitro* culture of petioles in *Prunus domestica* Lindl (European plum). *Biotechnology & Biotechnological Equipment*, *25*(3), 2458-2463.

Yuan, H.Y., Wu, Y.X., Liao, K., Geng, W.J., Li, J., Xu, Z. and Wang, T. (2009). *In Vitro* Propagation Of Wild European Plum (*Prunus ×domestica* L.), A Rare And Endangered Species. *Acta Hortic*., 839, 99-104

Zagrai, I., & Zagrai, L. A. (2015). A nuclear-stock plum collection established in Romania. *Bulletin UASMV Cluj-Napoca, Horticulture*, *72(*1): 205-208.

Zagrai, L. A., Zagrai, I., Guzu, G. M., & Moldovan, C. (2022). Towards a widening of Romanian nuclear-stock plum varieties. *Bulletin UASMV Cluj-Napoca, Horticulture, 79*(1), 89-95

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

EVALUATION OF SOME QUALITY PARAMETERS OF 'ENROSADIRA' RASPBERRY VARIETY UPON DIFFERENT GROWING SYSTEMS

Monica STURZEANU[1], Oana HERA[1], Loredana VÎJAN[2], Adrian ASĂNICĂ[3]

[1]Research Institute for Fruit Growing Pitești - Mărăcineni, 402 Mărului Street, Mărăcineni, Pitești, Argeș County, Romania
[2]National University of Science and Technology Politehnica Bucharest, Pitești University Centre, Faculty of Sciences, Physical Education and Computer Science, Pitești, 110142, Argeș County, Romania
[3]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăști Blvd, District 1, Bucharest, Romania

Corresponding author email: oana.hera@yahoo.com

Abstract

The increasing global trend in cultivating berries, particularly raspberries, is driven by their high content of bioactive nutrients. These fruits are not only very attractive for consumers but also offer numerous health and diet benefits. This study focuses on the cultivar 'Enrosadira', a recent Italian variety of raspberry known for its attractive flavor, good yield and high adaptability to different growing conditions. The objective of current research was to asses the influence of cultivation system upon some characteristics of raspberries after first growing season. Yield, fruit weight, total phenolic and flavonoids content, total tannins and antocyanins, total sugar content, soluble solid content (SSC) and carotenoids were evaluated during the trial. The fruit weight varied between 3.17 g to 4.30 g and the total phenolic content differed among growing conditions from 347.83 mg GAE/g FW to 443.76 mg GAE/g FW. The total sugar content ranged from 3.72 g glucose/100 g to 4.04 g glucose/100 g, indicating variability in the sweetness and caloric value of the fruit. The findings from the study enphasized significant variation in the fruits physico-chemical properties on different growing conditions.

Key words*: berries, bioactive nutrients, physicochemical properties, different growing condition.*

INTRODUCTION

The change in raspberry breeding towards sensory qualities and potential health benefits reflects growing interest in how fruit composition influences not only flavor and texture but also the nutritional and therapeutic properties of raspberries. This is in line with broader trends in agriculture where there is increasing recognition of the role that crops can play in promoting health and preventing disease (Motyleva et al., 2022). The adjust in raspberry breeding from traditional agronomic characteristics to those that focus on sensory qualities and health benefits, as noted by Jennings et al. (2016) and Mazzoni et al. (2016), highlights the increasing consumer demand for berries that not only taste good but also offer nutritional and therapeutic benefits. This change is also driven by the growing interest in functional foods-foods that provide health benefits beyond basic nutrition. As such, raspberries, with their high levels of antioxidants, vitamins, and other bioactive compounds, are at the forefront of this movement. Recent research has demonstrated a growing interest in the characterization of essential oils and secondary metabolites from both well-known and lesser-known plants. This trend underscores the untapped potential of natural resources to yield new scientific insights that can benefit human health (Viskelis et al., 2010; Al-Snafi et al., 2016; Bobinaitė et al., 2016; Perrino et al., 2021).

Berries, in particular, have emerged as a valuable source of a diverse array of secondary metabolites, which hold promise for applications in the pharmaceutical, agronomic, and food industries (Oomah et al., 2000; Sagar et al., 2018). The increasing global trend in

cultivating small fruits, particularly raspberries, is driven by their high content of bioactive nutrients. These fruits are not only delicious but also offer numerous health benefits, making them a valuable addition to the diet (Tosun et al., 2009).

The estimated global area of raspberry cultivated in 2022 was approximately 116,393 hectares (FAO, 2024). This figure highlights the extensive global interest in raspberries, which continue to grow in both consumption and production. Among the major raspberry producers, Poland stands out as a key player, with an area dedicated to raspberry farming of about 21,700 hectares. This makes Poland one of the top countries in terms of raspberry cultivation area, second only to Russia, which has an even larger area of cultivation. Poland's raspberry industry is significant not only because of the large area under cultivation but also due to its position as a leader in raspberry processing, with a significant portion of its production destined for frozen berries, juices, jams, and other processed products. The country has developed a strong export market for raspberries, particularly within the European Union, and increasingly to other regions (Maj et al., 2024). Raspberries are rich in various bioactive compounds, particularly antioxidants, which contribute to their health benefits (Arnold et al., 2022). There is a type of flavonoid, a class of plant compounds known for their antioxidant and anti-inflammatory properties. In raspberries, flavonols such as quercetin and kaempferol are present, which help neutralize free radicals and may contribute to reducing oxidative stress in the body. These are a type of flavonoid, a class of plant compounds known for their antioxidant and anti-inflammatory properties (Golovinskaia et al., 2021). Raspberries are an excellent source of vitamin C, an essential nutrient that acts as a powerful antioxidant. Vitamin C is involved in the protection of cells from oxidative damage, supports immune function, and aids in collagen synthesis for skin health. The recent findings highlighted the promising role of anthocyanins, a group of naturally occurring pigments found in many fruits, vegetables, and flowers, particularly in deeply colored foods like raspberry, purple sweet potatoes, and red cabbage. With higher levels of phenolics, flavonoids, and anthocyanins, raspberries are a great source of antioxidants, which can help protect against chronic diseases, including heart disease, cancer, and neurodegenerative disorders. Incorporating anthocyanin-rich foods into the diet may offer a natural, preventive approach to managing oxidative stress and reducing the risk of chronic diseases. These findings also encourage further research into anthocyanins as potential therapeutic agents (Bilawal et al., 2021).

Although raspberry fruits contain sugar, the relatively low sugar content in this variety makes it suitable for various health-conscious consumers, including those monitoring blood sugar levels (Drobek et al., 2024). The goal of this study is how various environmental factors and cultivation techniques can impact the physicochemical properties of raspberry fruits, which in turn affect their quality and nutritional value.

MATERIALS AND METHODS

Chemicals and Reagents

It is obtained various chemicals and reagents from Merck. Darmstadt. Germany. Including 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,6-dichloroindophenol (DCPIP). sodium hydroxide, sodium carbonate, sodium bicarbonate, sodium nitrite. disodium phosphate, aluminum chloride, methanol. acetone, n-hexane, ethanol, citrate/acetate buffer, gallic acid, catechin, vitamin C, cyanidin-3-O-glucoside, metaphosphoric acid, acetic acid, hydrochloric acid, citric acid, and Folin-Ciocalteu reagent (Stockhammer et al., 2009).

Plant material

The experiment was carried out in the Research Institute for Fruit Growing Pitesti - Mărăcineni (RIFG) in South of Romania 44°54'12" Northern latitude, and 24°52'18" Eastern longitude, 284 m altitude, over summer 2024. The biological material studied, the 'Enrosadira' variety, is a Italian primocane raspberry and the fruits ripen in two stages: in early June, on 1-year-old shoots, and in mid-July - early August on annual shoots. It is characterized by a high precocity, which allows obtaining an earlier production in the summer season (Figure 1).



Figure 1. 'Enrosadira' raspberry variety

Culture Systems

System I: Conventional irrigation in open field (1.5 L/day/plant).

System II: Controlled irrigation in open field using the Netafim system, based on the evapotranspiration (ET0) method (Păltineanu et al., 2018).

System III: Conventional irrigation in a solar (1.5 L/day/plant).

System IV: Controlled irrigation in a solar using methodologies from Hargreaves and Samani (1985), revised by Jaafar et al. (2019).

Conventional irrigation (system I and III): applies a fixed amount of water daily (1.5 liters per plant), regardless of the plant's specific water needs or environmental conditions.

Controlled irrigation (system II and IV) is adjusted based on evapotranspiration rates or other environmental data to optimize water use and prevent over- or under-watering. The methodology takes into account local climate conditions and plant growth to deliver more precise irrigation.

Fruit quality parameters

During the peak fruit harvesting period. we recorded various indicators from a sample of 50 fruits, with each measurement and analysis repeated 3 times.

Fruit production (g/plant) was calculated by summing the weights of fruits harvested per plant. Measurements were repeated across three harvests.

The average *fruit weight* was determined by using the HL-400 digital balance to weigh each fruit. The fruit shape index was calculated using the formula fruit length/fruit diameter (Titirică et al., 2023).

Various quality parameters were measured, such as total soluble solids, total sugar content, total polyphenols content (TPC), total flavonoids content (TFC), total tannins content (TTC), total anthocyanins content (TAC), as well as lycopene and β-carotene contents.

Total soluble solid content (TSS) was measured using a Kruss DR201-95 refractometer and the results expressed as °Brix at 20°C, indicating the sugar concentration in the fruit.

The total polyphenol content (TPC) was measured using the method recommended by Matić et al. (2017). Polyphenols react with phosphotungstic acid in an alkaline medium, forming a blue-colored complex. Absorbance measured at 760 nm. Results expressed as mg gallic acid equivalent (GAE) per 100 g FW. ()

The total flavonoid content (TFC) was measured using the method proposed by Tudor-Radu et al. Flavonoids react with aluminum chloride, producing a yellow-orange complex.

Absorbance was measured at 510 nm and the results were expressed as mg catechin equivalent (CE) per 100 g FW.

To determine *the total anthocyanin content (TAC)*, the pH differential method suggested by Di Stefano and Cravero was used. Structural changes in anthocyanins are pH-sensitive.

Absorbance was measured at 520 nm after preparing samples in pH 0.6 and 3.5 buffers (Sucu, et al., 2023) and the results were expressed as cyanidin-3-O-glucoside equivalent (C3-GE) per 100 g FW.

Based on Broadhurst et al.'s method (1978), *the tannin contents (TTC)* was determinated as total tannins (proanthocyanidins) which react with vanillin and hydrochloric acid, forming a red complex. Absorbance was measured at 500 nm and the results were expressed as g E. catechin per 100 g dry matter.

The levels of *lycopene and β-carotene* were determined using the method proposed by Tudor-Radu et al. Carotenoids were extracted using a mixture of hexane, ethanol. and acetone. Results expressed in mg per 100 g FW, with absorbance at 470 nm (β-carotene) and 503 nm (lycopene) (Cáceres, et al., 2014).

Statistical Analysis

The data collected from the experiments were summarized and presented as mean ± standard deviation (SD). This approach to data presentation provides a clear indication of both the central tendency (mean) and the variability (SD), making it easier to assess the consistency of the results. By employing a combination of ANOVA tests and post-hoc analyses, the methodology effectively identified significant trends, key differences, and interaction effects in the data. All statistical analyses were performed using Excel 2021 in conjunction with the XLSTAT add-in.

RESULTS AND DISCUSSIONS

The study highlights the influence of cultivation environments and irrigation technologies on fruit production. The best performance in terms of yield demonstrates that protected cultivation systems, combined with advanced irrigation methods, substantially outperform conventional open field conditions.

The experiment recorded a significant increase in fruit yield under solar conditions for both irrigation systems when compared to open-field growing. This suggests that protected spaces, with their ability to regulate environmental variables such as temperature, humidity, and pest exposure, create optimal growth conditions for crops. Additionally, the use of controlled irrigation technologies in these settings further enhanced production, underscoring the role of precise water management in agricultural efficiency.

In the open field with conventional irrigation, the yield was minimal, averaging 0.31 kg per bush (Figure 2). This reflects the limitations of uncontrolled environmental conditions and traditional irrigation methods.

In contrast, the solar with controlled irrigation achieved the highest yield of 0.87 kg per bush, nearly three times that of the field setup. This stark difference emphasizes the synergy between advanced technology and controlled environments.

These data confirm that integrating protected cultivation spaces with modern irrigation technologies is a robust strategy for achieving higher agricultural productivity. As global food demand grows, adopting such innovative practices will be essential for sustainable farming.

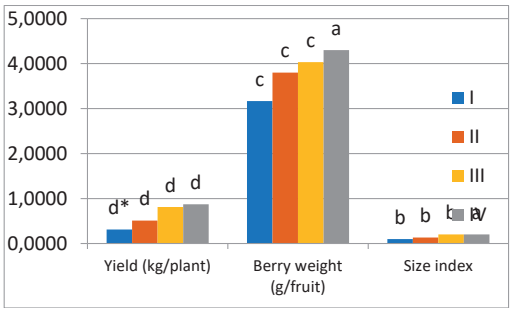


Figure 2. Fruit parameters of raspberry cultivar

Agricultural practices have evolved significantly with the advent of technology, particularly in controlled environments where factors like temperature, humidity, and irrigation can be precisely managed. One of the key indicators of plant health and productivity is the weight of the fruit, which directly correlates to environmental conditions and cultivation methods. The results from the experiment under discussion reveal how protected cultivation, combined with advanced irrigation systems, positively impacts fruit development, particularly in terms of fruit weight.

The study demonstrates that fruits grown under protected conditions, such as solar, consistently had higher average weights compared to those grown in open-field conditions. Protected spaces, such as solar, provide a stable environment that protects plants from extreme weather conditions, pests, and other stressors that can limit growth. These controlled environments not only ensure that plants have optimal growing conditions but also prevent environmental fluctuations that could negatively affect fruit quality. As a result, fruits grown in such spaces can allocate more energy towards development rather than survival, leading to larger, healthier fruits.

Irrigation is other important factor that determines fruit size and quality. This study compared two types of irrigation systems: conventional irrigation and digitized, or controlled, irrigation. It was found that controlled irrigation, especially in solar environments, led to the highest fruit weights. Controlled irrigation systems provide water in a more efficient and targeted way, ensuring that

plants receive the exact amount of water they need without waste or stress. This precise watering contributes to maintain healthier plants, with more resources available for fruit development rather than coping with water deficits or exceses.

The results of the experiment revealed significant differences in the average fruit weight across different cultivation and irrigation systems. In open-field conditions with conventional irrigation, the average fruit weight was 3.17 g. This relatively low value highlights the limitations of traditional farming methods, where environmental conditions are less controlled, and water distribution is less precise.

Under digitized irrigation in open field, fruit weight increased slightly to 3.8 g, suggesting that even in less controlled environments, modern irrigation techniques can lead to some improvement by delivering more consistent water levels to the plants.

In solar, using conventional irrigation, fruit weight increased to 4.03 g, showcasing the positive influence of the controlled environment. The higher weight in solar, even without the added benefit of controlled irrigation, suggests that protected spaces alone can significantly enhance fruit development.

The highest fruit weight was observed in solar with controlled irrigation, where the average fruit weight reached 4.30 g. This result confirms that the combination of both a protected environment and advanced irrigation technology offers the best conditions for optimal fruit development.

The size index reflects the overall growth and dimensional characteristics of fruits under different cultivation and irrigation conditions. The highest value (1.14) confirms the significant advantages of combining solar cultivation with digitized irrigation systems, which optimize both environmental conditions and resource use. Digitized irrigation in open fields produces a similar size index to conventional irrigation in solar (1.07). This suggests that while precise irrigation contributes significantly to growth, the added benefits of a protected environment further enhance fruit size.

The pH values reflect the acidity of the fruit, which impacts taste and shelf life.

The data indicates variability across the cultivation systems: lowest pH (3.57) recorded in systems I and IV, suggesting higher acidity. This is typical in open-field cultivation and under less optimized conditions. Highest pH (4.62) found in system II, indicating reduced acidity and potentially a milder taste. This could be attributed to the use of digitized irrigation in more controlled settings. Total soluble solids (TSS), measured in °Brix, represents the total sugars and other dissolved solids in the fruit, directly correlating with sweetness and flavor intensity: Highest TSS (12.50°Brix) recorded in system II, suggesting the sweetest fruits with enhanced flavor. This is likely due to digitized irrigation optimizing water and nutrient delivery. Lowest TSS (11.30°Brix) found in system IV, suggesting less sweetness compared to other systems.

Total sugar content (TSC) values measure the sugar content in the fruit, reflecting its energy reserves and sweetness: highest TSC (4.04 g/100 g), observed in system III, indicates superior sugar accumulation. Lowest TSC (3.71 g/100 g) found in system I, likely due to open-field conditions with conventional irrigation, which limits the plant's ability to maximize sugar synthesis (Figure 3).

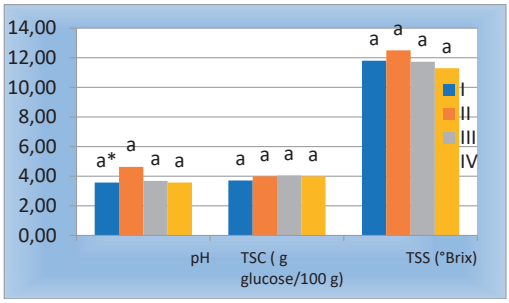


Figure 3. Fruit parameters of raspberry cultivar

The classic exterior method (I) is the most efficient at preserving phenolic compounds, which aligns with their role in protecting against oxidative stress. Systems III and IV show significant degradation of these compounds (-21.6% compared to the classic method). TPC is strongly positively correlated with TTC and TFC, suggesting that the processes affecting phenols also influence flavonoids. TTC is highest for the classic exterior method (I) but decreases significantly with digital and solar methods. Systems (III and IV) show the weakest retention of TTC, indicating that high-

temperature exposure negatively affects antioxidant compounds. The classic exterior method (I) preserves flavonoids the best (64.79 mg CE/100 g FW), while solar methods show significant losses. Flavonoid stability is slightly higher in the digital solar method (IV) compared to the classic solar method (III), suggesting minor improvements in stabilization. The classic exterior method (I) is the most efficient at preserving phenolic compounds, which aligns with their role in protecting against oxidative stress.

The open field growing system is the most efficient at preserving phenolic compounds, which aligns with their role in protecting against oxidative stress. Systems III and IV show significant degradation of these compounds (-21.6% compared to the classic method). TPC is strongly positively correlated with TTC and TFC, suggesting that the processes affecting phenols also influence flavonoids (Figure 4). TTC is highest for the classic exterior method (I) but decreases significantly with digital and solar methods. Solar systems (III and IV) show the weakest retention of TTC, indicating that high-temperature exposure negatively affects antioxidant compounds.

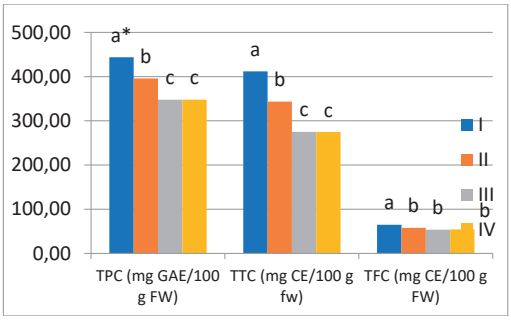


Figure 4. Fruit parameters of raspberry cultivar

The classic exterior method (I) preserves flavonoids the best (64.79 mg CE/100 g FW), while solar methods show significant losses. Flavonoid stability is slightly higher in the digital solar method (IV) compared to the classic solar method (III), suggesting minor improvements in stabilization.

The classic exterior method (I) retains the most lycopene (0.67 mg/100 g FW), an important carotenoid for heart health. There is moderate loss in the digital methods and significant degradation in the solar methods (-30%). Solar methods (III and IV) preserve beta-carotene the best (0.30 mg/100 g FW), which is unusual given the losses in other antioxidant components. The digital exterior method (II) shows a stabilization of beta-carotene (+17.1%) compared to the classic method.

TAC is lowest for the classic exterior method (1.11 mg C3-GE/100 g FW), but it increases dramatically in the solar methods (up to 4.78 mg C3-GE/100 g FW). TAC is negatively correlated with TPC, TTC, TFC, and lycopene, suggesting that the degradation of these compounds is associated with the increase in other antioxidants contributing to TAC.

Classic exterior method (I) is ideal for preserving phenolic compounds, flavonoids, and lycopene, but it does not maximize TAC.

Solar methods (III and IV) increase TAC, but at the cost of losing phenolic compounds, flavonoids, and lycopene (Figure5).

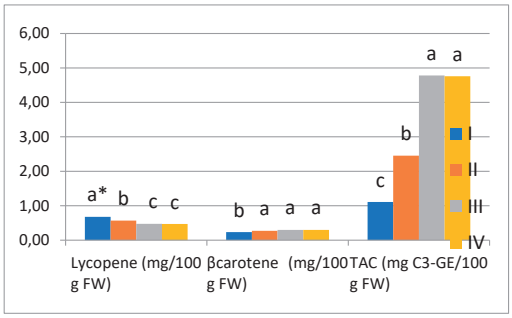


Figure 5. Fruit parameters of raspberry cultivar

β-carotene seems more stable in solar and digital methods but is negatively correlated with other antioxidants, suggesting a different degradation or stabilization mechanism.

The digital exterior method (II) offers a balance between preserving compounds and increasing TAC, making it a viable alternative.

CONCLUSIONS

The findings from this study demonstrated the substantial benefits of combining protected cultivation with controlled irrigation systems.

The increase in average fruit weight in solar environments with digitized irrigation underscores the importance of these innovations in modern agriculture.

Protected spaces create stable and optimal growing conditions, while controlled irrigation

ensures that plants receive consistent and adequate water, leading to improved fruit development.

The enhanced fruit weight observed in this study is a showcase of how controlled environments and advanced irrigation can help meet these challenges by improving productivity and ensuring higher-quality crops.

The progression of the size index underscores the critical role of advanced technologies in improving fruit growth.

Protected cultivation paired with digitized irrigation delivers the highest performance, making it a key strategy for achieving superior agricultural productivity. These findings highlight the potential for integrating these technologies to optimize fruit size and overall crop quality.

The study highlighted the importance of protected cultivation and digitized irrigation systems in boosting fruit yield, weight, and quality. While solar systems significantly enhance total antioxidant capacity, they may compromise the retention of certain beneficial compounds like phenolics and flavonoids.

The classic exterior method remains optimal for preserving these compounds but does not maximize TAC. The digital exterior method offers a balanced approach, stabilizing beta-carotene while preserving antioxidants, making it a promising alternative for sustainable fruit production in the future.

ACKNOWLEDGEMENTS

This research was carried out with the support of Ministry of Agriculture and Rural Development and it was financed from Project ADER 6.1.3/18.07.2023.

REFERENCES

Arnold, H., Dobson, G., Foito, A., Austin, C., Sungurtas, J., Allwood, J. W., McDougall, G. J. (2022). Assessing available phytochemicals from commercial blackcurrant and raspberry pomaces. *Journal of Berry Research*, *12*(3), 415–431.

Bilawal, Akhunzada, Muhammad Ishfaq, Munkh-Amgalan Gantumur, Abdul Qayum, Rujie Shi, Saqib Ali Fazilani, Asif Anwar, Zhanmei Jiang, and Juncai Hou (2021). A review of the bioactive ingredients of berries and their applications in curing diseases. *Food Bioscience*, 44: 101407.

Cáceres, J. O., Cámara, M., Manzoor, S., Cámara, R. M., Fernández-Ruiz, V., & Cortes Sánchez Mata, M. (2014, June). New developments in lycopene analysis by spectroscopic and chromatographic techniques, accompanied with mathematical modelling. In *XIII International Symposium on Processing Tomato*, 1081 (pp. 259–265).

Drobek, M., Cybulska, J., Zdunek, A., Sas-Paszt, L., & Frąc, M. (2024). Effect of microbial biostimulants on the antioxidant profile, antioxidant capacity and activity of enzymes influencing the quality level of raspberries (*Rubus idaeus* L.). *Food Chemistry*, *454*, 139746.

FAO (Food and Agriculture Organization of the United Nations). 2024. Available online: https://www.fao.org/ faostat/ en/#data/QI (accessed on 13 November 2024).

Golovinskaia, Oksana, and Chin-Kun Wang (2021). Review of functional and pharmacological activities of berries. *Molecules*, *26*, no. 13: 3904.

Jennings, S. N., Graham, J., Ferguson, L., & Young, V. (2015, June). New developments in raspberry breeding in Scotland. In *XI International Rubus and Ribes Symposium, 1133* (pp. 23–28).

Jaafar, H. H., & Ahmad, F. (2019). Determining reference evapotranspiration in greenhouses from external climate. *Journal of Irrigation and Drainage Engineering*, *145*(9), 04019018.

Hargreaves, G. H., & Samani, Z. A. (1985). Reference crop evapotranspiration from temperature. *Applied engineering in agriculture*, *1*(2), 96–99.

Maj, G., Buczyński, K., Klimek, K. E., & Kapłan, M. (2024). Evaluation of growth and energy parameters of one-year-old raspberry shoots, depending on the variety. *Energies*, *17*(13), 3153.

Matić, P., Sabljić, M., & Jakobek, L. (2017). Validation of spectrophotometric methods for the determination of total polyphenol and total flavonoid content. Journal of AOAC International, 100(6), 1795–1803.

Mazzoni, L., Perez-Lopez, P., Giampieri, F., Alvarez-Suarez, J. M., Gasparrini, M., Forbes-Hernandez, T. Y., ... & Battino, M. (2016). The genetic aspects of berries: from field to health. *Journal of the Science of Food and Agriculture*, *96*(2), 365–371.

Motyleva, S. M., Evdokimenko, S. N., Podgaetsky, M. A., Tumaeva, T. A., Burmenko, Y. V., Svistunova, N. Y., ... & Kulikov, I. M. (2022). Mineral composition of repair raspberry (*Rubus idaeu*s L.) fruits. *Vavilov Journal of Genetics and Breeding*, *26*(7), 622.

Tosun, M., Ercisli, S., Karlidag, H. Ü. S. E. Y. İ. N., Sengul, M. (2009). Characterization of red raspberry (*Rubus idaeus* L.) genotypes for their physicochemical properties. *Journal of Food Science*, *74*(7), C575-C579.

Viskelis, P.; Rubinskiene, M.; Bobinaite, R.; Dambrauskiene, E. Bioactive compounds and antioxidant activity of small fruits in Lithuania. *J. Food Agric. Environ.* 2010, *8*, 259–263.

Perrino, E.; Valerio, F.; Gannouchi, A.; Trani, A.; Mezzapesa, G. (2021). Ecological and Plant Community Implication on Essential Oils Composition in Useful Wild Officinal Species: A Pilot Case Study in Apulia (Italy). *Plants*, *10*, 574.

Al-Snafi, A.E. (2016). Medicinal plants possessed anti-inflammatory antipyretic and analgesic activities (part 2) - plant based review. *Sch. Acad. J. Pharm.*, *5*, 142–158.

Bobinaitė, R.; Viskelis, P.; Bobinas, Č.; Mieželienė, A.; Alenčikienė, G.; Venskutonis, P.R. (2016). Raspberry marc extracts increase antioxidative potential, ellagic acid, ellagitannin and anthocyanin concentrations in fruit purees. *LWT Food Sci. Technol.*, *66*, 460–467.

Păltineanu, C., Olga, V., Emil, C., Sorina, D. (2018). Irrigation regime in orchards and berries depending on the current climatic conditions and hydrophysical indices of the soils.

Sagar, N.A.; Pareek, S.; Sharma, S.; Yahia, E.M.; Lobo, M.G. (2018). Fruit and Vegetable Waste: Bioactive Compounds, Their Extraction, and Possible Utilization. Compr. *Rev. Food Sci. Food Saf*., 17, 512–531.

Stockhammer, S., Stolze, K., Rohr-Udilova, N., Chizzola, R., Zitterl-Eglseer, K., & Franz, C. (2009). Antioxidant activity of phytogenous industrial waste and derived extracts for the production of feed and food additives. *International Journal of food science & technology*, *44*(4), 702–710.

Sucu, S., Altıncı, N. T., Yağcı, A., Kılıç, D., Şahin, S., & Cangi, R. (2023). Effects of Selenium Biofortification on Phytochemical Characteristics of Some Table Grape Cultivars. *Black Sea Journal of Agriculture*, *6*(5), 558–563.

Titirică, I., Roman, I. A., Nicola, C., Sturzeanu, M., Iurea, E., Botu, M., Sestras, A. F. (2023). The main morphological characteristics and chemical components of fruits and the possibilities of their improvement in raspberry breeding. *Horticulturae*, *9*(1), 50.

Tudor-Radu, M., Vîjan, L. E., Tudor-Radu, C. M., Ion, T. I. Ț. A., Rodica, S. I. M. A., & Mitrea, R. (2016). Assessment of ascorbic acid, polyphenols, flavonoids, anthocyanins and carotenoids content in tomato fruits. Notulae botanicae horti agrobotanici Cluj-Napoca, *44*(2), 477–483.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

THE EFFECT OF PHOTOVOLTAIC SYSTEMS ON BERRIES PRODUCTION. A REVIEW

Lavinia-Mihaela UDREA (ILIESCU), Mirela Florina CĂLINESCU, Ivona Cristina MAZILU, Emil CHIȚU, Florin PLĂIAȘU

Research Institute for Fruit Growing Pitești - Mărăcineni,
402 Mărului Street, 117450, Mărăcineni, Piteşti, Argeş County, Romania

Corresponding author emails: elacalinescu@yahoo.com, icmazilu@yahoo.com

Abstract

Presently, there is a growing interest in berry production, particularly in developing innovative and resilient agricultural practices that align with consumer expectations for superior flavors and nutritional value. The yielding and quality of berries fruits are influenced by various abiotic and biotic stress factors, and closely monitoring of these can lead to significant improvements regarding production and fruit quality. Photovoltaic systems provide an innovative approach to improving the use of natural and anthropic resources, reducing the negative impacts of climate change, and enhancing environmental protection. In recent years, various studies have recognized the important benefits of photovoltaic systems in fruit production such as – reducing energy costs, decreasing carbon emissions at the farm level, preserving water resources, offer shelter for beneficial organisms, and improve fruit quality in terms of appearance and nutritional matter. This study aims to provide an extensive review of the current knowledge on the opportunities and advantages of photovoltaic systems for berry crops, with a particular focus on red currants. It explores their role in optimizing growth and fruiting, enhancing resource efficiency, and reducing abiotic stress – such as sunburn for example.

Key words*: abiotic stress factors, innovative cropping systems, red currant, resilient agriculture, sustainable farming.*

INTRODUCTION

Recent studies indicate a growing interest in berry production (Aguilera, 2024; Bezerra et al., 2024; Ertekin et al., 2024; Mezzetti, 2016), with a particular focus on developing innovative and resilient cropping systems (Andersson et al., 2024; Dane et al., 2016; Takeda & Soria, 2011; Udrea et al., 2024) that meet consumer demands for enhanced fruits taste and nutritional quality (Diamanti et al., 2012; Dobson et al., 2012; Fontanesi, et al., 2012; Heide & Sønsteby, 2012; Kruger et al., 2011; Mezzetti, 2016).

The production and quality of berries are influenced by various abiotic and biotic stressors (Alvarado-Raya et al, 2018; Dragišić Maksimovi et al, 2018; Laugale et al., 2018; Lepaja et al., 2018; Massetani et al., 2014; Poyraz Engin & Mert, 2018; Winardiantika et al., 2014; Yang et al., 2018; Zucchi et al., 2014), and closely monitoring of these factors can lead to significant improvements regarding yield and fruit quality (Bamouh et al, 2019; Çelik, 2018; Cho et al, 2019; Kandemir et al, 2018; Palha et al., 2018). Considering the challenges in berry production, climate change, and resource management, farmers are increasingly seeking new technological solutions (Okatan & Aşkın, 2017; Pitsioudis et al., 2008).

Also, in the context of the climate and energy targets, European Green Deal aims to achieve, with a long-term goal of net-zero greenhouse gas emissions by 2050, while supporting energy independence and economic growth (Chatzipanagi et al., 2023; Lee et al., 2023; Strapasson et al., 2017). Thus, in line with the post-2020 Common Agricultural Policy for climate adaptation and sustainability, solar photovoltaic power provides a sustainable solution (Mihălcioiu et al., 2024; Miloș et al., 2022; Mouhib et al., 2022a). Despite its benefits, photovoltaic systems require extensive land use, competing with agriculture (Ritchie & Roser, 2013; Victoria et al., 2021). To balance renewable energy expansion and food production, agrivoltaics systems offer an advanced solution (Dupraz et al., 2020; Roxani et al., 2023). By combining solar panels with

farmland, agrivoltaics systems enables dual land use for both agriculture and energy production, particularly beneficial in land-scarce regions (Bonomi et al., 2021; Sahu et al., 2020). This approach can improve land efficiency, reduce water consumption, enhance crop yields, and minimize soil erosion, contributing to food security and energy sustainability (Roxani et al., 2023; Trommsdorff et al., 2022a).

This review explores the various applications of photovoltaic systems in berry production, with a particular focus on red currants. It examines the potential benefits, while also addressing the challenges associated with implementation. By analyzing recent research and case studies, this study aims to provide insights into how photovoltaic systems can transform both the agricultural and energy sectors, paving the way for a more sustainable and resilient future.

MATERIALS AND METHODS

This review was conducted using a systematic approach to identify, analyze, and synthesize relevant scientific literature on the application of photovoltaic systems in berry production, with a particular focus on red currants.

Literature search strategy – a comprehensive literature search was conducted using scientific databases, including Web of Science, Scopus, PubMed, Open Access Journals, ResearchGate, MDPI, and Google Scholar. The search utilized keywords such as - photovoltaic systems in horticulture, agrivoltaics in berries production, solar energy and fruit crops, sunburn prevention in fruit trees plantation, among others.

Selection criteria – studies were included in the review based on their relevance to photovoltaic applications, especially in fruit production, and particularly in berry crops.

Data extraction and analysis – selected studies were categorized based on their primary focus on technological advances in the field of agrivoltaics systems; definition and classification of agrivoltaics systems; applications of photovoltaic systems technology in agriculture and a global perspective of this; challenges and limitations of implementing agrivoltaics system and selection of the optimal ones; the key characteristics for selecting crops in agrivoltaics systems and the performance evaluation indicators; types of agrivoltaics systems where studies were conducted and results; the leading role of photovoltaic systems in berries production improvement; abiotic and biotic stress factors affecting red currants cultivation; the primary benefits of photovoltaic systems in red currant cultivation technology.

Key findings were summarized in a comparative framework to highlight the current knowledge and the advantages of photovoltaic systems for berry crops.

RESULTS AND DISCUSSIONS

Agrivoltaics systems, which consist of combining energy production through photovoltaic systems and agricultural production in the same area, have emerged as a promising solution to the constraints related to the reduction of cultivated areas due to the use of solar panels instead of agricultural production systems (Chalgynbayeva et al., 2023; Thompson et al., 2020; Trommsdorff et al., 2022a).

Historical agrivoltaics systems development

The concept of photovoltaic systems (PV) was first introduced by Götzberger and Zastrow in 1981, combining PV modules with potato production (Goetzberger et al., 1981).

As photovoltaic technology evolved and became more accessible (Trommsdorff et al., 2022a), the way was opened for the development of more advanced agrivoltaics systems. In Table 1 it is presented a brief history of the agrivoltaics systems development.

Classification of agrivoltaics systems

In general, an agrivoltaics system consists of a photovoltaic solar panel, a mounting/support structure, a foundation, a control and monitoring system, and the cultivated crops (Sahu et al., 2016). In recent years, several classifications of agrivoltaics systems have emerged (Sirnik et al., 2023; Trommsdorff et al., 2022a). In Table 2 are categorizes agrivoltaics systems based on different criteria, providing types and relevant references.

SWOT analysis of agrivoltaics systems – a global perspective

The main environmental and economic benefits of agrivoltaics systems include sustainability advantages such as reducing the carbon footprint, generating renewable energy, and preserving biodiversity (Al Mamun et al., 2022; Zahrawi & Aly, 2024). Additionally, they enhance economic viability by providing cost-benefit advantages for farmers, lowering energy expenses, and improving land use efficiency (Al Mamun et al., 2022; Zahrawi & Ali, 2024). Moreover, agrivoltaics systems play a crucial role in balancing renewable energy production with food security, ensuring a sustainable and profitable agricultural model (Al Mamun et al., 2022; Zainol Abidin et al., 2021). Table 3 presents the Strengths, Weaknesses, Opportunities, and Threats of agrivoltaics utilization.

Selection of the optimal agrivoltaics system

Before selecting the solar panel design and crop type, agrivoltaics system design must be aligned with the climatic factors of the intended crop to determine the optimal configuration (Ali, 2024; Hermelink et al., 2024; Stallknecht et al., 2023; Zahrawi & Aly, 2024; Wielgat et al., 2024).

AVS design requires a transdisciplinary approach (Ioannidis et al., 2020; Lee et al., 2023; Sargentis et al., 2021), and different optimization strategies must be implemented depending on the system's primary objective (Macknick et al., 2013; Trommsdorff et al., 2022b; Zainol Abidin et al., 2021). Toledo and Scognamiglio (2021) identified three fundamental categories: minimizing crop shading, maximizing electricity generation, and ensuring social acceptance.

Table 1. Technological advances in the field of Agrivoltaics Systems (AVS)

Year	Country	Details	References
1981	Germany	Concept of dual land use for solar energy and agriculture	Goetzberger & Zastrow, 1981
2004	Japan	Experimental AVS for crop protection	Maruyama et al., 2004; Tajima & Iida, 2021; Toledo & Scognamiglio, 2021
2011	France	Large-scale agrivoltaics farm implementation	Dupraz et al., 2011a
2012	Italy	First commercial AVS installed	Amaducci et al., 2018; Roxani et al., 2023
2013	South Korea	Floating AVS for rice farming	Chae et al., 2013
2016	India	Agrivoltaics for water conservation in dry climates	Dinesh & Pearce, 2016b; Trommsdorff et al., 2022 a
2017	Italy	Integration of AVS in grapes plantations	Amaducci & Yin, 2017
2017 2018	Belgium	AVS in orchard and designs (KU Leuven)	Trommsdorff et al., 2022a; Willockx et al., 2020b
2018	Italy	Elevated AVS structures on different crops	Amaducci et al., 2018
2019	China	Implementation of shading strategies for vegetable crops	Liu et al., 2019
2020	USA	AVS for berries production	Sekiyama & Nagashima, 2020
2021	Australia	Dual-use of AVS for livestock and pastureland	Barron-Gafford et al., 2021
2022	Netherlands	AI and sensor-integrated agrivoltaics systems	Weselek et al., 2022
2023	Germany	The largest AVS in the world (13 hectares)	Chalgynbayeva et al., 2023
2023	Spain	Large-scale agrivoltaics for staple crops (wheat, corn)	Sarr et al., 2023a

Table 2. Classification of Agrivoltaics Systems (AVS)

Criteria	Type	References
PV panels design	Fixed-tilt	Dupraz et al., 2011b; Hassanpour et al., 2018a; Younas et al., 2019
	Dynamic/Tracking	Amaducci et al., 2018; Sekiyama & Nagashima, 2019 a
	Vertical bifacial	Weselek et al., 2021a; Trommsdorff et al., 2021a
PV panels transparency	Opaque	Dinesh & Pearce, 2016b; Domínguez et al., 2017; Sirnik et al., 2023
	Semi-transparent	Fernández et al., 2022; Hernandez et al., 2014; Mouhib et al., 2022b; Sirnik et al., 2023; Thompson et al., 2020
Integration level	Fully Integrated AVS	Barron-Gafford et al., 2019; Majumdar & Pasqualetti, 2021
	Semi-Integrated AVS	Santra et al., 2017; Schindele et al., 2020
	Adjacent PV Installations	Chiriacò et al., 2022, Dinesh & Pearce, 2016a
Aim of installation	Energy (priority on electricity production)	Maity et al., 2023a, b; Mouhib et al., 2022a
	Crop-integrated AVS (balanced crop & energy output)	Amaducci et al., 2018; Dupraz et al., 2011a, 2011b
	Yield optimization	Amaducci et al., 2018; Majumdar & Pasqualetti, 2021
	Water conservation	Barron-Gafford et al., 2019; Trommsdorff et al. 2021a
Scale of implementation	Small-scale AVS farms (localized energy & crop use)	Barron-Gafford et al., 2019; Maity et al., 2023a, b; Sekiyama & Nagashima 2019a, 2019b, 2019c
	Large-scale AVS projects (commercial-level production)	Amaducci et al., 2018; Weselek et al., 2019; Weselek et al., 2021a, 2021b, 2021c, 2021d

Criteria	Type	References
Geographical adaptation	Arid & Semi-Arid Regions	Barron-Gafford et al., 2019; Hassanpour et al., 2018a; Trommsdorff et al., 2021a
	Temperate & Humid Regions	Amaducci et al., 2018; Chiriacò et al., 2022; Maity et al., 2023a, b; Weselek et al., 2021c, 2021d, 2021e
Integration with crops	Elevated AVS (panels above crops)	Barron-Gafford et al., 2019; Dinesh & Pearce, 2016b
	Inter-row AVS (panels between rows)	Hassanpour Adeh et al., 2018; Hernandez et al., 2014
Crop type	Pastures & Forage	Hassanpour et al., 2018a; Weselek et al., 2021a, 2021b, 2021c, 2021d
	Vegetable & Row Crops	Dinesh & Pearce, 2016a, 2016b; Hernandez et al., 2014; Marrou et al., 2013b; Sekiyama & Nagashima 2019 a, 2019b, 2019c
	Vineyards, Berries & Orchards	Dupraz et al., 2011a, 2011b; Santra et al., 2017; Trommsdorff et al., 2021b; Weselek et al., 2021a, 2021b, 2021c, 2021d, 2021e

Table 3. SWOT analysis of Agrivoltaics Systems (AVS)

Strengths (Advantages)	Weaknesses (Disadvantages)
Dual land use for agriculture and energy production – AVS maximize land efficiency by enabling both crop cultivation and solar energy production (Dupraz et al., 2011b; Maity et al., 2023a, b; Naamandadin et al., 2018). **Contribution to reducing carbon emissions and promoting renewable energy** – AVS lower reliance on fossil fuels, reducing greenhouse gas emissions (Blakers et al., 2013; Leon & Ishihara, 2018). **Long-term financial benefits** – Farmers can benefit from lower energy costs and additional income from selling excess electricity (Mouhib et al., 2022a; Solangi et al., 2011). **Improved microclimate for plant growth** – Moderate shading and temperature regulation can create a favorable environment (Barron-Gafford et al., 2019; Weselek et al., 2021a, b, c, d). **Enhance water-use efficiency and reduction of evaporation** – PV systems provide shade, reducing water requirements for crops and evaporation (Dupraz et al., 2011b; Hassanpour Adeh et al., 2018). **Protection of crops from extreme weather conditions** – PV act as a physical barrier, reducing damage from weather extremes such as hail, excessive heat, heavy rainfall (Aroca-Delgado et al., 2018; Dupraz et al., 2011b; Hassanpour et al., 2018a, b; Jamil & Pearce, 2023; Ruiz-Canales et al., 2022; Weselek et al., 2021a, b, c). **Increasing efficiency of solar panels** – The crops under PV would circulate cool air to reduce the temperature to make panels work optimally. High insolation, light winds, moderate temperature, and low humidity keep the panel's microclimate suitable for growing crops and increase panel efficiency (Ezzaeri et al., 2018; Sirnik et al., 2023).	**High initial investment costs** – The installation and maintenance of AVS require significant capital investment, which may not be feasible for all farmers (Giri & Mohanty, 2022a, b; Weselek et al., 2019). **Unpredictable effects on long-term regarding plants growth and soil health** – Additional research is necessary to assess the long-term ecological AVS impact (Maity et al., 2023a, b; Weselek et al., 2021a). **Possible reduction in sunlight exposure affecting crop yields** – In some cases, excessive shading may limit photosynthesis and can reduce potential yield of certain crops (Barron-Gafford et al., 2019; Erwin et al., 2017; Maity et al., 2023a, b; Touil et al., 2021). **Requires specialized knowledge and management** – AVS demand expertise in both energy management and agricultural practices to optimize their benefits (Dinesh & Pearce, 2016a). **Potential challenges in farm equipment operation under panel structures** – The presence of solar panels may restrict access to mechanized farm equipment (Amaducci et al., 2018; Reasoner & Ghosh, 2022). **Regulatory and legal restrictions in some regions** – Land-use policies and zoning regulations may pose barriers to agrivoltaics expansion (Dupraz et al., 2011b; Maity et al., 2023a, b; Randle-Boggis et al., 2021; Reasoner & Ghosh, 2022). **Reduced solar energy output** – Installing solar panels over crops can limit direct sunlight exposure, decreasing electricity generation per unit area and, additionally, crop shading may cause temperature variations in the panels, reducing solar cell efficiency (Macdonald et al., 2022; Randle-Boggis et al., 2021).
Opportunities	**Threats**
Increasing global demand for sustainable farming solutions – Climate change concerns and sustainable agriculture initiatives drive interest in agrivoltaics (Hernandez et al., 2014; Maity et al., 2023a). **Technological advancements in solar panel efficiency and smart farming integration** – Ongoing research is improving the efficiency of agrivoltaics systems (Amaducci et al., 2018; Maity et al., 2023a, b). **Potential for subsidies and financial support for green energy projects** – Governments and international organizations increasingly support renewable energy adoption (Maity et al., 2023a, b). **Expansion to various crop types beyond berries** – Research shows that agrivoltaics systems are adaptable to different crops, including vegetables and cereals (Barron-Gafford et al., 2019; Corwin & Johnson, 2019; Gorjian et al., 2022). **Market advantage for sustainably produced food and energy** – Consumers increasingly prefer products with a lower carbon footprint (Hernandez et al., 2014). **Contribute to local agro-economics and generates work places** – Activities such regular maintenance, vegetation management, system monitoring, panel cleaning, and repairs provide employment opportunities (Corwin & Johnson, 2019; Elavarasan et al., 2020; Prehoda et al., 2019).	**Policy changes and uncertain government incentives** – Changes in subsidies and regulatory frameworks could impact the economic viability of AVS (Dinesh & Pearce, 2016a). **Competition with alternative renewable energy and agricultural technologies** (Hernandez et al., 2014). **Land use conflicts** – AVS require significant land, this could lead to conflicts with other land uses, such as urban development, natural habitats, or other agricultural activities (Weselek et al., 2019). **Human perceptivity and resistance to land-use modification** – Communities may oppose AVS due to concerns about landscape good-looking or land rights (Weselek et al., 2019). **Risk of financial losses if system design is not optimized for local conditions** (Giri & Mohanty, 2022a, b; Mouhib et al., 2022a). **Potential impact of climate change on PV efficiency and crop adaptability** – Rising temperatures and extreme weather may affect AVS performance/production (Dupraz et al., 2011b). **Extreme weather events** – AVS may be vulnerable to extreme weather events (Kumpanalaisatit et al., 2022) **Pest and disease infestations** – Introducing PV can create new microclimates that could attract pests and diseases (Elavarasan et al., 2020).

For an agrivoltaics system to be efficient, several conditions must be met, as highlighted by Maity et al. (2023a) and Al Mamun et al. (2022): adequate solar radiation (Amaducci et al., 2018); good soil quality (Toledo et al., 2021); flat or gently sloping terrain (Williams et al., 2023); access to water; low wind risk; favorable climate conditions (Dinesh & Pearce,

2016a); sufficient space availability (Sarr et al., 2023b); and suitable land characteristics (Lorencena et al., 2019). The key factors influencing the performance of an agrivoltaics system include the height at which the panels are installed (Goetzberger et al., 1982; Hussain et al., 2017; Sarr et al., 2023a; Sekiyama & Nagashima, 2019a; Trommsdorff et al., 2022b), the panel orientation and tilt angle based on location, time of day, and period of the year (Amaducci et al., 2018; Gorjian et al., 2022; Macknick et al., 2022; Marrou et al., 2013a; Roxani et al., 2023; Sarr et al., 2023a; Scognamiglio et al., 2016; Valle et al., 2017), and the array density (Campana et al., 2021; Macknick et al., 2022; Moreda et al., 2021; Sarr et al., 2023a).

The key characteristics for selecting crops in agrivoltaics systems include the following: shade tolerance (Marrou et al., 2013b), water stress and irrigation requirements, plant height, crop lifespan (considering that photovoltaic systems typically have an operational lifespan of 30 years), pollination systems, biodiversity conservation, and resilience to climate change (Roxani et al., 2023).

The performance evaluation indicators for agrivoltaics systems are divided into two main categories (Lu et al., 2024):

Metric Indicators: these include incident radiation at ground level (Brutsaert, 1982; Roxani et al., 2023), energy efficiency (Roxani et al., 2023), crop yield (Campillo et al., 2012; Sinclair & Muchow, 1999), crop water requirements (Blackett et al., 2019; Roxani et al., 2023), and overall system performance (Roxani et al., 2023; Willockx et al., 2020a).

Economic Indicators: these include the price-performance ratio and electricity price capping (Schindele et al., 2020).

Types of agrivoltaics systems – some studies and results

In recent years, numerous researches have been carried out on the effects of different agrovoltaic systems, on **cereals** (Dupraz et al., 2011a; Jo et al., 2022; Kim et al., 2021; Lee 2022; Moreda et al., 2021; Sekiyama & Nagashima, 2019c;), ***green vegetable*** (Adomavicius, 2021; Chae et al., 2022; Erwin et al., 2017; Ezzaeri et al., 2018; Jo et al., 2022; Marrou et al., 2013b; Trommsdorff et al., 2022a, b; Weselek et al., 2021d, e), ***grapevines plants*** (Ahn et al., 2022; Malu et al.,2017; Maity et al., 2023b) and ***fruit trees*** (Claudia Hanisch, 2022; Juillion et al., 2020; Magarelli et al., 2024; Trommsdorff et al., 2022a).

In recent years, productivity assessment of some fruit growing species such as apple (Juillion et al., 2021; 2022), pear (Gim et al., 2025; Bellini, 2025; Willockx et al., 2024), peach, nectarine, cherry (Magarelli et al., 2024), kiwifruit (Basile et al., 2008; Jiang et al., 2022), have been evaluated for their suitability for growth under the photovoltaic systems.

The leading role of photovoltaic systems in berries production improvement are: optimizing growth and fruiting processes (microclimate regulation, shade effects, and photosynthesis optimization); improving resource efficiency (soil moisture retention, water conservation, and reduced irrigation needs); protection against abiotic stress (reducing sunburn, buffering temperature extremes, and wind/hail protection); enhancing fruit quality **(**effects on taste, texture, color, and nutritional value). In Table 4 are synthetized some results regarding the effect of photovoltaic systems on berries production. Figure 1 illustrates some berries plantations cultivated beneath solar panels.



(Source: BayWa r.e)

Figure 1. Photovoltaic systems in berry crop:
a) Raspberries; b) Red Currants

Abiotic and biotic stress factors affecting red currants cultivation

The primary factors impacting red currant production include: abiotic stressors: sunburn, drought, extreme temperatures, and soil degradation (Panfilova et al., 2021) and biotic stressors: pests and diseases influenced by environmental changes.

In Figure 2 are presented red currant cultivation climatic limiting factors.

The primary benefits of photovoltaic systems on red currant cultivation technology

Analyzing SWOT analysis presented in Table 3 and red currant requirements for vegetation factors (Figure 2), can be concluded that integrating solar panels into an agrivoltaics system offers numerous benefits for its cultivation such as growth and fruiting, improving resource efficiency, and reducing abiotic stress (Blando et al., 2018; Jung & Salmon, 2022; Jung et al., 2023).

Table 4. Effect of photovoltaic systems on berries production

Species	Results	References
Strawberries	The panels with higher transparency, demonstrated promising results regarding fruit quality for 'Sibilla' variety	Dragomir et al., 2024
	The shading from the semi-transparent PV increased the total phenolic content, as well as the free radical-scavenging activity of the harvested fruits	Petrakis et al., 2024
	Increased fruit quality due to optimized light exposure and reduced heat stress	Weselek et al., 2021d, e
	Enlarged yield and reduced water consumption under agrivoltaics shading	Amaducci et al., 2018
Blackberries	Improved productivity due to regulated microclimate and extended growing season	Magarelli et al., 2024; Maity et al., 2023a; Sarr et al., 2023 c
	Extended harvesting period, improved fruit color and sugar content	Dinesh & Pearce, 2016 a, b;
Raspberries	Enhanced photosynthesis efficiency, reduced heat stress, and increased fruit size	Marrou et al., 2013; Sekiyama & Nagashima, 2019b
	Decreased sunburn damage and better preservation of fruit firmness and color	Dupraz et al., 2011; Jamil & Pearce, 2023
Blueberries	Enhanced fruit quality, improved water-use efficiency, and protect against extreme weather conditions	Adeh et al., 2019; Barron-Gafford et al., 2019; Hassanpour et al., 2018b; Hermelink et al., 2024; Majidi et al., 2023
Red currants	Higher antioxidant content, improved resource efficiency, and better fruit appearance	Weselek et al., 2021d, e
	Enhanced photosynthetic efficiency and improved fruit size and taste	Aroca-Delgado et al., 2018; Ruiz-Canales et al., 2022
Black currants	Higher sugar content and improved fruit coloration under agrivoltaics systems	Maity et al., 2023a, b; Weselek et al., 2021a, b, c
Gooseberries	Protection from sunburn and increased shelf life	Obergfell et al., 2021
	Better protection from frost and extreme weather, leading to stable yields	Lorencena et al., 2019; Toledo et al., 2021
Cranberries	Lower water consumption and stabilized temperature in cultivation areas	Touil et al., 2022
	Increased resistance to drought stress and improved water conservation	Dinesh et al., 2016b; Williams et al., 2023
Lingonberries	Higher vitamin C content and improved frost resistance	Trommsdorff et al., 2021b
	Enhanced fruit quality and antioxidant content due to optimized shading	Sahu et al., 2016; Sarr et al., 2023c
Elderberries	Improved fruit set and resistance to pests due to favorable microclimate	Hassanpour et al., 2018a

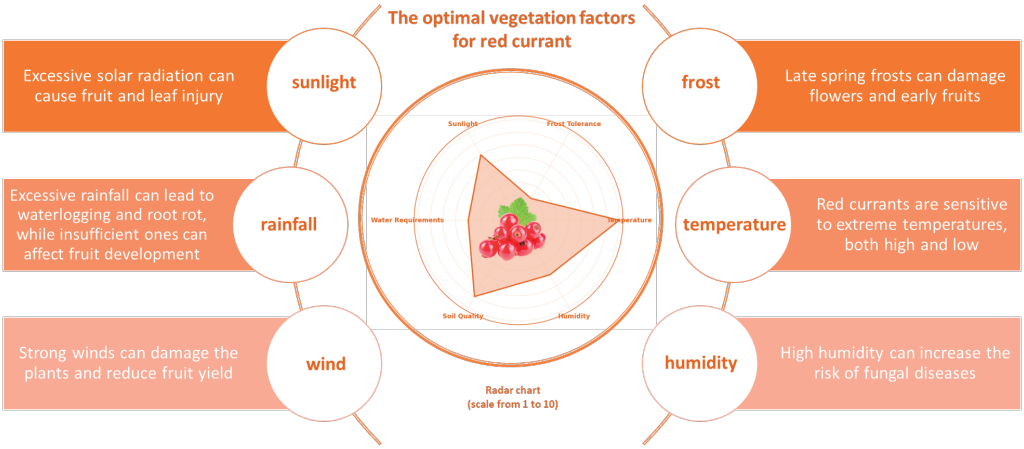


Figure 2. The main climatic limiting factors for red currant cultivation (Source: original)

The primary benefits of PV systems in fruit berries growing technology include protection against hail, frost, and drought, as well as providing partial shading, which is essential for species sensitive to direct sunlight, such as currants.

CONCLUSIONS

The integration of photovoltaic systems in berry production offers a promising path toward sustainable and resilient agriculture. By optimizing resource use, reducing climate-related stress, and improving fruit quality, agrivoltaics solutions can play a vital role in the future of horticulture. Advancements in technology, including smart PV systems, AI-driven monitoring, and precision agriculture, will be essential for enhancing efficiency, scalability, and long-term benefits. These innovations will further support sustainability, making berry production more adaptable to environmental challenges while ensuring economic viability for farmers.

ACKNOWLEDGEMENTS

This research was carried out with the support of the Ministry of Agriculture and Rural Development, financed through Project 'The use of autonomous energy systems for digitalization of technological sequences specific to precision fruit growing', ADER 6.3.23/2023.

REFERENCES

Adeh, E. H., Good, S. P., Calaf, M., Higgins, C. W. (2019). Solar PV power potential is greatest over croplands. *Scientific Reports*, 9, 11442.

Adomavicius, V. (2021). Review of results of agro-photovoltaic system implementation in agriculture. *Proceedings of International Conference on Engineering for Rural Development*, 605-610.

Aguilera, J. M. (2024). Berries as foods: processing, products, and health implications. *Annual Review of Food Science and Technology*, *15*.

Ahn, S. Y., Lee, D. B., Lee, H. I., Myint, Z. L., Min, S. Y., Kim, B. M., Oh, W., Jung, J.H., Yun, H. K. (2022). Grapevine growth and berry development under the agrivoltaic solar panels in the vineyards. *Journal of Bio-Environment Control*, 31(4), 356-365.

Al Mamun, M. A., Dargusch, P., Wadley, D., Zulkarnain, N. A., Aziz, A. A. (2022). A review of research on agrivoltaic systems. *Renewable and Sustainable Energy Reviews*, 161, 112351.

Ali, N. (2024). Agrivoltaic system success: A review of parameters that matter. *Journal of Renewable and Sustainable Energy*, 16(2). DOI 10.1063/5.0197775.

Alvarado-Raya, H. E., Rivera-del-RÃo, R., RamÃrez-Arias, A., CalderÃ³n-Zavala, G. (2018). Vegetative growth for three Mexican strawberry cultivars grown in two bed systems. *XXX Int. Hort. Congr.: III International Berry Fruit Symposium,* 1265, 171-178.

Amaducci, S., Yin, H. (2017). Integration of agrivoltaics in viticulture: Effects on crop growth and solar energy generation. *Renewable Energy*, 112, 487-496. https://doi.org/10.1016/j.renene.2017.05.070.

Amaducci, S., Yin, X., Colauzzi, M. (2018). Agrivoltaic systems to optimise land use for electric energy production. *Applied Energy*, 220, 545–561.

Andersson, J., Plummer, P., Lennerfors, T. et al. (2024). Socio-techno-ecological transition dynamics in the re-territorialization of food production: the case of wild berries in Sweden. *Sustainability Science*, 1-16.

Aroca-Delgado, R., Pérez-Alonso, J., Callejón-Ferre, Á. J., Velázquez-Martí, B. (2018). Compatibility between crops and solar panels: An overview from shading systems. *Agricultural Systems*, 167, 92-100.

Bamouh, A., Bouras, H., Nakro, A. (2019). Effect of foliar potassium fertilization on yield and fruit quality of strawberry, raspberry and blueberry. *Acta Hortic*, *1265*, 255-262.

Barron-Gafford, G. A., Pavao-Zuckerman, M. A., Minor, R. L., Sutter, L. F., Barnett-Moreno, I., Blackett, D. T., Thompson, M., Dimond, K. S., Gerlak, A. K., Nabhan, G. P., Macknick, J. (2019). Agrivoltaics provide mutual benefits across the food–energy–water nexus in drylands. *Nature Sustainability*, 2(9), 848–855.

Barron-Gafford, G. A., et al. (2021). Dual-use agrivoltaics for livestock and pastureland. *Nature Sustainability*, 4(3), 232-239.

Basile, B.; Romano, R. Giaccone, M. Barlotti, E. Colonna, V. Cirillo, C. Shahak, Y. Forlani, M. (2008). Use of Photo-Selective Nets for Hail Protection of Kiwifruit Vines in Southern Italy. *Acta Hortic.*, 770, 185–192.

Bellini, E. Agrivoltaics for Pear Orchards. Available online: https://www.pv-magazine.com/2020/10/02/ agrivoltaics-for-pear orchards/.

Bezerra, M., Ribeiro, M., Cosme, F., Nunes, F. (2024). Overview of the distinctive characteristics of strawberry, raspberry, and blueberry in berries, berry wines, and berry spirits. Comprehensive Reviews. *Food Science and Food Safety*, 23, e13354.

Blackett, D. T., Thompson, M., Dimond, K. S., Gerlak, A. K., Nabhan, G. P., Macknick, J. (2019). Agrivoltaics provide mutual benefits across the food–energy–water nexus in drylands. *Nat. Sustain.*, 2(9), 848–855.

Blakers, A., Zin, N., McIntosh, K., Fong, K. (2013). High efficiency silicon solar cells. *Energy Procedia*, 33.

Blando, F., Gerardi, C., Renna, M., Castellano, S., Serio, F. (2018). Characterisation of bioactive compounds in berries from plants grown under innovative photovoltaic greenhouses. *Journal of Berry Research*, 8(1), 55-69.

Bonomi, S., Sarti, D., Sordi, L. (2021). Agrivoltaics as an integrated approach for energy and food security. *Sustainability,* 13(9), 5159.

Brutsaert, W. (1982). Evaporation into the Atmosphere; Springer: Dordrecht, The Netherlands.

Campana, P.E., Stridh, B., Amaducci, S., Colauzzi, M. (2021). Optimisation of vertically mounted agrivoltaic systems. *J. Clean. Prod.*, 325, 129091.

Campillo, C., Fortes, R., Prieto, M.D.H., Babatunde, E.B. (2012). Solar radiation effect on crop production. *Solar radiation*, *1*(494), 167-194.

Çelik, H. (2018). The productivity of some southern highbush and rabbiteye blueberry cultivars under Turkey conditions. *XXX Int. Hort. Congr.: III International Berry Fruit Symposium*, 1265, 11-18.

Chae, K. H., et al. (2013). Floating agrivoltaic systems for rice farming. *Renewable and Sustainable Energy Reviews*, 24, 265-272.

Chae, S.H., Kim, H.J., Moon, H.W., Kim, Y.H., Ku, K.M. (2022). Agrivoltaic systems enhance farmers' profits through broccoli visual quality and electricity production without dramatic changes in yield, antioxidant capacity, and glucosinolates. *Agronomy,* 12, 1415. https://doi.org/10.3390/ agronomy12061415

Chalgynbayeva, A., Gabnai, Z., Lengyel, P., Pestisha, A., Bai, A. (2023). Worldwide research trends in agrivoltaic systems - A bibliometric review. *Energies*, 16(2), 611.

Chatzipanagi, A., Taylor, N., Jaeger-Waldau, A. (2023). Overview of the potential and challenges for agri-photovoltaics in the European Union. *Luxembourg: Publications Office of the European Union*.

Chiriacò, M. V., et al. (2022). Agrivoltaic systems: A sustainable synergy between agriculture and energy production. *Renewable Energy*, 190, 1256-1267.

Cho, W., Park, Y., Na, M. H., Choi, D. W. (2019). Exploring environmental factors affecting strawberry yield using pattern recognition techniques. *Journal of Internet Computing and Services*, *20*(1), 39-46.

Claudia Hanisch, M.A. Communications. First Agrivoltaic System for Carbon-Neutral Orcharding Being Tested. Available online: https://www.ise. fraunhofer.de/content/dam/ise/en/documents/press-releases/2021/2121_ISE_e_PR_Agri_PV_ Orcharding.pdf.

Corwin, S., Johnson, T.L. (2019). The role of local governments in the development of China's solar photovoltaic industry. *Energy Policy*, 130, 283–293.

Dragomir, D., Ofrim, D.V., Oltenacu, V. C., Oltenacu, N., Călinică, C., Dogaru, M. (2024). Impact of semi-transparent photovoltaic panels on the quality of 'Sibilla' strawberries in Southeastern Romania. *Fruit Growing Research*, Vol. XXXX, 72-77.

Dane, S., Laugale V., Lepse L. and Šterne D. (2016). Possibility of strawberry cultivation in intercropping with legumes: a review. *Acta Hortic.*, 1137, 83-86.

Diamanti, J., Capocasa, F., Balducci, F., Battino, M., Hancock, J., and Mezzetti, B. (2012). Increasing strawberry fruit sensorial and nutritional quality using wild and cultivated germplasm. *PLoS ONE 7* (10).

Dinesh, H., Pearce, J. M. (2016a). Agrivoltaics for water conservation in dry climates. *Energy for Sustainable Development*, 31, 19-26.

Dinesh, H., Pearce, J. M. (2016 b). The potential of agrivoltaic systems. *Renewable and Sustainable Energy Reviews*, 54, 299-308.

Dobson, P., Graham, J., Stewart, D., Brennan, R., Hackett, C.A., and McDougall, G.J. (2012). Over-seasons analysis of quantitative trait loci affecting phenolic content and antioxidant capacity in raspberry. *J. Agric. Food Chem.*, 60 (21), 5360–5366.

Domínguez, C., Jost, N., Askins, S., Victoria, M., Antón, I. (2017). A review of the promises and challenges of micro-concentrator photovoltaics. *AIP Publ. LLC*, 1881, 080003.

Dragišić Maksimovi, J., Urošević, S., Koron, D., Žnidaršič Pongrac, V., Milivojević, J., Radivojević, D. (2018). Field performance of 'Bluecrop' highbush blueberry in a soilless growing system by using

different fertilizers. *XXX Int. Hort. Congr.: III International Berry Fruit Symposium,* 1265, 187-194.

Dupraz, C., et al. (2011a). Large-scale agrivoltaic farm implementation. *Nature Communications*, 2, 262-267.

Dupraz, C., Marrou, H., Talbot, G., Dufour, L., Nogier, A., Ferard, Y. (2011b). Combining solar photovoltaic panels and food crops for optimising land use: Towards new agrivoltaic schemes. *Renewable Energy*, 36(10), 2725–2732.

Dupraz, C., Vagliasindi, F., Montalbano, P. (2020). *Agrivoltaics:* Synergies and trade-offs between renewable energy and food production. *Renewable and Sustainable Energy Reviews*, 119, 109586.

Elavarasan, R.M., Afridhis, S., Vijayaraghavan, R.R., Subramaniam, U., Nurunnabi, M. (2020). SWOT analysis: A framework for comprehensive evaluation of drivers and barriers for renewable energy development in significant countries. *Energy Rep.*, 6, 1838–1864.

Ertekin, C., Comart, A., Ekinci, K. (2024). Energy Analysis for Global Berry Fruit Production. *Sustainability*, *16*(6), 2520, 1-22.

Erwin, J., Gesick, E. (2017). Photosynthetic responses of swiss chard, kale, and spinach cultivars to irradiance and carbon dioxide concentration. *HortScience*, 52, 706–712.

Ezzaeri, K., Fatnassi, H., Bouharroud, R., Gourdo, L., Bazgaou, A., Wifaya, A., Demrati, H., Bekkaoui, A., Aharoune, A., Poncet, C., et al. (2018). The effect of photovoltaic panels on the microclimate and on the tomato production under photovoltaic canarian greenhouses. *Sol. Energy,* 173, 1126–1134.

Fernández, E.F., Villar-Fernández, A., Montes-Romero, J., Ruiz-Torres, L., Rodrigo, P.M., Manzaneda, A.J., Almonacid, F. (2022). Global energy assessment of the potential of photovoltaics for greenhouse farming. *Appl. Energy*, 309, 118474.

Fontanesi, Y., Rambla, J.L., Cabeza, A., Medina, J.J., Sanchez-Sevilla, J.F., Valpuesta, V., Botella, M.A., Granell, A., and Amaya, I. (2012). Genetic analysis of strawberry fruit aroma and identification of O-methyltransferase FaOMT as the locus controlling natural variation in mesifurane content. *Plant Physiol.* 159(2), 851–870.

Gim,G.H., Park, J., Young Kim, T., Deok, S., Kim, W., Nam, J., Lim, C. Investigation of Pear Growth under Foldable Agrivoltaic Structure. Available online: https://www.agrisolarclearinghouse.org/wp-content/uploads/2022/02/Investigation-of-Pear Growth-Under-Foldable-Agrivoltaic-Structure.pdf.

Giri, N.C., Mohanty, R.C. (2022a). Agrivoltaic system: Experimental analysis for enhancing land productivity and revenue of farmers. *Energy Sustain.*, 70, 54–61.

Giri, N.C., Mohanty, R.C. (2022b). Design of agrivoltaic system to optimize land use for clean energy-food production: A socio-economic and environmental assessment. *Clean Technol Env. Policy*, 24, 2595–2606.

Goetzberger, A., Zastrow, A. (1981). The concept of dual land use for solar energy and agriculture. *Renewable Energy*, 1(1), 53-57.

Goetzberger, A.; Zastrow, A. (1982). On the Coexistence of Solar-Energy Conversion and Plant Cultivation. *Int. J. Sol. Energy*, 1, 55–69.

Gorjian, S., Bousi, E., Özdemir, E., Trommsdorff, M., Kumar, N.M., Anand, A., Kant, K., Chopra, S.S. (2022). Progress and challenges of crop production and electricity generation in agrivoltaic systems using semi-transparent photovoltaic technology. *Renew. Sustain. Energy Rev.*, 158, 112126.

Hassanpour Adeh, E., Selker, J. S., Higgins, C. W. (2018). Remarkable agrivoltaic influence on soil moisture, micrometeorology, and water-use efficiency. *PLOS ONE*, 13(11), e0203256.

Hassanpour, H., Shamsi, K., Mousavi, A. (2018a). Effect of agrivoltaic shading on elderberry (*Sambucus nigra*) production. *Agricultural Systems*, 162, 102-113.

Hassanpour, H., Shirmohammad, M., Ghasemnezhad, M. (2018 b). Effects of different shading treatments on quality and antioxidant properties of highbush blueberry. *Scientia Horticulturae*, 234, 1-8.

Heide, O.M., and Sønsteby, A. (2012). Floral initiation in black currant cultivars (*Ribes nigrum* L.): effects of plant size, photoperiod, temperature, and duration of short day exposure. *Sci. Hortic.,* 138, 64–72.

Hermelink, M. I., Maestrini, B., de Ruijter, F. J. (2024). Berry shade tolerance for agrivoltaics systems: A meta-analysis. *Scientia Horticulturae*, 330, 113062.

Hernandez, R. R., Hoffacker, M. K., Field, C. B. (2014). Efficient use of land to meet sustainable energy needs. *Nature Climate Change*, 4(5), 373–377.

Hussain, A., Batra, A., Pachauri, R. (2017). An experimental study on effect of dust on power loss in solar photovoltaic module. *Renewables: Wind, Water, and Solar*, *4*, 1-13.

Ioannidis, R.; Koutsoyiannis, D. (2020). A review of land use, visibility and public perception of renewable energy in the context of landscape impact. *Appl. Energy*, 276, 115367.

Jamil, T., Pearce, J. M. (2023). Enhancing berry production through agrivoltaics. *Journal of Agricultural Science and Technology*, 45(3), 325-341.

Jiang, S., Tang, D., Zhao, L., Liang, C., Cui, N., Gong, D., Wang, Y., Feng, Y., Hu, X., Peng, Y. (2022). Effects of different photovoltaic shading levels on kiwifruit growth, yield, and water productivity under "agrivoltaic" system in Southwest China. *Agricultural Water Management*, 269, 107675.

Jo, H., Asekova, S., Bayat, M.A., Ali, L., Song, J.T., Ha, Y.S., Hong, D.H., Lee, J.D. (2022). Comparison of Yield and Yield Components of Several Crops Grown under Agro-Photovoltaic System in Korea. *Agriculture*, 12, 619.

Juillion, P., Lopez, G., Fumey, D., Génard, M., Lesniak, V., Vercambre, G. (2020). Water Status, Irrigation Requirements and Fruit Growth of Apple Trees Grown under Photovoltaic Panels. *Agrivoltaics 2020 Conference and Exhibition*.

Juillion, P. (2021). Impact of full sun tracking with photovoltaic panels on subsequent year bloom density and fruit drop in apple trees. *Fraunhofer-*

Institut für Solare Energiesysteme ISE, AgriVoltaics 2021 -Connecting Agrivoltaics Worldwide, 14.

Juillion, P., Lopez, G., Fumey, D., Lesniak, V., Génard, M., Vercambre, G. (2022). Shading Apple Trees with an Agrivoltaic System: Impact on Water Relations, Leaf Morphophysiological Characteristics and Yield Determinants. *SSRN Electron. J.*, 306, 111434.

Jung, D., Salmon, A. (2022). Price for covering cropland with an agrivoltaic system: PV panels replacing shading nets in Chilean blueberry cultivation. *AIP Conference Proceedings*, 2635(1), AIP Publishing.

Jung, D., Schönberger, F., Moraga, F. (2023). Agrivoltaics Over Berries in Chile: Potential for Clean Energy Generation and Climate Change Adaption. *AgriVoltaics Conference Proceedings*, 2.

Kandemir, A., Mısır, D., Demirsoy, L., Soysal, D., Demirsoy, H. (2018). Harvest season and some fruit quality characteristics of strawberry under protected and open field conditions. *XXX Int. Hort. Congr.: III International Berry Fruit Symposium,* 1265, 195-202.

Kim, S., Kim, S.,Yoon, C.Y. (2021). An Efficient Structure of an Agrophotovoltaic System in a Temperate Climate Region. *Agronomy*, 11, 1584.

Kruger, E., Dietrich, H., Hey, M., and Patz, C.D. (2011). Effects of cultivar, yield, berry weight, temperature and ripening stage on bioactive compounds of black currants. *J. Appl. Bot. Food Qual.,* 84, 40–46.

Kumpanalaisatit, M., Setthapun, W., Sintuya, H., Pattiya, A., Jansri, S. N. (2022). Current status of agrivoltaic systems and their benefits to energy, food, environment, economy, and society. *Sustainable Production and Consumption*, *33*, 952-963.

Laugale, V., Lepsis, J., Dane, S., Strautiņa, S. (2018). Performance of seven blackcurrant cultivars under two soil maintenance systems. *XXX Int. Hort. Congr.: III International Berry Fruit Symposium, 1265*, 43-50.

Lee, H.J., Park, H.H., Kim, Y.O., Kuk, Y.I. (2022). Crop Cultivation Underneath Agro Photovoltaic Systems and Its Effects on Crop Growth, Yield, and Photosynthetic Efficiency. *Agronomy*, 12, 1842.

Lee, S., Lee, J. H., Jeong, Y., Kim, D., Seo, B. H., Seo, Y. J., ... & Choi, W. (2023). Agrivoltaic system designing for sustainability and smart farming: Agronomic aspects and design criteria with safety assessment. *Applied Energy*, 341, 121130.

Leon, A.; Ishihara, K.N. (2018). Influence of allocation methods on the LC-CO2 emission of an agrivoltaic system. *Resour. Conserv. Recycl.*, 138, 110–117.

Lepaja, K., Lepaja, L., Kullaj, E. (2018). Effect of water stress on blueberry cultivation in pots. *XXX Int. Hort. Congr.: III International Berry Fruit Symposium,* 1265, 51-54.

Liu, W., et al. (2019). Implementation of shading strategies for vegetable crops. *Agricultural Systems*, 170, 145-152.

Lorencena, C., Pérez, R., Almeida, D. (2019). The impact of agrivoltaic systems on gooseberry cultivation. *Sustainable Agriculture Research*, 8(2), 112-123. https://doi.org/10.1016/j.sar.2019.06.004.

Lu, S. M., Amaducci, S., Gorjian, S., Haworth, M., Hägglund, C., Ma, T., ... & Campana, P. E. (2024). Wavelength-selective solar photovoltaic systems to enhance spectral sharing of sunlight in agrivoltaics. *Joule*. 8, 2483-2522,

Macdonald, J.; Probst, L.; Cladera, J.R. (2022). Opportunities and challenges for scaling agrivoltaics in rural and Urban Africa. *AIP Conf. Proc.*, 2635, 070002.

Macknick, J., Beatty, B., Hill, G. (2013). Overview of opportunities for co-location of solar energy technologies and vegetation. *National Renewable Energy Lab.(NREL), Golden, CO*, United States.

Macknick, J., Hartmann, H., Barron-Gafford, G., Beatty, B., Burton, R., Seok-Choi, C., ... & Walston, L. (2022). The 5 Cs of agrivoltaic success factors in the United States: Lessons from the InSPIRE research study. *National Renewable Energy Lab. (NREL), Golden, CO*, United States.

Magarelli, A., Mazzeo, A., Ferrara, G. (2024). Fruit Crop Species with Agrivoltaic Systems: A Critical Review. *Agronomy*, 14, 722.

Maity, P., Sarr, M., Dupont, J. (2023a). Agrivoltaic technology for sustainable agriculture: A case study on berry crops. *Renewable Energy Reports*, 12, 85-102.

Maity, R., Sharma, A., Raj, A. (2023b). Role of agrivoltaics in sustainable food and energy production. *Journal of Renewable and Sustainable Energy*, 15(2), 025101. https://doi.org/10.1063/5.0086822

Majidi, M., Naserian, S., Rezaei, E. (2023). Improving blueberry production under agrivoltaic shading systems. *Horticultural Science and Biotechnology*, 98(1), 87-95.

Majumdar, D., Pasqualetti, M. J. (2021). Dual-use of agricultural land: A review of agrivoltaic systems. *Renewable and Sustainable Energy Reviews*, 148, 111290.

Malu, P.R.; Sharma, U.S.; Pearce, J.M. (2017). Agrivoltaic Potential on Grape Farms in India. *Sustain. Energy Technol.*, 23, 104–110.

Marrou, H., Dufour, L., Wery, J. (2013a). How does a shelter of solar panels influence water flows in a soil–crop system?. *European Journal of Agronomy*, *50*, 38-51.

Marrou, H., Wéry, J., Dufour, L., Dupraz, C. (2013b). Productivity and radiation use efficiency of lettuces grown in the partial shade of photovoltaic panels. *European Journal of Agronomy*, *44*, 54-66.

Maruyama, H., et al. (2004). Experimental agrivoltaic systems for crop protection. *Solar Energy Materials & Solar Cells*, 84(1-4), 213-221.

Massetani, F., Palmieri, J., Neri, D. (2014). Misshapen fruits in'Capri'strawberry are affected by temperature and fruit thinning. *XXIX International Horticultural Congress on Horticulture: Sustaining Lives, Livelihoods and Landscapes,* II (1117), 373-379.

Mezzetti, B. (2016). The sustainable improvement of European berry production, quality and nutritional value in a changing environment: strawberries, currants, blackberries, blueberries and raspberries – the EUBerry project. *Acta Hortic.*, 1117, 309-314.

Mihălcioiu, I. M., Olteanu, A. L. G., Tabacu, A. F., Lorentz, E. D., Butcaru, A. C., Mihai, C. A., Stănică, F. (2024). Hortivoltaics-A road to go or not? A

review. *Scientific Papers. Series B. Horticulture*, 68(2).788 – 794.

Miloș G.C., Georgescu M.I., Petra S.A., Peticila G.A., Costache N., Toma F. (2022). Research On Modular Hortivoltaic Solutions. *Scientific Papers. Series B, Horticulture*, LXVI(1), 718-723.

Moreda, G.P., Muñoz-García, M.A., Alonso-García, M.C., Hernández-Callejo, L. (2021). Techno-Economic Viability of Agro-Photovoltaic Irrigated Arable Lands in the EU-Med Region: A Case-Study in Southwestern Spain. *Agronomy*, 11, 593.

Mouhib, T., Al-Shetwi, A. Q., Hossain, M. A. (2022a). Agrivoltaics: Challenges, current status, and future trends. *Renewable and Sustainable Energy Reviews*, 162, 112391.

Mouhib, E., Micheli, L., Almonacid, F.M., Fernández, E.F. (2022b). Overview of the Fundamentals and Applications of Bifacial Photovoltaic Technology: Agrivoltaics and Aquavoltaics. *Energies*, 15, 8777.

Naamandadin, N.A.; Ming, C.J.; Mustafa, W.A. (2018). Relationship between Solar Irradiance and Power Generated by Photovoltaic Panel: Case Study at UniCITI Alam Campus, Padang Besar, Malaysia. *J. Adv. Res. Eng. Knowl.,* 5, 16–20.

Obergfell, J., Schindele, S., Becker, G. (2021). Performance and microclimatic effects of agrivoltaic shading on gooseberry crops. *Sustainability*, 13(4), 2214.

Okatan, V., Aşkın, M. A. (2017). The effects of different growing systems on the yield and quality of currant cultivation. *Scientific Papers. Series B, Horticulture,* LXI, 21-26.

Palha, M. G., Pestana, F., Oliveira, C. M. (2018). Plant growth, yield and fruit quality of *Fragaria ananassa* genotypes under supplemental LED lighting system and substrate cultivation. *XXX Int. Hort. Congr.: III International Berry Fruit Symposium,* 1265, 91-98.

Panfilova, O., Tsoy, M., Golyaeva, O., Knyazev, S., Karpukhin, M. (2021). Agrometeorological and Morpho-Physiological Studies of the Response of Red Currant to Abiotic Stresses. *Agronomy*, 11, 1522.

Petrakis, T., Ioannou, P., Kitsiou, F., Kavga, A., Grammatikopoulos, G., Karamanos, N. (2024). Growth and Physiological Characteristics of Strawberry Plants Cultivated under Greenhouse-Integrated Semi-Transparent Photovoltaics. *Plants*, 13, 768.

Pitsioudis, F., Odeurs, W., Meesters, P. (2008). Early and late production of raspberries, blackberries and red currants. Workshop on Berry Production in Changing Climate Conditions and Cultivation Systems. COST-Action 863: Euroberry Research: from 838, 33-38.

Poyraz Engin, S., Mert, C. (2018). Determination of fruit growth in'Nero'and'Viking'aronia cultivars. *XXX Int. Hort. Congr.: III International Berry Fruit Symposium,* 1265, 179-186.

Prehoda, E.; Pearce, J.M.; Schelly, C. (2019). Policies to Overcome Barriers for Renewable Energy Distributed Generation: A Case Study of Utility Structure and Regulatory Regimes in Michigan. *Energies*, 12, 674.

Randle-Boggis, R.J., Lara, E., Onyango, J., Temu, E.J., Hartley, S.E. (2021). Agrivoltaics in East Africa: Opportunities and challenges. *AIP Conf. Proc.*, 2361, 090001.

Reasoner, M., Ghosh, A. (2022). Agrivoltaic Engineering and Layout Optimization Approaches in the Transition to Renewable Energy Technologies: A Review. *Challenges*, 13, 43.

Ritchie, H., Roser, M. (2019). Land use. Our world in data. Available online: https://ourworldindata.org/land-use.

Roxani, A., Zisos, A., Sakki, G. K., Efstratiadis, A. (2023). Multidimensional Role of Agrovoltaics in Era of EU Green Deal: Current Status and Analysis of Water–Energy–Food–Land Dependencies. *Land*, 12(5), 1069.

Ruiz-Canales, A., Padilla-Gasco, L., López-López, M. (2022). Red currant production in agrivoltaic environments. *Agricultural Research*, 10(4), 412-427.

Sahu, A., Yadav, N., Sudhakar, K. (2016). Agrivoltaic system: A dual purpose smart agricultural approach for sustainable food and energy production. *Renewable and Sustainable Energy Reviews*, 54, 299-308. https://doi.org/10.1016/j.rser.2015.10.024

Sahu, S. K., Patel, R., Tiwari, G. N. (2020). Agrivoltaics: A new paradigm for food and energy security. *Renewable Energy*, 148, 1-10.

Santra, P., et al. (2017). Agrivoltaics: Prospects for renewable energy and sustainable agriculture. *Current Science*, 112(10), 1992-2001.

Sargentis, G. F., Ioannidis, R., Chiotinis, M., Dimitriadis, P., Koutsoyiannis, D. (2021). Aesthetical issues with stochastic evaluation. *Data Analytics for Cultural Heritage: Current Trends and Concepts*, 173-193.

Sarr, A., Soro, Y. M., Tossa, A. K., Diop, L. (2023a). Agrivoltaic, a Synergistic Co-Location of Agricultural and Energy Production in Perpetual Mutation: A Comprehensive Review. *Processes*, 11(3), 948.

Sarr, A., et al. (2023b). Large-scale agrivoltaics for staple crops. *Renewable and Sustainable Energy Reviews*, 173, 112891.

Sarr, M., Diallo, B., Ndiaye, P. (2023c). Enhancing berry growth in arid regions using agrivoltaic solutions. *Environ. Sci. Technol.*, 57(5), 678-689.

Schindele, S., et al. (2020). Implementation of agrivoltaics: Techno-economic analysis. *Applied Energy*, 265, 114737.

Scognamiglio, A. (2016). 'Photovoltaic landscapes': Design and assessment. A critical review for a new transdisciplinary design vision. *Renew. Sustain. Energy Rev.*, 55, 629–661.

Sekiyama, T., Nagashima, A. (2019a). Solar sharing for sustainable agriculture and energy. *Sustainability*, 11(2), 588.

Sekiyama, T., Nagashima, A. (2019b). Solar sharing for both food and clean energy production: Performance of agrivoltaic systems for strawberries in Japan. *Renewable Energy*, 140, 620-632.

Sekiyama, T., Nagashima, A. (2019c). Solar Sharing for Both Food and Clean Energy Production:

Performance of Agrivoltaic Systems for Corn, A Typical Shade-Intolerant Crop. *Environments*, 6, 65.
Sekiyama, K., Nagashima, T. (2020). Agrivoltaic systems for berry production. *Renewable Energy*, 158, 1010-1017.
Sinclair, T.R., Muchow, R.C. (1999). Radiation Use Efficiency. *Advances in Agronomy; Sparks, D.L., Ed.; Academic Press: Cambridge,* MA, USA, 65, 215–265.
Sirnik, I., Sluijsmans, J., Oudes, D., Stremke, S. (2023). Circularity and landscape experience of agrivoltaics: A systematic review of literature and built systems. *Renewable and Sustainable Energy Reviews*, 178, 113250.
Solangi, K.H., Islam, M.R., Saidur, R., Rahim, N.A., Fayaz, H. (2011). A review on global solar energy policy. *Renew*. *Sustain*. *Energy Rev.*, 15, 2149–2163.
Stallknecht, E. J., Herrera, C. K., Yang, C., King, I., Sharkey, T. D., Lunt, R. R., Runkle, E. S. (2023). Designing plant–transparent agrivoltaics. *Scientific reports*, 13(1), 1903.
Strapasson, A., Woods, J., Chum, H., Kalas, N., Shah, N., Rosillo-Calle, F. (2017). On the global limits of bioenergy and land use for climate change mitigation. *GCB Bioenergy*, 9, 1721–1735.
Tajima, M., Iida, T. (2021). Evolution of agrivoltaic farms in Japan. *AIP Publ. LLC*, 2361, 030002.
Takeda F, Soria J. (2011). Method for producing longcane blackberry plants. *HortTechnology*, 21, 563- 568.
Thompson, E. P., Bombelli, E. L., Shubham, S., Watson, H., Everard, A., D'Ardes, V., ... & Bombelli, P. (2020). Tinted semi-transparent solar panels allow concurrent production of crops and electricity on the same cropland. *Advanced Energy Materials*, *10*(35), 2001189.
Toledo, C., Scognamiglio, A. (2021). Agrivoltaic systems design and assessment: A critical review, and a descriptive model towards a sustainable landscape vision (three-dimensional agrivoltaic patterns). *Sustainability*, *13*(12), 6871.
Toledo, M., García, R., Morán, L. (2021). Soil quality improvements under agrivoltaic systems in fruit orchards. *Geoderma*, 400, 115-130.
Touil, S.; Richa, A.; Fizir, M.; Bingwa, B. (2021). Shading effect of photovoltaic panels on horticulture crops production: A mini review. *Rev. Environ. Sci. Biotechnol.*, 20, 281–296.
Touil, S., Benmamar, S., Bouguerra, A. (2022). Agrivoltaic technology: Impacts on cranberry production and energy sustainability. *Energy Reports*, 8, 1123-1134.
Trommsdorff, M., et al. (2021a). Combining photovoltaic power and food production: An overview of agrivoltaic systems. *Renewable and Sustainable Energy Reviews*, 141, 110794.
Trommsdorff, M., Kang, J., Schindele, S. (2021b). Agrivoltaic potential for improving berry crop quality and environmental sustainability. *Frontiers in Sustainable Food Systems*, 5, 456-472.
Trommsdorff, M., Dhal, I. S., Özdemir, Ö. E., Ketzer, D., Weinberger, N., Rösch, C. (2022a). Chapter 5. Agrivoltaics: solar power generation and food production. In Solar energy advancements in agriculture and food production systems. *Academic Press*., 159-210.
Trommsdorff, M., Gruber, S., Keinath, T., Hopf, M., Hermann, C., Schönberger, F., Zikeli, S., Ehmann, A., Weselek, A., Bodmer, U., et al. (2022b). Agrivoltaics: Opportunities for Agriculture and the Energy Transition. *Fraunhofer Institute for Solar Energy Systems ISE, 2nd ed.,* Freiburg, Germany.
Udrea, L. M., Violeta Alexandra, I. O. N., Barbu, A., Frîncu, M., Petre, A. C., Bădulescu, L. A., Bujor, O. C. (2024). Sensorial evaluation of organic strawberries and raspberries: effects of companion plants. Scientific Papers. *Series B, Horticulture*, LXVIII(2), 212-218.
Valle, B., Simonneau, T., Sourd, F., Pechier, P., Hamard, P., Frisson, T., Ryckewaert, M., Christophe, A. (2017). Increasing the total productivity of a land by combining mobile photovoltaic panels and food crops. *Appl. Energy*, 206, 1495–1507.
Victoria, M., Haegel, N., Peters, I.M., Sinton, R., Jäger-Waldau, A., del Cañizo, C., Breyer, C., Stocks, M., Blakers, A., Kaizuka, I.; et al. (2021). Solar photovoltaics is ready to power a sustainable future. *Joule*, 5, 1041–1056.
Weselek, A., Bauerle, A., Hartung, J., Zikeli, S. (2019). Agrivoltaics: High tech meets agriculture. Sustainable Agriculture Reviews, 36, 303–334.
Weselek, A., Ehmann, A., Zikeli, S., Lewandowski, I., Schindele, S., Högy, P. (2021a). Agrivoltaic systems: Applications, challenges, and opportunities. *Renewable and Sustainable Energy Reviews*, 144, 110-125. https://doi.org/10.1016/j.rser.2021.111280
Weselek, A., Ehmann, A., Zikeli, S., Lewandowski, I., Schindele, S., Högy, P. (2021b). Agrivoltaic systems: Applications and potentials for organic farming. *Renewable Agriculture and Food Systems*, 36(1), 1-15.
Weselek, A., et al. (2021c). Agrivoltaics: Synergies and trade-offs between land, food, and energy. *Renewable and Sustainable Energy Reviews*, 148, 111299.
Weselek, A., Bauerle, A., Hartung, J., Zikeli, S., Lewandowski, I., Högy, P. (2021d). Agrivoltaic system impacts on microclimate and yield of different crops within an organic crop rotation in a temperate climate. *Agron. Sustain. Dev.*, 41, 59.
Weselek, A., Bauerle, A., Zikeli, S., Lewandowski, I., Högy, P. (2021e). Effects on Crop Development, Yields and Chemical Composition of Celeriac (*Apium graveolens L.* var. Rapaceum) Cultivated Underneath an Agrivoltaic System. *Agronomy*, 11, 733.
Weselek, A., et al. (2022). AI and sensor-integrated agrivoltaic systems. Solar Energy, 245, 92-103.
Wielgat, R., Kołodziej, A., Candela, L., Lisowska-Lis, A., Jasielski, J., Chlastawa, Ł., Touhami, M, Jaramillo, M. F. (2024). A concept of smart agro-photovoltaic tunnels. *IEEE Access*, 12, 40765-40794.
Williams, R., Brown, T., Li, X. (2023). Agrivoltaic farming: Future perspectives for sustainable agriculture. *Journal of Renewable Energy and Agriculture*, 29(2), 55-72.

Willockx, B., Herteleer, B., Ronsijn, B., Uytterhaegen, B., Cappelle, J. (2020a). A standardized classification and performance indicators of agrivoltaic systems. *EU PVSEC Proceedings,* 1–4.

Willockx, B., Herteleer, B., Cappelle, J. (2020b). Combining photovoltaic modules and food crops: first agrovoltaic prototype in Belgium. *Renewable Energy & Power Quality Journal*, *18*.

Willockx, B., Reher, T., Lavaert, C., Herteleer, B., Van de Poel, B., Cappelle, J. (2024). Design and evaluation of an agrivoltaic system for a pear orchard. *Applied Energy*, 353, 122166.

Winardiantika, V., Lee, Y. H., Choi, I. Y., Yoon, C. S., Yeoung, Y. R. (2014). Effects of high temperature on pollen performance in ever-bearing strawberry cultivars. *XXIX International Horticultural Congress on Horticulture: Sustaining Lives, Livelihoods and Landscapes,* II (1117), 365-372.

Yang, H. Y., Zhang, C. H., Wu, W. L., Lyu, L. F. (2018). Influence of drought stress on the leaf morphology and physiological characteristics in blackberry (*Rubus* L.) seedlings. *XXX Int. Hort. Congr.: III International Berry Fruit Symposium,* 1265, 27-34.

Younas, R., Imran, H., Riaz, M. H., Butt, N. Z. (2019). Agrivoltaic farm design: Vertical bifacial vs. tilted monofacial photovoltaic panels. *Appl Phys*, 1-29. https://arxiv.org/abs/1910.01076v1

Zahrawi, A.A., Aly, A.M. (2024). A Review of Agrivoltaic Systems: Addressing Challenges and Enhancing Sustainability. *Sustainability*, 16, 8271.

Zainol Abidin, M.A., Mahyuddin, M.N., Mohd Zainuri, M.A.A. (2021). Solar Photovoltaic Architecture and Agronomic Management in Agrivoltaic System: AReview. *Sustainability*, 13, 7846.

Zucchi, P., Martinatti, P., Pergher, A. (2014). Photoperiod extension effect on nursery tray-plants of everbearing strawberry. *XXIX International Horticultural Congress on Horticulture: Sustaining Lives, Livelihoods and Landscapes*, II (1117), 359-364.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

PROXIMATE COMPOSITION AS A FORM OF NUTRITIONAL QUALITY ASSESSMENT OF BANANA PEEL

Ariana-Bianca VELCIOV[1], Antoanela COZMA[2], Georgeta-Sofia POPESCU[1], Virgil-Dacian LALESCU[1], Daniela STOIN[1], Laura RĂDULESCU[1], Maria RADA[3]

[1]University of Life Sciences "King Mihai I" from Timişoara, Faculty of Food Engineering, Food Science Department, 119 Calea Aradului, 300645, Timişoara, Romania
[2]University of Life Sciences "King Mihai I" from Timişoara, Faculty of Agriculture, 119 Calea Aradului, 300645, Timişoara, Romania
[3]"Victor Babeş" University of Medicine and Pharmacy, 2 Eftimie Murgu Sq., 300041, Timişoara, Romania

Corresponding authors emails: radamariam@gmail.com, dlalescu@usvt.ro

Abstract

Banana peel, obtained as by-product of banana processing, contains sufficient amounts of nutritive and phytochemical constituents that have positive effects in human nutrition. Previous studies have shown that banana peels are rich in nutritional and bioactive compounds, which provides a number of benefits, that include antibacterial, antihypertensive, antidiabetic and anti-inflammatory properties. Proximate analysis provides valuable information about the nutritional composition of banana peels and helps to evaluate the sample quality. This work aims to determine the proximate composition of banana peels, with a view to their valorization for the development of value-added food products. The obtained results show that the nutritional parameters of banana peel provide values that vary depending on the origin of the fruit from which they were obtained: 5.21-8.21% moisture, 12.86-8.17% ash, 5.28-7.84% protein, 3.14-4.11% fat, 10.23-14.65% fiber, and 57.93- 62.72% carbohydrate. These values show that the analyzed banana peels have considerable nutritional value and could be effectively integrated into a diverse range of value-added food products

Key words*: banana peel, by-products, nutritional constituents, proximate composition.*

INTRODUCTION

Bananas (*Musa* spp., Musaceae family) are one of the most widely consumed fruits in the world, being an important source of nutrients, including carbohydrates, fibre, vitamins (especially vitamin B_6 and vitamin C), minerals (especially potassium), and various bioactive compounds such as phenols, carotenoids, and biogenic amines (Ogo et al., 2024; Jandal, 2024; Ansari et al., 2023; Chimdi et al., 2024; Munir et al., 2024: Rawat et al., 2024). These fruits are widely known for their high nutritional value, containing dietary fiber, pectin, high levels of minerals (potassium and phosphorus), phenolic compounds (e.g., catechins, trichothecenes, lignans, tannins, and anthocyanins), vitamins (A, B, C, and E), β-carotene, and phytosterols (Huang et al., 2024). Naturally ripened bananas contain 77.78% moisture, 0.81% fat, 2.59% ash, 2.32% fiber, 2.25% protein, 25.23% carbohydrates, 0.02 ppm Pb, 0.78 ppm Cu, 6.08 ppm Zn, 0.14 ppm Mn, 9.12 ppm Mg, 0.01ppm Cu, 0.98 ppm Cd (Ogo et al., 2024). Bananas vary in colour, size, and firmness but are usually elongated and curved, with soft, starchy flesh and skin that can be green, yellow, red, purple, or brown, depending on the variety (Edima-Nyah et al., 2024). The nutritional content of bananas varies widely in concentration, depending on the banana variety, soil conditions, climate, cultivation, and stage of fruit development and storage conditions (Huang et al., 2024). The edible part of the banana fruit represents about 60-70% of the fruit weight (Chimdi et al., 2024; Jandal, 2024; Ansari et al., 2023; Rawat et al., 2024; Akhter et al., 2024), is usually consumed raw or processed into a variety of products, on a large or small scale, including dried fruits, snacks, smoothies, ice cream, bread, wheat, wine, and functional food additives (Sahoo & Lenka, 2024; Ansari et al., 2023).

Banana fruit processing activities generate significant quantities of banana peels, which

are considered significant by-products (Jandal, 2024). Banana peel, the outer shell of the banana fruit, is a by-product of household consumption and banana processing (Hikal et al., 2022) and is rich in nutrients (fiber, protein, crude fat, lipids, pectin, essential amino acids, polyunsaturated fatty acids, and micronutrients) (Kusumasari et al., 2024; Youssef et al., 2024) and bioactive compounds that provide many health benefits. Even though its nutritional content and bioactive compounds vary depending on the banana variety, environmental conditions, extraction methods, and evaluation methods (Ansari et al., 2023), the amount of ash, protein, crude fiber, and digestible starch in banana peel flour was reported to be significantly higher than that of pulp, which makes the banana peel flour more effective as a functional additive (Kusumasari et al., 2024; Amini Khoozani et al., 2019). In addition to its excellent nutritional value, banana peels have a variety of health benefits, treating enteritis, diarrhea, dysentery, ulcerative colitis, nephritis, gout, heart disease, hypertension, and diabetes (Youssef et al., 2024). Banana peels are also rich in phenolic compounds, with high antioxidant activity that prevents heart disease and cancer. Banana peels contain a wide range of bioactive constituents, including flavonoids, tannins, alkaloids, glycosides, anthocyanins, and terpenoids, which confer many benefits to banana peels, including antibacterial, antihypertensive, antidiabetic, and anti-inflammatory properties (Rawat et al, 2024; Kusumasari et al, 2024; Ansari et al., 2023). Banana peels are considered richer in phytochemicals compared to pulp, which has antifungal and antibiotic properties and is beneficial for human health (Ansari et al., 2023). However, it should be noted that banana peels may also contain antinutrient compounds, such as glycosides, alkaloids, oxalates, and phytate (Bhavani et al., 2023).

Several studies have shown that banana peel has a rich nutritional content that depends on the banana variety, environmental circumstances, cultivation techniques, fruit maturity stage, and evaluation methods (Chimdi et al., 2024; Sahoo and Lenka, 2024; Hikal et al., 2022; Ansari et al., 2023). The values for some nutritional parameters of banana peels reported by researchers in the field are presented in Table 1.

Table 1. Proximate composition of banana peels according to various authors

Sample	Proximate composition (%)						References
	Moisture	Ash	Protein	Fat	Crude fibre	Carbohydrates	
Banana peel	3.97	9.07	7.23	2.90	-	76.83	Jandal, 2024
Banana (*Musa acuminata)* peel	7.36	12.11	16.42	10.43	30.53	23.15	Chimdi et et al., 2024
Banana (*Musa acuminata Cavendish* subgroup*)* peels	4.42	12.73	9.56	5.04	19.49 NS	46.76	Issara et al., 2024
Banana (*Musa sapientum)* peel	-	5.93	1.95	-	-	11.82	Hikal et al., 2024
Banana (*Musa* x *paradisiaca* L.) organically grown peel	10.50	4.61	7.70	1.28	3.81	72.1	Mahomud et al., 2024
Bantal variety banana (*Musa paradisiaca*) peel flour	8.65	6.79	11.81	6.7	11.5	66.05	Sahoo and Lenka, 2024
Ripe banana (*Musa sapientum*) peel	89.34	17.79,	8.72	8.22	34.80	-	Youssef et al., 2024
Malbhog (*Musa paradisiaca* AAB group) banana peel	7.05	12.19	9.29	3.92	11.70	67.53	Akhter et al., 2024
Yellow banana peels (*Musa* sp.)	-	11.58	10.76	6.58	19.48 NS	51.6	Alshehri et al., 2024
Nam wa mali-ong banana peels	4.93	8.20	3.76	5.22	16.56	61.34	Khamsaw et al., 2024
Ripe banana (*Musa* spp.) peel powder	15.32	14.33	3.46	5.89	15.57	-	Ahsan et al., 2024
Bananas at stage 3 of ripening supermarket	8.9	7.0	6.5	13.0	16.6	71.0	Dom et al, 2024

Sample	Proximate composition (%)						References
	Moisture	Ash	Protein	Fat	Crude fibre	Carbohydrates	
Banana (Musa gen) peel pwders	7.50	6.70	3.22	1.81	29.52	51.25	Saeed et al., 2024
Banana (*Musa* spp.) peel waste powder	13.90	14.93	8.60	7.41	13.88	43.28	Taha et al. 2024
Banana (Cavendish var.) peel, dried	-	12.45	10.44	8.40	11.81	43.40	Gupta, T., & Abdollah M., 2022
Banana (*Musa cavendish*) peel powder	3.56	11.86	6.41	10.22	14.38	57.13	Segura-Badilla et al., 2022
Banana peel	-	9 – 11	5.5 –7.87	2.24 –11.6	-	59.51 – 76.58	Zaini et al., 2022
Banana (*Musa sapientum*) peels termed waste	63.33	9.60	1.95	5.93	8.37	11.82	Joy et al., 2022.
Banana peels from market	9.83	9.56	3.23	0.89	12.67	63.82	Tsado et al., 2021
Banana (*Musa cavendish*) peel, drying	10.03	25.19	15.10	7.04	–	44.64	Ahmed et al., 2021
Banana (*Musa sapientum*) peels	49.5	8.8	5.30	1.06	19.2	-	Pyar et al, 2018
Banana (*Musa sapientum*) peel	88.10	13.42	7.57	10.44	-	68.31	Aboul-Enein et al., 2016
Banana (*Musa sapientum*) peel	6.70	8.50	0.90	1.70	28.56	59.00	Anhwange et al., 2009

The nutritional benefits of banana peels offer ample opportunities for their use as food additives, which can produce value-added food products (Kusumasari et al., 2024; Ansari et al., 2023). The literature reviewed shows that the use of banana peels in the development of value-added food products has been explored in several studies that have shown potential formulations for obtaining functional foods (Ansari et al., 2023; Zaini et al., 2022; Jandal, 2024; Kusumasari et al., 2024). Several studies have been conducted to improve and fortify various food products using banana peels, for example: dried plant-based meat products like chicken sausages (Issara et al., 2024) or fish and beef patties (Bin Mohd Zaini et al., 2019; Dom et al., 2024), pasta and bakery products (Akhter et al., 2024; Youssef et al., 2024; Segura-Badilla et al., 2022), biscuits (Paramitasari et al., 2024; Jandal, 2024; Owuno & Wabali, 2024), muffin (Ahsan et al., 2024), chapatti as an ingredient in gluten-free cake formulation (Türker & Savlak, 2022), functional puddings (Sadek, 2024), salted noodles and yellow noodles (Pasha et al., 2022; Dom et al., 2024), anxiolytic bagels (Manzoor et al., 2024), probiotic yogurt (Mahomud et al., 2024), in recipes such as stews, soups, and curries (Kusumasari et al., 2024).

Considering the above, in this paper, the proximate composition of banana peels was analyzed, which provides valuable information about the nutritional composition of banana peels to use in the formulation of value-added foods, respectively, in obtaining functional foods. The following parameters were evaluated: water, ash, crude protein, lipids, crude fiber, and carbohydrate content of banana peels sold in local agri-food markets.

MATERIALS AND METHODS

For the experiment, samples of ripe yellow bananas (quality I) "Vela rosa Premium Banana" - of the Cavendish variety (Figure 1 a) imported from a Latin American country, sold by three traders in local agro-industrial markets, were taken. Three sets of banana samples (about 1 kg each) were made, corresponding to the three sellers from whom they were purchased. The banana fruits, well washed under cold tap water, were peeled by hand, and the peels obtained (Figure 1 b), marked with BP1, BP2, and BP3 - corresponding to the three suppliers, were cut into strips 2-3 mm wide. To prevent the initial enzymatic browning reaction, banana peels were immersed in a 0.5% (w/v) citric acid solution at a cold temperature for 10 min, after which they were rinsed with distilled water (Mahomud et al., 2024). After draining the water, the peels were dried in a ventilated oven at 50^0C until a constant moisture content was reached (Figure 2 a). After drying, the banana peel samples were ground using a coffee grinder. The banana peel powder thus prepared (Figure 2 b) was stored at cold temperature in plastic boxes until analysis.

The nutritional composition of the banana peels taken in the experiment was determined in accordance with the AOAC Official Methods of

Analysis, 2000 (AOAC, 2000) and the recommendations according to the recommendations of Velciov et al. (2024) and Sahoo & Lenka (2024). The moisture content was determined by the oven drying method at 105^0C to constant mass (Velciov et al., 2024).



a b

Figure 1. Ripe banana fruit (a) and peel (b)



a b

Figure 2. Dried banana peel (a) and ground (powder, b)

The ash (mineral) content was determined by the calcination method at 550°C (Velciov et al., 2024). The protein content was determined by the Kjeldahl method, using a nitrogen conversion factor of 6.25. (Velciov et al., 2024; Sahoo & Lenka, 2024). Crude fat was determined using the Soxhlet method with hexane as solvent (Velciov et al., 2024; Sahoo & Lenka (2024). Crude fiber was determined by acid-base digestion method. Carbohydrate content was obtained by difference (Velciov et al., 2024).

All analyses were performed using Rstatistical Software (v4.3.3; RCoreTeam2023).

The comparisons for means were done using Duncan's Multiple Range Tests (DMRT). Duncan's Multiple Range Tests or Duncan's New Multiple Range Tests provide significance levels for the difference between any pair of means, regardless of whether a significant F resulted from an initial analysis of variance. The Shapiro-Wilk test was used to assess the normality of the data (Ghosh and Mitra, 2020).

RESULTS AND DISCUSSIONS

Proximate analysis provides valuable information about the nutritional composition and helps to assess the quality of the sample, providing information about the content of water, protein, lipids, ash, fiber and carbohydrates. (Saeed et al, 2024; Pyar & Peh, 2018).

The proximate composition of banana peels, which includes moisture, ash, protein, fat, fiber, and carbohydrates are present in Table 2.

The experimental results obtained show that the analyzed BPs have a considerable nutritional value that depends on the location and nature of the analyzed nutritional parameter: 5.21-8.21% moisture, 8.17-12.86% ash (minerals), 5.28-7.84% protein, 3.14-4.11% fats, 10.23-14.65% crude fibre, and 57.93-62.72% carbohydrate.

This can also be confirmed by numerous studies reporting that the proximal composition and nutritional properties of banana peels vary between different banana varieties and even between the same varieties from different cultivation areas (Sahoo and Lenka, 2024; Ansari et al., 2023).

The moisture content of the analyzed BPs indicates that the examined banana peels could be used in food processing, as well as in cosmetics, medicine, the textile industry, paper manufacturing, bio-absorbents, biofuel production, and the agricultural sector (Bhavani et al., 2023).

Table 2. Proximate composition (mean values) of banana peels

Specification	Nutritional parameter (% dry matter) *					
	Moisture	Ash	Proteins	Fats	Fibres	Carbohydrates
Provider 1 (BP1)	8.210.31a	8.17±0.31a	7.84±0.47a	4.11±0.29a	13.28±0.41b	57.93±2.41a
Provider 2 (BP2)	7.58±0.27a	8.42±0.27a	7.35±0.34a	3.62±0.26ab	14.65±0.42a	58.16±2.25a
Provider 3 (BP3)	5.21±0.31b	12.86±0.55a	5.28±0.37b	3.14±0.22b	10.23±0.35c	62.72±2.97a
Mean value	*7.00±1.29*	*9.82±2.15*	*6.82±11*	*3.62±0.40*	*12.72±1.85*	*59.60±2.21*

*Values are expressed as means ± standard deviation of triplicate determination. Different letters within a column signify significant differences (p< 0.001).

The moisture content of a product provides information about the amount of water contained in a given product and the amount of dry matter in that product (Velciov et al., 2024). Foods with a low moisture content, especially those with less than 10%, have a longer shelf life with limited quality deterioration due to microbial activities (Velciov et al., 2024). The moisture content of BPs varies between 5.21% (BP3) and 8.21% (BP1). Compared to these, the BP3 sample shows a much lower moisture content.

The assumption of normality moisture content distributions was assessed by using the Shapiro-Wilks test. Results indicated that the moisture contents are normally distributed (for BP1: W=0.989, p=0.79; for BP2: W=0.976, p =0.70; for GP3: W=0.89, p=0.76). A one-way ANOVA was performed to compare the effect of the three different providers on moisture contents. It revealed that there was a statistically significant difference in mean moisture contents between at least two groups (F = 65.68, p < 0.001, Eta2=0.96, 95% C.I. [0.84, 1.00]). Duncan's MRT for multiple comparisons found that the mean value of moisture content was significantly different between provider 1 and provider 3 (p < 0.001, 95% C.I. = [2.29, 3.69]), and between provider 2 and provider 3 (p < 0.001, 95% C.I. = [1.69, 3]). There was no statistically significant difference in mean moisture contents between provider 1 and provider 2 (p=0.06).

These values show that the analyzed BPs are rich in organic matter, which is a good source of nutrients (Anhwange et al., 2009). In addition, the low moisture content (less than 10%) gives the analyzed BPs a longer shelf life without limited changes in their quality. (Velciov et al, 2024) The moisture values of the analyzed BPs (Table 2), as well as their average value (7.00%), are comparable to the values reported by Chimdi et al. (2024), Akhter et al. (2024), Saeed et al. (2024) and Anhwange et al. (2009) (see Table 1).

Ash content represents the inorganic residues (mineral composition), which remain after the complete oxidation of organic materials with the removal of water by calcination of a food product (Rawat et al., 2024; Ansari et al., 2023; Pyar & Peh, 2018).). Ash content provides information on the content of mineral elements in the food (Rawat et al., 2024). High ash values indicate a high content of mineral elements (Saeed et al., 2024; Tsado et al., 2021). Banana peel is a rich source of minerals such as Ca, Fe, Mg, Zn, Na, K, P, and Cu (Jandal, 2024; Bhavani et al., 2023; Tsado et al., 2021; Rawat et al., 2024). They are essential in many physiological functions, such as metabolic pathway management, important organ formation, pH balancing, and muscular action (Ansari et al., 2023).

The ash content (Table 2) of BPs ranges from 8.17% (BP1) to 12.86% (BP3). Lower but relatively close values were determined in BP1 and BP2 (8.17, respectively, 8.72%); the BP3 sample is the richest in minerals (12.86%).

The assumption of normality ash content distributions was assessed by using the Shapiro-Wilks test. Results indicated that the ash contents are normally distributed (for BP1: W= 0.992, p=0.83; for BP2: W=0.994, p=0.84; for BP3: W=1, p=0.97). A one-way ANOVA was performed to compare the effect of the three different providers on ash contents. It revealed that there was a statistically significant difference in mean ash contents between at least two groups (F = 87.21, p < 0.001, Eta2=0.97, 95% C.I. [0.88, 1.00]). Duncan's MRT for multiple comparisons found that the mean value of ash content was significantly different between provider 1 and provider 3 (p < 0.001, 95% C.I. = [3.66, 5.69]), and between provider 2 and provider 3 (p < 0.001, 95% C.I. = [3.45, 5.40]). There was no statistically

significant difference in mean ash contents between provider 1 and provider 2 (p=0.55).

These values show that the analyzed BPs, especially BP3, contain increased amounts of mineral elements, being sources of valuable biominerals for obtaining foods with added mineral value. Comparing the ash content values of the analyzed BP samples with the values reported by different authors (see Table 1), it can be seen that it falls within the range of values determined by Jandal (2024), Chimindi et al. (2024), Issara et al. (2024), Khamsaw et al. (2024), Alshehri et al., 2024), Akhter et al. (2024), Zaini et al. (2022), Joy et al. (2022), Tsado et al. (2021), Pyar & Peh, (2018), and Anhwange et al. (2009).

Proteins are essential component of the diet needed for the survival of animals and human, they serve as source of nitrogen in the body (Velciov et al., 2024). Protein provides amino acids which are the substrates required for the support of body protein synthesis and maintenances of cell and organ protein content; thus, it furnishes amino acid the building block of all protein (Ahmed et al., 2021; Pyar & Peh, 2018). Protein deficiency is closely related to a number of diseases such as mental disorders, insufficiency of different organs, edema and immune system weakness and increased protein intake plays an important role in diseases related to diabetes and the cardiovascular system (Velciov et al., 2024). The analyzed BP samples contain relatively low amounts of protein but sufficient to contribute to their nutritional intake. The crude protein content of fruits is lower because fruits are generally not important protein sources (Pyar & Peh, 2018). The concentration of crude fibers in analyzed BPs (Table 2) shows different values, ranging between 5.28% (BP3) and 7.84% (BP1).

The assumption of normality protein content distributions was assessed by using the Shapiro-Wilks test. Results indicated that the protein contents are normally distributed (for BP1: W= 0.808, p=0.73; for BP2: W=0.916, p=0.73; for BP3: W=0.999, p=0.96). A one-way ANOVA was performed to compare the effect of the three different providers on fat contents. It revealed that there was a statistically significant difference in mean protein contents between at least two groups ($F = 23.54$, $p < 0.001$, Eta2=0.89, 95% C.I. [0.60, 1.00]). Duncan's MRT for multiple comparisons found that the mean value of protein content was significantly different between provider 1 and provider 3 ($p < 0.001$, 95% C.I. = [1.55, 3.56]), and between provider 2 and provider 3 ($p < 0.01$, 95% C.I. = [1.10, 3.03]). There was no statistically significant difference in mean ash contents between provider 1 and provider 2 (p=0.26).

Although BPs are not important sources of protein, the protein concentration in the analyzed BPs shows that they can be helpful in the preparation of foods with added nutritional value (Dom et al., 2024) and are relatively good protein sources. The protein values determined in BP1, BP2, BP3 and their average (6.82%, see Table 1) are comparable to those reported by Jandal (2024), Mahomud et al. (2024), Dom et al. (2024), Segura-Badilla et al. (2022), Zaini et al. (2022), Pyar & Peh (2018), Aboul-Enein et al. (2016).

Fats, along with carbohydrates and proteins, are macronutrients that are considered the main components in proximate composition analysis (Ansari et al., 2023). Fats play important physiological and biochemical roles in the functioning of the human body, such as energy storage, structural components of biological membranes, electron carriers and cofactors of enzymes, light-absorbing pigments, hydrophobic anchors for proteins, and emulsifying agents in the digestive tract (Velciov et al., 2024). BPs have a low-fat content and therefore cannot be considered a good source of fat-soluble vitamins, but can contribute to the energy content of foods (Ahmed et al., 2021; Pyar & Peh, 2018). In addition, the low-fat content increases the shelf life of BP powders by reducing the risks of rancidity. The peel is cholesterol-free and low in calories, sugar, and fat (Joy et al., 2022).

The results obtained from the analysis of BPs taken in the experiment (Table 2) show that they contain amounts of fat ranging between 3.14% (BP3) and 4.11% (BP1).

The assumption of normality fat content distributions was assessed by using the Shapiro-Wilks test. Results indicated that the fat contents are normally distributed (for BP1: W= 0.887, p=0.54; for BP2: W=0.997, p=0.89; for BP3: W=0.996, p=0.93). A one-way ANOVA was performed to compare the effect of the three different providers on fat contents. It revealed that there was a statistically significant

difference in mean fat contents between at least two groups ($F = 6.91$, $p < 0.05$, Eta2=0.70, 95% C.I. [0.11, 1.00]).

Duncan's MRT for multiple comparisons found that the mean value of fat content was significantly different between provider 1 and provider 3 ($p < 0.051$, 95% C.I. = [0.30, 1.63]), There was no statistically significant difference in mean fat contents between provider 1 and provider 2 (p=0.10), and between provider 2 and provider 3 (p=0.11).

These values show that the analyzed BPs contain small amounts of fat and cannot be considered primary sources but could be used as ingredients to improve the texture, taste, and aroma of new food formulations (Velciov et al., 2024) or as an addition to low-fat foods (Ismail et al., 2024). The fat concentrations determined in BP1, BP2, and BP3 experimentally determined, and their average (3.62%) are comparable to those reported by: Akhter et al., 2024, Jandal, 2024, Issara et al., 2024, Khamsaw et al., 2024, and Zaini et al., 2022 (see Table 1).

Crude fiber, the portion of indigestible carbohydrates in food, consists mainly of cellulose, hemicellulose, pectin, and lignin (Velciov et al., 2024; Piar and Peh, 2018). Cellulose, hemicellulose, and lignin are well known for water absorption and regulation of the intestinal tract, while pectin and gums are important in cholesterol reduction and glucose regulation (Velciov et al., 2024). Crude fiber from BPs helps maintain normal peristaltic movement of the intestinal tract and, therefore, aids in food digestion and can be widely used in the food industry when incorporated into many foods to enrich their nutritional and sensory properties (Saeed et al., 2024). Therefore, diets that include foods with appreciable fiber additions may be beneficial for eliminating potential mutagens, steroids, and xenobiotics (Tsado et al., 2021).

Among the analyzed nutritional parameters, after carbohydrates (59.60%, mean value), crude fibers were determined in the highest amounts (12.72%, mean value). The concentration of crude fibers (Table 2) in BPs presents values ranging between 10.23% (BP3) and 14.65% (BP2).

The assumption of normality fibre content distributions was assessed by using the Shapiro-Wilks test. Results indicated that the fibre contents are normally distributed (for BP1: W= 0.997, p=0.90; for BP2: W=0.972, p=0.67; for BP3: W=0.994, p=0.84). A one-way ANOVA was performed to compare the effect of the three different providers on crude fiber contents. It revealed that there was a statistically significant difference in mean crude fiber contents between at least two groups ($F = 64.95$, $p < 0.001$, Eta2=0.96, 95% C.I. [0.84, 1.00]). Duncan's MRT for multiple comparisons found that the mean value of crude fibre content was significantly different between provider 1 and provider 2 ($p < 0.05$, 95% C.I.= [0.39, 2.34]), and between provider 1 and provider 3 ($p < 0.001$, 95% C.I. = [2.07, 4.02]), and between provider 2 and provider 3 ($p < 0.001$, 95% C.I. = [3.41, 5.42]).

These values, but also the fact that crude fiber represents only a part of dietary fiber (Yang et al., 2017), show that the analyzed BPs contain significant amounts of fiber and could constitute a viable and cheap source of dietary fiber that promotes health, thus contributing to improving digestion (Saeed et al., 2024). In addition, incorporating BP powder into some foods can increase the total dietary fiber content and decrease their protein content, slowing down the digestion of starch and decreasing the glycemic index of foods (Tan et al., 2024). Therefore, BP powders can be helpful additives to improve foods' texture, taste, and nutritional properties (Wani & Dhanya, 2025). The values obtained for the determination of crude fibers in analyzed BPs are comparable to those reported by Sahoo and Lenka (2024), Akhter et al. (2024), Taha et al. (2024), Gupta, T., and Abdollah M. (2022), Tsado et al. (2021).

Carbohydrates are important macronutrients for the human body that act as a source of energy, help and control the metabolism of blood sugar and insulin, participate in cholesterol and triglyceride metabolism, and help in fermentation (Velciov et al., 2024; Ahmed et al., 2021). In the nutritional analysis of food, only total carbohydrates are considered, representing the totality of carbohydrates in food after other components, such as proteins, fats, moisture, and ash, have been removed (Velciov et al., 2024). Banana peel contains a significant amount of carbohydrates, indicating good sources of energy for humans and animals (Tsado et al., 2021). Banana peels'

carbohydrates consist mainly of dietary fiber and sugars, especially pectin, hemicellulose, and cellulose, which contribute to their potential as a functional ingredient in foods (Rawat et al., 2024; Chimdi et al., 2024). The presence of carbohydrates, especially indigestible fibers, can support digestive health and act as prebiotics, favoring the growth of beneficial bacteria in the gut (Chimdi et al., 2024).

The results of the present study showed that carbohydrates are the most abundant components in banana peel. The concentrations of carbohydrates determined in the analyzed BPs (Table 2) show similar values, ranging from 57.97% (BP1) to 62.72% (BP3).

The assumption of normality carbohydrate content distributions was assessed by using the Shapiro-Wilks test. Results indicated that the carbohydrate contents are normally distributed (for BP1: W= 0.844, p=0.52; for BP2: W=0.907, p=0.50; for GP3: W=1, p=0.97). A one-way ANOVA was performed to compare the effect of the three different providers on carbohydrate contents. It revealed that there was not a statistically significant difference in mean carbohydrate contents between the groups (F = 2.22, p = 0.19, Eta2 = 0.43, 95 % C.I. [0, 1]).

These values and their average show that the analyzed BPs contain increased amounts of carbohydrates and can be used as additives to improve the texture and general quality of foods (Saeed et al., 2024). Therefore, increased carbohydrate content is recommended for use in the formulation of value-added and functional foods. Comparing the carbohydrate content values of BP1, BP2, BP3 and their average with the values determined by: Sahoo & Lenka, 2024, Khamsaw et al., 2024, Segura-Badilla et al., 2022, Zaini et al., 2022, Tsado et al., 2021 and Anhwange et al., 2009, no apparent differences are recorded.

From what is presented, it can be stated that the analyzed BPs contain important amounts of nutritional compounds, especially carbohydrates and minerals, moderate amounts of crude fiber and proteins, and lower amounts of fats. Considering the proximate analysis of the analyzed BPs, it can be stated that they could be considered for improving the nutritional quality and obtaining foods with added value. In addition, apart from the analyzed nutritional parameters, BPs contain increased amounts of biologically active compounds with anti-allergenic, anti-atherogenic, anti-inflammatory, anti-microbial effects and antioxidant, anti-thrombotic, cardio-protective, and anti-carcinogenic properties that present numerous health benefits, such as the prevention of diabetes, immunological disorders, and neurogenerative diseases (Taha et al., 2024). However, it must be pointed out that using banana peels in powder form requires a preliminary operation necessary to remove unwanted impurities and any pesticides or other substances used in banana cultivation techniques.

CONCLUSIONS

The values obtained from the proximate analysis of banana peels, which provide information on their nutritional value, show that banana peels obtained by peeling ripe yellow bananas "Vela rosa Premium Banana" (Cavendish variety), sold in local markets, contain increased amounts of nutritional compounds, especially carbohydrates, minerals, and organic compounds, moderate amounts of crude fiber and protein and relatively low amounts of fat.

The nutritional parameters of the analyzed banana peels show that this by-product could be used to develop food products with added value, having potential applications for obtaining functional foods.

We consider that the use of banana peels to improve the nutritional quality of foods requires further investigations to improve the procedures for obtaining powders from banana peels with increased contents of carbohydrates, fiber, minerals, protein, fat, and biologically active substances and the development of efficient, promising ways to open new horizons in the field of food fortification.

The use of banana peels, after a prior operation to remove impurities and possible pesticide residues, in food formulations creates opportunities to reduce waste and generate additional income for the banana processing industry.

REFERENCES

Aboul-Enein, A. M., Salama, Z. A., Gaafar, A. A., Aly, H. F., Abou-Elella, F., & Ahmed, H. A. (2016). Identification of phenolic compounds from banana peel (*Musa paradaisica L.*) as antioxidant and antimicrobial agents. *Journal of chemical and pharmaceutical research*, *8*(4), 46-55.

Ahmed, Z., El-Sharnouby, G. & El-Waseif, M. (2021). Use of banana peel as a by-product to increase the nutritive value of the cake. *Journal of Food and Dairy Sciences, 12,* 87–97.

Ahsan, M., Ashraf, H., Liaquat, A., Nayik, G. A., Ramniwas, S., Alfarraj, S., Ansari, M.J, & Gere, A. (2024). Exploring pectin from ripe and unripe Banana Peel: A novel functional fat replacer in muffins. *Food Chemistry:* X, 101539.

Akhter, M. J., Al-Amin, M., Hossain, M. A., & Kamal, M. M. (2024). Enriching Wheat Bread with Banana Peel Powder: Impact on Nutritional Attributes, Bioactive Compounds, and Antioxidant Activity. *International Journal of Food Science*, *2024*(1), 2662967.

Alshehri, A. A., Younes, N. M., Kamel, R., & Shawir, S. M. (2024). Characterization and potential health benefits of millet flour and banana peel mixtures on rats fed with a high-fat diet. *Heliyon*.

Amini Khoozani, A., Birch, J., & Bekhit, A. E. D. A. (2019). Production, application and health effects of banana pulp and peel flour in the food industry. *Journal of food science and technology*, *56*, 548-559.

Anhwange, B. A., Ugye, T. J., & Nyiaatagher, T. D. (2009). Chemical composition of *Musa sapientum* (banana) peels, *EJEAFChe, 8*(6), 437-442.

Ansari, N. A. I. M., Ramly, N., Faujan, N.H., & Arifin, N. (2023). Nutritional Content and Bioactive Compounds of Banana Peel and Its Potential Utilization: A Review. *Malaysian Journal of Science Health & Technology*, *9*(1), 74-86.

AOAC. Official Methods of Analysis, Association of Official Analytical Chemist. EUA; 2000.

Bhavani, M., Morya, S., Saxena, D., & Awuchi, C. G. (2023). Bioactive, antioxidant, industrial, and nutra-ceutical applications of banana peel. *International Journal of Food Properties*, *26*(1), 1277-1289.

Bin Mohd Zaini, H., Bin Sintang, M. D., Dan, Y. N., Ab Wahab, N., Bin Abdul Hamid, M., & Pindi, W. (2019). Effect of addition of banana peel powder (Musa balbisiana) on physicochemical and sensory properties of fish patty. *British Food Journal*, *121*(9), 2179-2189.

Chimdi, E. E., Iheme, C. I., Njoku, O. C., Agwu, L. O., & Airaodion, A. I. (2024). Investigation of Proximate Composition and Bioactive Components in Banana (Musa acuminata) Peels Using Advanced Analytical Techniques. *J. Nutrition and Food Processing*, *7*(10).

Dom, Z.M., Chan, W.Q. and Ab Aziz, N.A. (2024). Utilising Banana Peel as a Food Ingredient for Enhancing Nutritional Value of Yellow Noodles and Beef Patties. *Advances in Agricultural and Food Research Journal*, *5*(2).

Edima-Nyah, A. P., Ntukidem, V. E., & Ogbonna, C. V. (2024). Chemical, Microbial, and Sensory Properties of Breakfast Cereals Made from Yellow Maize and Soybean Flour Blends with Firmly Ripe Banana Flavoring. *IPS Journal of Nutrition and Food Science*, *3*(3), 227-233.

Ghosh, S. & Mitra J. (2020). Importance of Normality Testing, Parametric and Non-Parametric Approach, Association, Correlation and Linear Regression (Multiple & Multivariate) of Data in Food & Bio-Process Engineering. *Mathematical and Statistical Applications in Food Engineering*. CRC Press, 112-126.

Gupta, T., and Abdollah, M. (2022). Proximate and mineral composition of some fruit peels in the environment. *International Contemporary Journal of Science Education and Technology*, *1*, 1-12

Hikal, W. M., Said-Al Ahl, H. A., Bratovcic, A., Tkachenko, K. G., Sharifi-Rad, J., Kačániová, M., Elhourri M., & Atanassova, M. (2022). Banana peels: A waste treasure for human being. *Evidence-Based Complementary and Alternative Medicine*, *1*, 7616452

Huang, P.-H., Cheng, Y.-T., Lu,W.-C., Chiang, P.-Y., Yeh, J.-L., Wang, C.-C., Liang, Y.-S., & Li, P.-H. (2024). Changes in Nutrient Content and Physicochemical Properties of Cavendish Bananas var. Pei Chiao during Ripening. *Horticulturae*, *10*, 384. https://doi.org/10.3390/horticulturae10040384.

Ismail, S., Dubey, P. K., Mishra, A. A., & Ashka, F. (2024). Valorisation of banana peel and mango peel as functional ingredients in baked products: a review. *International Journal of Food Science and Technology*, *59*(9), 5938-5950.

Issara, U., Tiwasiri, N., Keawwong, P., Lamphao, O., Chana, S., Sangketkit, W., & Changpasert, W. (2024). The utilization of banana peels (*Musa acuminata* Cavendish subgroup) as an alternative ingredient for producing dried plant-based meat products. *Food Research*, *8*(4), 384-39

Jandal, M. J. (2024). Study of the physical, chemical, and sensory properties of the cookie product resulting from replacing different proportions of banana peel powder. *Mesopotamia Journal of Agriculture*, *52*(3), 71-0.

Joy, G. F. F., Sunday, O. Z., Remilekun, A. and Olusegun, A. D. (2022). Waste-to-wealth; nutritional potential of five selected fruit peels and their health benefits: A review. *African Journal of Food Science, 16*(7), 172-183.

Khamsaw, P., Sommano, S.R., Wongkaew, M., Willats, W.G.T., Bakshani, C.R., Sirilun, S., Sunanta, P. (20240. Banana Peel (*Musa ABB cv. NamWa Mali-Ong*) as a Source of Value-Adding Components and the Functional Properties of Its Bioactive Ingredients. *Plants, 13*, 593. https://doi.org/10.3390/plants13050593]

Kusumasari, S., Syabana, M. A., Pamela, V. Y., & Meindrawan, B. (2024). Potential use of food waste in food processing to add nutritional value. in *E3S Web of Conferences, 483*, 02006). EDP Sciences.

Mahomud, M. S., Islam, M. N., Hossen, D., Wazed, M. A., Yasmin, S., & Sarker, M. S. H. (2024). Innovative probiotic yogurt: Leveraging green banana peel for enhanced quality, functionality, and sensory attributes. *Heliyon*, *10*(19).

Manzoor, S., Rakha, A., Rasheed, H., Munir, S., Abdi, G., Bhat, Z. F., & Aadil, R. M. (2024). Development and evaluation of anxiolytic potential of bagels incorporated with banana peel flour and lavender. *Journal of Agriculture and Food Research*, *15*, 101029.

Munir, H., Alam, H., Nadeem, M. T., Almalki, R. S., Arshad, M. S., & Suleria, H. A. R. (2024). Green banana resistant starch: A promising potential as functional ingredient against certain maladies. *Food Sci Nutr, 12*, 3787–3805.

Ogo, O., Amos, F., Ijeoma, P., & El-Khalid, A. (2024). Post-harvest Treatment of Banana with Artificial Ripening Agent Influences Its Nutritional Quality and Antioxidant Potential. *European Journal of Nutrition & Food Safety*, *16*(5), 1-10.

Owuno, F., & Wabali, V. (2024). Effect of banana peel addition on the chemical, functional and organoleptic properties of cookies, *RJFSQC, 10*(2). www.iiardjournals.org.

Pasha, I., Basit, A., Ahsin, M., & Ahmad, F. (2022). Probing nutritional and functional properties of salted noodles supplemented with ripen Banana peel powder. *Food Production, Processing and Nutrition*, *4*(1), 22.

Paramitasari, D., Pramana, Y. S., Suparman, S., Putra, O. N., Musa, M., Pudjianto, K., ... & Ulinuhayani, M. (2024). Valorization of Lampung Province banana peel cultivars: nutritional and functional characterizations for biscuit production and wheat flour substitution. *Journal of Food Measurement and Characterization*, 1-15.

Pyar, H., & Peh, K. K. (2018). Chemical compositions of banana peels (*Musa sapientum*) fruits cultivated in Malaysia using proximate analysis. *Res. J. Chem. Environ, 22*(2), 108-111

RCoreTeam. R: A Language and Environment for Statistical Computing; R Foundation for Statistical Computing: Vienna, Austria, 2023; Available online: https://www.R-project.org/ (accessed on 14 March 2024).

Ra, R., Chakraborty, A., Menon, C. K. M., & Verma, S. (2023). Banana peel: precious waste & its astounding properties. *Journal Of Advanced Applied Scientific Research*, *5*(5):158-174.

Rawat, N., Das, S., Ab Waheed Wani, K. J., & Qureshi, S. N. (2024). Antioxidant potential and bioactive compounds in banana peel: A review. *International Journal of Research in Agronomy, 7*(7), 07-16.

Saeed, M. K., Zahra, N., Saeed, A., Quratulain, S. Y. E. D., & Abidi, S. H. I. (2024). Banana peels a contemptible source of dietary fiber and natural antioxidants. *ACTA Pharmaceutica Sciencia*, *62*(1).

Sadek, N.F. (2024). Utilization of banana peel as functional ingredient in pudding: physicochemical and sensory aspects. In *IOP Conference Series: Earth and Environmental Science,* 1324(1), 012117. IOP Publishing.

Sahoo, A. and Lenka, C. (2024). A Comparative Study on Proximate Composition of Odisha's Local Bantal Variety Banana Peel (Musa Paradisiaca) Flour with Cereals Flour for Their Value Addition. *Asian Food Science Journal 23*(10), 17-27. https://doi.org/10.9734/afsj/2024/v23i10745.

Segura-Badilla, O., Kammar-García, A., Mosso-Vázquez, J., Sánchez, R. Á. S., Ochoa-Velasco, C., Hernández-Carranza, P., & Navarro-Cruz, A. R. (2022). Potential use of banana peel (Musa cavendish) as ingredient for pasta and bakery products. *Heliyon*, *8*(10), e11044.

Taha, Soad H., Fouad M.F. Elshaghabee, and Mostafa Ahmed M. A. (2024). Nutritional Value, Antioxidant and Anticancer Activities of Some Nano Fruit Wastes. *Egyptian Journal of Chemistry 67*(8), 187-201.

Tan, C. Y., Arifin, N. N. M., & Sabran, M. R. (2024). Banana Peels as Potential Prebiotic and Functional Ingredient. *Jurnal Gizi dan Pangan*, *19*(Supp. 1), 119-126.

Tsado, A. N., Okoli, N. R., Jiya, A. G., Gana, D., Saidu, B., Zubairu, R., & Salihu, I. Z. (2021). Proximate, minerals, and amino acid compositions of banana and plantain peels. *BIOMED natural and applied science*, *1*(01), 032-042).

Türker, B., & Savlak, N. (2022). Gluten-free cake with unripe banana peel flour substitution: impact on nutritional, functional and sensorial properties. *Nutrition & Food Science*, *52*(6), 980-995.

Velciov, A. B., Danci, M., Cozma, A., Lalescu, V. D., Rădulescu, L., Popescu, G. S., & Rada, M. (2024). Evaluation Of Some Nutritional Compounds of Garlic (*Allium Sativum* L.) Peel Waste, *Scientific Papers. Series B, Horticulture, LXVIII*(2). Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653.

Zaini, H. M., Roslan, J., Saallah, S., Munsu, E., Sulaiman, N. S., & Pindi, W. (2022). Banana peels as a bioactive ingredient and its potential application in the food industry. *Journal of Functional Foods*, *92*, 105054.

Youssef, E., Shaltout, O., & Abouel -Yazeed, A. (2024). Chemical and Nutritional Evaluation of Banana Peels and Their Potential Use in Improving the Egyptian Balady Bread. *Journal of the Advances in Agricultural Researches*, *29*(1), 107-115.

Yang, Y.Y., Ma, S., Wang, X.X. & Zheng, X.L. (2017). Modification and application of dietary fiber in foods. *Journal of Chemistry*, *2017*.

Wani, K. M., & Dhanya, M. (2025). Unlocking the potential of banana peel bioactives: extraction methods, benefits, and industrial applications. *Discover Food*, *5*(1), 1-25.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

GRAFTING EFFICIENCY OF APPLE TREES IN THE NURSERY BY APPLYING DIFFERENT TECHNOLOGY ELEMENTS

Adelina VENIG[1], Florin STĂNICĂ[2], Adrian PETICILĂ[2]

[1]University of Oradea, Faculty of Environmental Protection, 26 Gen. Magheru Blvd, Oradea, Romania
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăști Blvd, District 1, Bucharest, Romania

Corresponding author email: adelina_venig@yahoo.com

Abstract

Considering the close relationship between soil, water and the plant, the current research aims to establish the influence of irrigation, fertilization, and cultivar on some morphological characters of the apple trees in the nursery. The nursery research sector in our country has demonstrated the necessity of specialization and modernization of this field, due to the growing demand for high-quality certified planting materials. The main aim is to increase continuously the quality and quantity of valuable planting material and guaranteed in terms of authenticity, health, and suitability for the new orchard systems with large plots per hectare and high potential. The research was carried out in the climate conditions of the northwestern Romania and the studied apple cultivars were Florina and Idared, organized on five repetitions, with irrigation as the primary factor, cultivar as the secondary factor and fertilization as the tertiary factor. Following the research, it is found that both the irrigation and the application of different doses of NPK have allowed a significant increase in the survival rate of grafting trees of the two apple cultivars.

Key words*: apple cultivar, fertilization, grafting, irrigation, nursery.*

INTRODUCTION

The production of high-quality tree planting material associated with an economically efficient activity at the level of nurseries, requires considering both the pedo-climatic conditions in the area and the applied technology elements.

In Romania, due to its geographical location at the confluence of continental and Mediterranean climates, generally offers favourable climate and soil conditions for many fruit nurseries. Numerous autochthonous cultivars attest to the presence of fruit growing on the lands of Romania since ancient times. Initially, the fruit nurseries were concentrated in areas with a rich rainfall regime, so that the capture of rootstocks depended to a greater extent on the rainfall regime, human intervention at the beginning being modest in this sense.

Water is indispensable for plants, being a basic component of living cells and tissues. Only in the presence of water can the processes of assimilation and dissimilation and gas exchange take place. Once inside the plant, water keeps the cell walls stretched and thus gives the cells and tissues turgidity, which ensures the mechanical balance of the various organs (Venig & Stănică, 2024). Together with dissolved substances, water determines the osmotic pressure of cells and tissues, ensuring intercellular exchanges. As a participant in plant metabolism, water is involved in the fundamental processes of the living world, photosynthesis, respiration, transpiration.

The importance of water as a vegetation factor in plants' life is known and appreciated since humans began to practice agriculture and horticulture (Waller, 2016). Corresponding to the interaction of influencing factors, the water consumption requirements specific to fruit plants vary within relatively wide limits according to the species and cultivar, rootstocks, the age period of the plants and the vegetation phase, climatic factors through the precipitation regime, thermal and wind regime, eco-pedological factors through the orography of the land, the state of fertility and technological factors through the culture system and the agrophytotechnical measures applied.

With fruit trees in the nursery, as with all cultivated plants, the growth process depends to the greatest extent on the climate and soil conditions. Out of these, along heat, light, air and mineral substances, water plays a very important role (Smith, 2022). It enters the composition of various tree organs in proportion of 75-85% and sometimes even more, of their total weight. In addition to the fact that water ensures the circulation of fertilizing elements from the soil to the plant, water participates as a basic element in the synthesis of all the organic substances that make up the tissues of the rootstocks, respectively of the trees. This is the reason why it is necessary for the trees to always have water available, in sufficient quantity, for the growth processes to take place with as much intensity as possible (Arthur & Bejaei, 2025).

In the climatic conditions of Romania, irrigation is a measure to supplement the amounts of water that come naturally, from precipitation, in periods when they are insufficient compared to the requirements of the crops (Stănică & Peticilă, 2012). It is, in fact, the mean used to correct a natural factor, which, as it appears, results in large fluctuations in harvest from one year to another. The use of irrigation systems aims to obtain as stable productions as possible, close to the productive potential of the plants in the given pedo-climatic conditions (Branișteet al., 2010). This, more since, during the current research, it was found that there were years in which, due to insufficient rainfall in certain periods, harvests were greatly reduced, going as far as total compromise.

The most common method of irrigation in fruit nurseries is the drip irrigation method. Drip irrigation consists of the distribution of small amounts of water over a long period of time (Cronin, 2019). Spot irrigation is known as a method by which limited amounts of water are distributed from a point source directly to the roots of the plant (Żarski & Kuśmierek-Tomaszewska, 2023). However, the advantages of drip irrigation systems lie in the saving of water and the possibility of obtaining rich harvests thanks to the particularly favourable conditions created in the soil in terms of the humidity regime, aeration process, etc. The economy of water is achieved by the fact that it can be directed to the area where the plant can consume it most easily, and can be easily dosed in relation to the needs of the plant. In the same time, losses through evaporation and infiltration are almost completely reduced. Drip irrigation also contributes to the transport of harmful salts to the surface of the soil, below the root zone (Patzwahl, 2023). As a direct effect on the plant, a faster growth rate is observed, which leads to obtaining earlier harvests than those obtained with irrigation by other methods. Other advantages of localized irrigation are: it allows the free movement of machinery for all maintenance work, thus avoiding soil settlement, it reduces weed control treatments, it requires much less energy consumption compared to sprinkling (1 bar at the end of the watering pipe) (Megh, 2021).

The important fact that must be remembered is that the way in which the researches carried out in the field of plant water consumption and their results were designed, fully correspond to the requirements of both the sizing of irrigation systems, including feasibility calculations (which also include production data), and those regarding the exploitation of irrigated lands in the context of the rational use of irrigation water (Faulkner, 2022). It is also important to emphasize that the soil is the most important component of the environment and, as such, in this direction, all attention must be channelled to maintain and permanently increase the fertility of the soils from the irrigation facilities (Stănică, 2004). When discussing the use of irrigation systems, a set of measures must be considered, intended to accompany this action, such as fertilization, maintenance works etc. It should be remembered that irrigated horticulture is not only the application of watering rules at the most appropriate time. This type of horticulture must be seen as a concept that sums up a complex of interventions integrated into a whole, including a series of components, that substantially differentiates it from conventional horticulture, applied to unirrigated lands (Maurer, 2016). With the introduction of water artificially, through irrigation, essential changes occur, primarily in the soil, but also in the plant, which must not escape the attention of the producer who has this facility (Neupane et al., 2024).

For irrigated crops, additional fertilization becomes one of the main links of the fertilization system (Schmid, 2021). To improve the physical and chemical characteristics of the soil and

increase its fertility, the nursery's fertilization system consists of several long-term activities. These activities are designed to meet the needs of the species, rootstocks, and cultivar/rootstock associations in relation to the age and vegetation phases of the plants (Jäger, 2021). The application of mineral fertilizers containing nitrogen, phosphorus, and potassium; the buildup of organic matter in the soil through crop rotations and the addition of unique plant residues for green fertilizers are some of the primary components of the fertilization system in a contemporary fruit tree nursery. Fertilizers must be used in horticulture to replenish the supply of nutrients that plants consume or that have leached deeply, as well as to enhance the soil's overall physical condition, and fertilization status. Fertilizers are regarded as the most crucial component of the soil fertilization system. When horticultural plants are growing, they are the primary source of nutrients for the plants (Mayer, 2019). Thus far, it has been established that when fertilizing, factors such as species, cultivar, rootstock, density, predicted production, and soil conditions must be considered (Soman, 2022). After examining several soil characteristics and being aware of the demands made by the culture as well as those associated with guaranteeing a particular level of production, both quantitative and qualitative, the ideal dosages for each situation in the field must be determined (Ladikou et al., 2025). Fertilization work in nurseries must provide workable solutions for each unique situation because conditions and situations vary greatly (Otto et al., 2015). To increase the synthesis of organic matter and achieve large, cost-effective productions with higher quality indices in the nursery, fertilizers are used to optimize the nutritional conditions without polluting the environment or reducing the plants' resistance to disease and pest attacks (Fischer et al., 2025). Only when fertilizers are incorporated into a system of carefully ranked technological measures and their dosages are correlated with the plant, soil, climate, and culture technology they can contribute optimally to increased fruit tree production (Ozherelieva et al., 2025).

Therefore, the main aim of this research was to establish the influence of technology elements, such as irrigation and fertilization, on a morphological character in the apple species, namely grafting efficiency.

MATERIALS AND METHODS

This research was carried out in a private nursery, in the pedo-climatic conditions of the Northwestern part of Romania. The study was conducted based on a 4 x 2 x 4 trifactorial experiment, organized in five repetitions, with plots comprising four rootstocks planted at 0.7 x 0.25 m, the entire number of used rootstocks was 480. The biological material was represented by wax cherry rootstock seedlings and the two apple cultivars were Florina and Idared. Irrigation was the primary factor, variety was the secondary factor and fertilization was the tertiary factor. Irrigation was carried out using a drip hose with a diameter of 16 mm and a thickness of 0.4 mm, equipped with droppers spaced at 25 cm, each dropper having four exit holes and a flow rate of 2 l/ha at a pressure of 1 bar. The daily duration of watering for the administration of the different watering rates was 3.5 hours for irrigation with the 10 mm rate, 7 hours for the 20 mm rate and 10.5 hours for the 30 mm rate. Fertilization treatments were applied together with mechanical tillage, respectively in the spring of 2024.The amounts of fertilizers used were $N_8P_8K_8$ in the amount of 50 kg, $N_{16}P_{16}K_{16}$ in amount of 100 kg and 150 kg for $N_{24}P_{24}K_{24}$. Florina cultivar is almost immune to turnip, tolerant to flouring, precocious and extremely productive. It does not need a lot of spraying. The fruit is medium to large, spherical-flattened, about 150-200 g, slightly truncated with 5 ribs, striped, light red with whitish dots all over the surface, like some freckles. The pulp is juicy, suitably sweet, and slightly acidic, crunchy, very pleasantly aromatic, but becomes floury when overcooked. The ripening period is October. Idared cultivar is an autumn apple cultivar, obtained by crossing the award-winning Ionahan "Jonathan" x Wagener cultivars. It is a tree of medium vigour with a height of approximately 200-300 cm. The leaves have a sharp oval shape with slightly jagged edges in a dark green, semi-glossy colour. The fruits have a very asymmetric flattened spherical shape with a weight of approximately 170-200 g. The skin of the fruit is thin in shades of red-burgundy on a yellow-

green background. The pulp is whitish, smooth, and juicy. The taste of the fruit is acidic to sweet, with an intense aroma close to the Jonathan cultivar. Idared is an apple cultivar sensitive to the attack of *Erwinia amylovora*. The harvest period is September.

The grafting method used during in this research was chip budding. This grafting method is the most widespread used in the nursery (first field) and when replanting young trees in the orchard. During this research, grafting took place in August of 2023 and the observations regarding the grafting efficiency were made in the spring of 2024, after winter frosts. The grafting success has been evaluated by a simple touch of the petiole portion of the bud. If this fell easily and left a scar grafting was declared successful. In the contrary, if the petiole portion did not fall when touched, and the graft bud and bark shield were dehydrated (wrinkled) and browned, indicated that grafting was not successful.

The research results were statistically processed using the least significant difference (LSD) test.

RESULTS AND DISCUSSIONS

The main purpose of grafting is the vegetative propagation of trees. In addition, this process improves the trees' resistance to external factors, such as drought, frost, or various diseases. By grafting, a new cultivar of tree and new seeds are obtained, adapted to the climatic conditions specific to the area. Grafting trees is beneficial and even necessary for obtaining healthy, high-quality, and large-sized fruits. In addition, it is an efficient method to control the height and shape of the trees, thus facilitating the maintenance and harvesting works. Grafting is widespread because it ensures the faithful transmission of characters to offspring, the material has a great uniformity, and the stability of planting material production is maintained year after year, unlike propagation by seeds and kernels, which can be affected by unfavourable conditions during flowering. Grafting also has the advantages of early fruiting, as it bypasses the juvenile stage.

The relationship between the dose of NPK and the percentage of grafted trees in Florina cultivar is highlighted with a precision of approximately 87% by means of an exponential function (Table 1). Thus, against the background of a grafting percentage of 93% in the absence of fertilization, the average growth rate of this indicator was 0.093%/kg NPK applied, with different values from one dose to another (-0.02-0.26%/kg NPK).

Table 1. The effect of cultivar and fertilization on survival rate of grafted trees

Cultivar	NPK dose					
	$N_0P_0K_0$	$N_8P_8K_8$	$N_{16}P_{16}K_{16}$	$N_{24}P_{24}K_{24}$	$\bar{x} \pm s_{\bar{x}}$	$S_{\%}$
Idared	y 90.75a	y 92.50 b	y 92.25 b	x 94.75 a	92.56 ±0.68	5.37
Florina	y 92.50 a	xy 94.55 a	xy 94.40 a	x 94.75 a	94.05 ±0.48	4.60
$\bar{x} \pm s_{\bar{x}}$	91.62 ±0.91	93.53 ± 0.88	93.33 ±0.82	94.75±0.67	93.31 ±0.42	
$S_{\%}$	6.29	5.96	5.55	4.49	5.68	

Cultivar - DL $(LSD)_{5\%}$=1.92
DL $(LSD)_{1\%}$=2.54 DL $(LSD)_{0.1\%}$=3.29 (a, b)
Fertilization - DL $(LSD)_{5\%}$=2.09
DL $(LSD)_{1\%}$=2.77 DL $(LSD)_{0.1\%}$=3.57

After comparing the grafted trees of the two cultivars on different fertilization treatments (Table 2), it was found that the unfertilized trees exhibited a reduced grafting percentage which was not statistically ensured. Under the effect of 8 and 16% NPK doses, the grafting rate in Florina cultivar was significantly higher than that recorded in Idared by 2.05-2.15%. The grafted trees of the two cultivars were equally fertilized with 24% NPK.

Table 2. The effect of cultivar on the survival rate of grafted trees under different fertilization schemes

Cultivars x $N_0P_0K_0$	Average (%)		Relative values (%)	Difference/Significance
Florina - Idared	92.50	90.75	101.93	1.75
Cultivars x $N_8P_8K_8$	Average (%)		Relative values (%)	Difference/Significance
Florina - Idared	94.55	92.50	102.22	2.05*
Cultivars x $N_{16}P_{16}K_{16}$	Average (%)		Relative values (%)	Difference/Significance
Florina - Idared	94.40	92.25	102.33	2.15*
Cultivars x $N_{24}P_{24}K_{24}$	Average (%)		Relative values (%)	Difference/Significance
Florina - Idared	94.75	94.75	100.00	0.00

DL $(LSD)_{5\%}$ = 1.92 DL $(LSD)_{1\%}$ = 2.54 DL $(LSD)_{0.1\%}$ = 3.29

Considering the interaction between fertilization and the percentage of grafted seedlings in the Idared cultivar (Table 3), it was observed that only fertilization with 24% NPK allowed a significant increase (4.0%) of this indicator, while the effects of the other treatments were reduced and insignificant (1.5-1.75%). Also, changing the dose from 8 and 16 % respectively to 24% NPK determined significant increases of 2.25-2.50% in the grafting percentage.

Table 3. The effect of fertilization on survival rate of grafting trees of the two apple cultivars

NPK dose x Idared	Average (%)		Relative values (%)	Difference/Significance
$N_8P_8K_8 - N_0P_0K_0$	92.50	90.75	101.93	1.75
$N_{16}P_{16}K_{16} - N_0P_0K_0$	92.25	90.75	101.65	1.50
$N_{24}P_{24}K_{24} - N_0P_0K_0$	94.75	90.75	104.41	4.00***
$N_{16}P_{16}K_{16} - N_8P_8K_8$	92.25	92.50	99.73	-0.25
$N_{24}P_{24}K_{24} - N_8P_8K_8$	94.75	92.50	102.43	2.25*
$N_{24}P_{24}K_{24} - N_{16}P_{16}K_{16}$	94.75	92.25	102.71	2.50*
NPK dose x Florina	Average (%)		Relative values (%)	Difference/Significance
$N_8P_8K_8 - N_0P_0K_0$	94.55	92.50	102.22	2.05
$N_{16}P_{16}K_{16} - N_0P_0K_0$	94.40	92.50	102.05	1.90
$N_{24}P_{24}K_{24} - N_0P_0K_0$	94.75	92.50	102.43	2.25*
$N_{16}P_{16}K_{16} - N_8P_8K_8$	94.40	94.55	99.84	-0.15
$N_{24}P_{24}K_{24} - N_8P_8K_8$	94.75	94.55	100.21	0.20
$N_{24}P_{24}K_{24} - N_{16}P_{16}K_{16}$	94.75	94.40	100.37	0.35

DL (LSD)5% = 2.09 DL (LSD)1% = 2.77 DL (LSD)0.1% = 3.57

Under the effect of different fertilizing treatments, trees of Florina cultivar recorded a grafting percentage with its limits ranging from 92.50% in the case of the unfertilized variant, to 94.75% in the variant with 24% NPK, against the background of a variability between treatments of 4.60%. Compared to the unfertilized agricultural background, the treatment with 24% had a significantly higher efficiency materialized by an increase of 2.25%. The progressive modification of NPK doses from 8 to 16 and 24%, respectively, was not associated with significant effects on grafting.

Considering the combined effect of irrigation and fertilization on the percentage of grafting (Table 4), in the absence of watering, fertilization had the highest influence on this indicator, while in the variant related to the watering norm of 30 mm, the effect of fertilization was less.

Table 4. The effect of irrigation and fertilization on survival rate of grafting trees

Watering norm	NPK dose					
	$N_0P_0K_0$	**$N_8P_8K_8$**	**$N_{16}P_{16}K_{16}$**	**$N_{24}P_{24}K_{24}$**	$\bar{x} \pm s_{\bar{x}}$	S%
0 mm	y 84.50 c	x 90.00 b	x 89.30 c	x 91.00 c	88.70 ±0.85	6.07
10 mm	xy 91.00 b	y 89.10 b	xy 92.00 bc	x 93.00 bc	91.28 ±0.58	3.99
20 mm	xy 94.50 a	x 96.50 a	y 92.50 b	x 95.50 b	94.75 ±0.56	3.77
30 mm	y 96.50 a	xy 98.50 a	x 99.50 a	x 99.50 a	98.50 ±0.39	2.91
$\bar{x} \pm s_{\bar{x}}$	91.62 ±0.91	93.53 ±0.88	93.33 ±0.82	94.75 ±0.67	93.31 ±0.42	
S%	6.29	5.96	5.55	4.49	5.68	

Irrigation - DL $(LSD)_{5\%} = 2.91$ DL $(LSD)_{1\%} = 3.85$ DL $(LSD)_{0,1\%} = 4.96$ (a, b, c)
Fertilization - DL $(LSD)_{5\%} = 2.96$ DL $(LSD)_{1\%} = 3.91$ DL $(LSD)_{0,1\%} = 5.05$ (x, y)

Considering the results presented in Figure 1, based on the exponential regression, it can be observed that in the case of the unirrigated variant, the percentage of grafting showed an average growth rate of 0.027% for each kg of NPK, with the limits from -0.09%/kg to 0.69%/kg. The respective estimates have a precision of 71%, given a catch percentage of approximately 85.86% in the absence of fertilization. For the watering norm of 10 mm, the effect of fertilization on the grafting of grafted trees is expressed by an exponential regression that is based on a determination coefficient of 77.71% and indicates an irregular variation of this indicator related to an average rate of 0.096%/kg. Under the effect of the

watering norm of 20 mm, fertilization showed a reduced influence on grafting, associated with a sinuous variation from one dose to another.

Under the conditions of irrigation with 30 mm, there is an increase in the catching rate proportional to the dose of NPK only up to the level of 16%, associated with a rhythm with values of 0.1-0.0.2%/kg.

In the conditions of the unfertilized agricultural -background (Table 5), a variation of the catching rate can be observed from 84.50% for the unirrigated variant to 96.50% for the watering rate of 30 mm.

On this agricultural land, all three watering norms showed significant increases of 6.5-12% compared to the unirrigated variant. Only changing the watering rate from 10 to 20 mm was materialized by a significant effect on the grafted trees, while increasing the watering rate from 20 to 30 mm had a statistically uncertain effect.

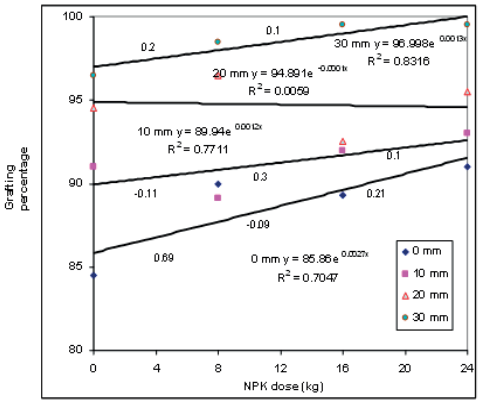


Figure 1. Variation of survival rate of grafting trees under the effect of different watering norms and fertilizations

Table 5. The effect of irrigation on survival rate of grafted trees under different fertilizations

Watering norm x NPK	Average (%)		Relative values (%)	Difference/Significance
$N_0P_0K_0$				
10 mm – 0 mm	91.00	84.50	107.69	6.50***
20 mm – 0 mm	94.50	84.50	111.83	10.00***
30 mm – 0 mm	96.50	84.50	114.20	12.00***
20 mm – 10 mm	94.50	91.00	103.85	3.50*
30 mm – 10 mm	96.50	91.00	106.04	5.50***
30 mm – 20 mm	96.50	94.50	102.12	2.00
$N_8P_8K_8$				
10 mm – 0 mm	89.10	90.00	99.00	-0.90
20 mm – 0 mm	96.50	90.00	107.22	6.50***
30 mm – 0 mm	98.50	90.00	109.44	8.50***
20 mm – 10 mm	96.50	89.10	108.31	7.40***
30 mm – 10 mm	98.50	89.10	110.55	9.40***
30 mm – 20 mm	98.50	96.50	102.07	2.00
$N_{16}P_{16}K_{16}$				
10 mm – 0 mm	92.00	89.30	103.02	2.70
20 mm – 0 mm	92.50	89.30	103.58	3.20*
30 mm – 0 mm	99.50	89.30	111.42	10.20***
20 mm – 10 mm	92.50	92.00	100.54	0.50
30 mm – 10 mm	99.50	92.00	108.15	7.50***
30 mm – 20 mm	99.50	92.50	107.57	7.00***
$N_{24}P_{24}K_{24}$				
10 mm – 0 mm	93.00	91.00	102.20	2.00
20 mm – 0 mm	95.50	91.00	104.95	4.50**
30 mm – 0 mm	99.50	91.00	109.34	8.50***
20 mm – 10 mm	95.50	93.00	102.69	2.50
30 mm – 10 mm	99.50	93.00	106.99	6.50***
30 mm – 20 mm	99.50	95.50	104.19	4.00**

$DL\ (LSD)_{5\%} = 2.91$ $DL\ (LSD)_{1\%} = 3.85$ $DL\ (LSD)_{0.1\%} = 4.96$

Under the effect of the applied treatments with 8% NPK, the grafting percentage was between 90 and 98.5%. As such, in this case only the watering norms of 20 and 30 mm showed a significant effect of 6.5-8.5% on the grafting of the grafted trees. The addition of irrigation from 10 to 20 mm allowed a significant increase of 7.4% in the catch percentage.

Against the background of fertilization with 16% NPK, the application of irrigation with 20 and 30 mm favored a significant increase in the percentage of grafting by 3.2-10.2%. In the case

of this agricultural land, the increase in the watering rate from 20 to 30 mm showed a high efficiency materialized by an increase of 7.50%. In the conditions of the agricultural fund fertilized with 24% NPK, the same trends are manifested, but the irrigation effect is less.

Considering the interaction between fertilization and grafting the rootstocks in the absence of irrigation (Table 6), it was observed that the applied fertilization variants allowed obtaining values between 84.50% for the unfertilized agricultural background and 91% for the dose of 24% NPK, with an amplitude of variation of 6.50% and a variability of 6.07% between treatments. In these conditions of soil moisture, all three fertilization treatments generated significant increases in the percentage of capture. But the increase of fertilization dose from 8 to 16 and respectively 24% NPK reduced the efficiency and showed an insignificant impact on grafting. Under the effect of irrigation with 10 and 20 mm, fertilization showed a reduced and insignificant efficiency on grafting. In the case of the agricultural background where the 30 mm watering norm was applied, the grafted trees efficiently utilized only the doses of 16 and 24% NPK, registering significant increases in the catch percentage of 3%.

Table 6. The effect of fertilization on survival rate of grafting trees under different watering norms

NPK dose x Watering norm	Average (%)		Relative values (%)	Difference/Significance
0 mm				
$N_8P_8K_8 - N_0P_0K_0$	90.00	84.50	106.51	5.50***
$N_{16}P_{16}K_{16} - N_0P_0K_0$	89.30	84.50	105.68	4.80**
$N_{24}P_{24}K_{24} - N_0P_0K_0$	91.00	84.50	107.69	6.50***
$N_{16}P_{16}K_{16} - N_8P_8K_8$	89.30	90.00	99.22	-0.70
$N_{24}P_{24}K_{24} - N_8P_8K_8$	91.00	90.00	101.11	1.00
$N_{24}P_{24}K_{24} - N_{16}P_{16}K_{16}$	91.00	89.30	101.90	1.70
10 mm				
$N_8P_8K_8 - N_0P_0K_0$	89.10	91.00	97.91	-1.90
$N_{16}P_{16}K_{16} - N_0P_0K_0$	92.00	91.00	101.10	1.00
$N_{24}P_{24}K_{24} - N_0P_0K_0$	93.00	91.00	102.20	2.00
$N_{16}P_{16}K_{16} - N_8P_8K_8$	92.00	89.10	103.25	2.90
$N_{24}P_{24}K_{24} - N_8P_8K_8$	93.00	89.10	104.38	3.90*
$N_{24}P_{24}K_{24} - N_{16}P_{16}K_{16}$	93.00	92.00	101.09	1.00
20 mm				
$N_8P_8K_8 - N_0P_0K_0$	96.50	94.50	102.12	2.00
$N_{16}P_{16}K_{16} - N_0P_0K_0$	92.50	94.50	97.88	-2.00
$N_{24}P_{24}K_{24} - N_0P_0K_0$	95.50	94.50	101.06	1.00
$N_{16}P_{16}K_{16} - N_8P_8K_8$	92.50	96.50	95.85	-4.00^{00}
$N_{24}P_{24}K_{24} - N_8P_8K_8$	95.50	96.50	98.96	-1.00
$N_{24}P_{24}K_{24} - N_{16}P_{16}K_{16}$	95.50	92.50	103.24	3.00*
30 mm				
$N_8P_8K_8 - N_0P_0K_0$	98.50	96.50	102.07	2.00
$N_{16}P_{16}K_{16} - N_0P_0K_0$	99.50	96.50	103.11	3.00*
$N_{24}P_{24}K_{24} - N_0P_0K_0$	99.50	96.50	103.11	3.00*
$N_{16}P_{16}K_{16} - N_8P_8K_8$	99.50	98.50	101.02	1.00
$N_{24}P_{24}K_{24} - N_8P_8K_8$	99.50	98.50	101.02	1.00
$N_{24}P_{24}K_{24} - N_{16}P_{16}K_{16}$	99.50	99.50	100.00	0.00

$DL\ (LSD)_{5\%} = 2.96$ $DL\ (LSD)_{1\%} = 3.91$ $DL\ (LSD)_{0.1\%} = 5.05$

Regarding the combined effect of the three factors (Table 7), in the case of Idared cultivar, fertilization generally showed a reduced and insignificant influence on the grafting percentage. In the case of the agricultural background where irrigation was applied with 10 mm, fertilization with 24% NPK favored a significant increase in the catch percentage compared to the unfertilized variant or the one where 8% NPK was applied. Under the conditions of fertilizing trees of the Idared cultivar with reduced doses of up to 8% NPK, it can be observed that irrigation with 20- 30 mm caused a significant increase in the percentage of grafting by 6-13% compared to the other irrigation options. The highest effect of irrigation on the grafting of trees is found on the agricultural background fertilized with 16%

NPK, where all three watering norms generated significant increases of this indicator. In the case of trees fertilized with 24% NPK, the highest values of the grafting percentage are recorded for this cultivar, against the background of significant effects of 5-11% of irrigation. Against the background of irrigation with the three watering norms, the seedlings of the Florina cultivar capitalized to a reduced extent the fertilization with NPK, registering insignificant variations of 3.8-4% of the percentage of capture.

Table 7. The effect of irrigation and fertilization on survival rate of grafting trees of the two apple cultivars

Watering norm	**0 mm**			
	NPK dose			
Cultivar	**$N_0P_0K_0$**	**$N_8P_8K_8$**	**$N_{16}P_{16}K_{16}$**	**$N_{24}P_{24}K_{24}$**
Idared	y 84.00 a	xy 86.00 b	xy 86.00 b	x 89.00 a
Florina	y 85.00 a	x 94.00 a	x 92.60 a	x 93.00 a
Watering norm	**10 mm**			
	NPK dose			
Cultivar	**$N_0P_0K_0$**	**$N_8P_8K_8$**	**$N_{16}P_{16}K_{16}$**	**$N_{24}P_{24}K_{24}$**
Idared	y 88.00 b	y 88.00 a	xy 91.00 a	x 94.00 a
Florina	x 94.00 a	x 90.20 a	x 93.00 a	x 92.00 a
Watering norm	**20 mm**			
	NPK dose			
Cultivar	**$N_0P_0K_0$**	**$N_8P_8K_8$**	**$N_{16}P_{16}K_{16}$**	**$N_{24}P_{24}K_{24}$**
Idared	x 94.00 a	x 97.00 a	x 93.00 a	x 96.00 a
Florina	x 95.00 a	x 96.00 a	x 92.00 a	x 95.00 a
Watering norm	**30 mm**			
	NPK dose			
Cultivar	**$N_0P_0K_0$**	**$N_8P_8K_8$**	**$N_{16}P_{16}K_{16}$**	**$N_{24}P_{24}K_{24}$**
Idared	x 97.00 a	x 99.00 a	x 99.00 a	x 100.00 a
Florina	x 96.00 a	x 98.00 a	x 100.00 a	x 99.00 a

Cultivar - DL $(LSD)_{5\%}$ = 4.12 DL $(LSD)_{1\%}$ = 5.44 DL $(LSD)_{0.1\%}$ = 7.01 (a, b)
Fertilization - $DL_{5\%}$ = 4.18 $DL_{1\%}$ = 5.53 $DL_{0,1\%}$ = 7.15 (x, y)
DL $(LSD)_{5\%}$ = 4.04 DL $(LSD)_{1\%}$ = 5.35 DL $(LSD)_{0.1\%}$ = 6.89

Only in the case of unirrigated agricultural land, it was observed that fertilization with NPK had a significant effect on the grafted trees, associated with increases of 7.6-9%. On the unfertilized agricultural background, irrigation caused a significant increase in grafting by 9-11%, under the conditions of small and insignificant variations between the three watering norms.

In the case of fertilizing with 8 % NPK, a significant increase in the percentage of grafting was observed by 20-30 mm compared to the norm of 10 mm. Under the effect of fertilization with 16 and 24 % NPK, irrigation showed a considerably lower influence on grafting, so that only the application of the 30 mm rule allowed a significant increase.

CONCLUSIONS

Compared to the unfertilized variant, it was found that the application of different doses of NPK allowed a significant increase in the grafting percentage by 1.71-3.13%. On the other hand, increasing the nutrient concentrations from 8 to 16 and respectively 24% NPK had reduced and statistically uncertain effects on grafting. Fertilization with 24% NPK was equally utilized by the grafted trees of the two apple cultivars.

Future research directions that can be considered include studying tree production, as well as an analysis of economic efficiency under irrigation and fertilization conditions.

REFERENCES

Arthur, J. & Bejaei, M. (2025). Evaluating and Classifying Apple Brand Names: Criteria and Trends over a Century, *MDPI Horticulturae Journal*, *11*(2), 127.

Braniște et al. (2010). *Pomicultură generală*, Bucharest, RO: Invel Multimedia Publishing House.

Cronin, D. (2019). *Irrigation and Soil Nutrition*, New York, USA: Syrawood Publishing House.

Faulkner, N. (2022). *Principles of Irrigation*, New York, USA: Syrawood Publishing House

Fischer, P.T.B., Carella, A., Massenti, R., Fadhilah, R., Lo Bianco, R. (2025). Advances in Monitoring Crop and

Soil Nutrient Status: Proximal and Remote Sensing Techniques, *MDPI Horticulturae Journal*, *11*(2), 182.
Jäger, H. (2021). *Die Baumschule*, Paderborn, DE: Salzwasser Publishing House.
Ladikou, E.-V., Daras, G., Landi, M., Chatzistathis, T., Sotiropoulos, T., Rigas, S., Papadakis, I. E. (2025) Physiological and Biochemical Effects of Potassium Deficiency on Apple Tree Growth, *MDPI Horticulturae Journal*, *11*(1), 42.
Maurer, J. (2016). *Handbuch Bio- Obst Sortenvielfalt erhalten*, Innsbruck, AT: Löwenzahn Publishing House.
Mayer, J. (2019). *Mein kleiner Obstbaum*, Stuttgart, DE: Franckh Kosmos Publishing House.
Megh, R. G. & co. (2021). *Micro Irrigation Scheduling and Practices*, Florida, USA: Apple Academic Press.
Neupane, K., Witcher A., Baysal- Gurel, F. (2024). Evaluation of the Effect of Fertilizer Rate on Tree Growth and the Detection of Nutrients Stress in Different Irrigation Systems, *MDPI Horticulturae Journal*, *10*(7), 767.
Otto, S., Wolff, G., Hatch, P. (2015). *The Backyard Orchardist: A Complete Guide to Growing Fruit Trees in the Home Garden*, London, UK: Ottographics Publishing House.
Ozherelieva, Z.E., Nikitin, A.L., Prudnikov, P.S. (2025). The Effect of Organomineral Fertilizers on the Yield and Quality of Apples After Long-Term Storage, *MDPI Horticulturae Journal, 11*(1), 13.
Patzwahl, W. (2023). *Wassermanagement und Bewässerung im Weinbau*, Stuttgart, DE: Eugen Ulmer Publishing House.
Schmid, A. (2021). *Obstbäume verstehen*, Frankfurt, DE: Hauptverlag Publishing House.
Smith, P. (2022). *Trees: From Root to Leaf*, Chicago, USA: University of Chicago Press.
Soman, P. (2022). *Fertigation Scheduling of Field Crops*, New Delhi, IN: New India Publishing Agency.
Stănică, F. (2004). *Cercetări experimentale privind fertilizarea în livezi*, Bucharest, RO: Invel Multimedia Publishing House.
Stănică, F., Peticilă, A. G. (2012). *Înființarea plantațiilor pomicole*, Târgoviște, RO: Valahia University Press.
Venig, A. & Stănică, F. (2024). Irrigation and Fertilization: A Comprehensive Analysis of Their Influences on Qualitative Indices in Two Plum Cultivars*, MDPI, Sustainability Journal*, *16*(6), 2496.
Waller, P. (2016). *Irrigation and Drainage Engineering*, Heidelberg, DE: Springer Press.
Żarski, J., & Kuśmierek-Tomaszewska, R. (2023). Effects of Drip Irrigation and Top-Dressing Nitrogen Fertigation on Maize Grain Yield in Central Poland, *MDPI Agriculture Journal*, *13*(2), 360.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

INVESTIGATING THE RELATIONSHIP BETWEEN WATER/NUTRIENT AVAILABILITY AND YIELD EFFICIENCY IN GRAFTED CHERRY TREES

Adelina VENIG[1], Florin STĂNICĂ[2], Adrian PETICILĂ[2]

[1]University of Oradea, Faculty of Environmental Protection, 26 Gen. Magheru Blvd, Oradea, Romania
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăști Blvd, District 1, Bucharest, Romania

Corresponding author email: adrian.peticila@horticultura-bucuresti.ro

Abstract

This research aims to establish the connection between irrigation, fertilization, and economic efficiency in nurseries. This link significantly impacts nursery profitability, as optimal water and nutrient management leads to the production of higher-quality planting material at reduced costs, and consequently, greater profitability. Managing costs related to irrigation and fertilization is a pivotal aspect of achieving economic efficiency in nursery operations. These inputs, while fundamental for plant growth and quality, represent significant expenditures that demand meticulous planning and execution. The aim of academic research in this domain is to quantify these costs and identify strategies for their optimization, ultimately enhancing the profitability and sustainability of nursery enterprises. The research was conducted in a private nursery in northwestern Romania, utilizing planting material obtained from grafting with two cherry cultivars, Katalin and Kordia. Four irrigation rates and four fertilization levels were applied as technological elements. Considering the economic indicators for the two cultivars, a small variation in the cost price was observed, ranging from 5.79 Ron/tree for the Katalin cultivar down to 5.07 Ron/tree for Kordia grafted trees. Correspondingly, the profit margin varied from 38.08% to 57.72%. Thus, the profit per grafted tree for the two cultivars was between 2.21 and 2.93 Ron.

Key words*: water, primary macronutrients, nursery, economic efficiency, fruit trees production.*

INTRODUCTION

Achieving profitability in a nursery is crucial for its ongoing viability and growth. It reflects the disparity between the total income earned from selling plants and the overall production and operational expenses. A profitable nursery not only provides financial benefits for its owners but also enables reinvestment in modernization, expansion, and enhancements in product quality. The profitability of a nursery is greatly and directly affected by the technological factors used. While investing in technology may come with higher upfront costs, it typically leads to increased revenue, lower operational expenses, and improved quality of products, resulting in better long-term profitability (Wallin, 2020). Irrigation in a nursery provides the essential water needed for the proper growth and development of seedlings. The significance of irrigation in a nursery is crucial, acting as a key horticultural practice that directly supports the biological and economic viability of plant propagation and cultivation. Irrigation guarantees the accurate delivery of the ideal amount of water to plants during key growth phases, thus supporting crucial physiological functions like photosynthesis, nutrient absorption, and cell turgor, which are all essential for robust and healthy development (Smith, 2022). Water needs differ based on factors such as species, growth stage, type of substrate or soil, and environmental conditions like temperature, humidity, and wind. From a business standpoint, managing irrigation effectively leads to the cultivation of strong, evenly sized, and visually attractive nursery plants. Plants that suffer from a lack of water will show restricted growth, inferior leaf quality, and a generally less appealing appearance, which necessitates longer cultivation times to reach marketable sizes and quality. This, in turn, raises production expenses and delays the generation of revenue (Fasani et al., 2025). Consequently, optimizing irrigation practices is not merely a matter of plant survival but a critical factor influencing the marketability of the material, the efficiency of resource

utilization, and ultimately, the financial profitability of the nursery enterprise (Jäger, 2021). Insufficient water (water stress) hampers growth, disrupts photosynthesis, decreases plant turgor, and increases vulnerability to diseases and pests. On the other hand, too much water can cause root suffocation, the onset of diseases (such as root rot), and the leaching of nutrients (Thomas, 2021). Water-stressed plants will be smaller, less attractive, and will require a longer growing period to achieve the desired size and quality (Ghorbanian et al., 2025). Fertilization is especially important in a nursery because it is the main source of vital macroelements (e.g. G. microelements - such as potassium, phosphorus, and nitrogen) and microelements to plants in amounts and ratios specifically designed for the best possible growth and development. Key physiological processes such as root proliferation, shoot elongation, foliar expansion, and the overall structural integrity and resilience of the plant are all directly impacted by nutrient availability (Ferrara et al., 2025) Suboptimal plant development is always the consequence of inadequate or unbalanced fertilization regimes, which show up as obvious nutrient deficiencies (e.g. G. reduced vitality, stunted growth, chlorosis, and weakened general health). Such malnourished seedlings are naturally less appealing to potential customers and need to be cultivated for a longer period to satisfy market requirements, which lengthens production cycles and raises operating expenses (Mayer, 2019). On the other hand, overfertilization can result in nutrient toxicities, which can harm plants and cause root burn. It can also pose environmental hazards by leaking nutrients into runoff or groundwater, which calls for careful management. Economically speaking, producing high-quality planting material—which is defined by strong growth, a well-developed root system, and the lack of stress symptoms - requires careful fertilization (Khan et al., 2025). Due to higher post-planting survival rates, such superior quality stock fetches higher market prices, improves customer satisfaction, and eventually lowers nursery losses. Furthermore, nutrient use efficiency is greatly increased by sophisticated fertilization techniques like controlled-release fertilizers and fertigation, which delivers soluble nutrients through the irrigation system. By minimizing nutrient losses, lowering application frequency, and optimizing labour and material costs, these techniques directly improve the nursery's financial success and environmental sustainability. The effective control of production costs, which calls for the prudent use of resources, is a key component of profitability. For example, using sophisticated fertigation and drip irrigation systems greatly reduces the amount of water and fertilizer used. Significant savings are achieved by precisely applying water and nutrients to the root zone, minimizing losses from evaporation and leaching (Megh, 2021). Additionally, automating these procedures lessens the need for manual labour, which is a significant operational expense in any nursery. Utilizing controlled-release fertilizers optimizes labour and material costs by reducing application frequency and promoting effective nutrition. In the end, a fruit tree nursery's success depends on careful operational execution, strategic planning, integrating contemporary technologies, optimizing resources, and keeping a laser-like focus on quality.

The primary objective of this research is to comprehensively analyse the economic efficiency of fruit tree production within a nursery setting, specifically under varying conditions of irrigation and fertilization. This investigation aims to establish the intricate relationship between these critical technological inputs and the overall profitability and sustainability of nursery operations. By quantifying the direct and interactive effects of water and nutrient management on both production costs and generated revenues, the research seeks to determine optimal practices that lead to the cultivation of superior quality planting material at reduced unit costs. Ultimately, the goal is to provide evidence-based insights that can inform strategic decision-making for nursery managers, enabling them to enhance resource use efficiency, maximize profit margins, and foster long-term economic viability in the horticultural sector.

MATERIALS AND METHODS

This research was conducted at a private nursery in the north-western region of Romania, under its specific soil and climatic conditions. The study employed a trifactorial experimental design to analyse key influences on nursery

operations. Irrigation served as the primary experimental factor, with the cultivar as the secondary factor, and fertilization as the tertiary factor. For fertilization, three distinct amounts of NPK were applied: 50 kg of $N_8P_8K_8$, 100 kg of $N_{16}P_{16}K_{16}$, and 150 kg of $N_{24}P_{24}K_{24}$. The four irrigation rates employed were non-irrigated, and irrigation at 10 mm, 20 mm, and 30 mm. For grafting, dormant buds from the Kordia and Katalin cherry cultivars were used.

A highly regarded sweet cherry cultivar, the Kordia cherry has become increasingly popular among amateur gardeners and commercial farmers. Its outstanding fruit quality and appealing tree traits make it especially prized. Kordia cherries are very big; they usually weigh 10-12 g and measure 28-30 mm. When completely mature, they have a gorgeous dark crimson to black, glossy skin and a heart-shaped, prominent apex. The flesh is dark crimson and firm. This cherry cultivar is well known for their excellent, sweet, and fragrant flavour; they are frequently characterized as having a strong scent and a lengthy, fulfilling finish. They provide a delicate acidity and a nice mix of sweetness. Because of its firm flesh, the fruit is very attractive when eaten fresh. Its good transportability and capacity to be stored in cold circumstances for several days are also factors.

The Katalin cherry cultivar is a lesser-known yet promising sweet cherry cultivar. Although it is not as widely available as Kordia, it has several advantageous qualities that make it desirable for processing as well as fresh eating. To satisfy the current market demands for delicious cherries, Katalin usually produces huge fruits, frequently measuring 26-28 mm or even larger. It is renowned for its high sugar content, good firmness, and well-balanced sugar-to-acid ratio, all of which contribute to its superb flavour. Katalin's low susceptibility to rain-induced fruit cracking is a major benefit as it preserves fruit quality and lowers post-harvest losses (Blind, 2020).

RESULTS AND DISCUSSIONS

Regarding the economic efficiency indicators for different irrigation norms (Table 1), it is observed that the cost price per tree varied from 4.23 Ron for the 30 mm norm, up to 10.62 Ron for the non-irrigated variant. Similarly, the profit margin ranged from -24.67% for trees grown under non-irrigated conditions to 89.13% for trees obtained with the 30 mm irrigation norm.

Table 1. Economic efficiency indicators for grafted fruit tree production per experimental factor

Factors	Grafted trees/ ha	Income (Ron/ ha)	Costs (Ron/ ha)	Profit (Ron/ ha)	Profit margin /ha (%)	Cost price (Ron/ tree)	Profit (Ron/ tree)
Irrigation							
0 mm	19950	159600	211860	-52260	-24.67	10.62	-2.62
10 mm	42023	336184	216759	119425	55.10	5.16	2.84
20 mm	46230	369840	217498	152342	70.04	4.70	3.30
30 mm	51594	412752	218238	194514	89.13	4.23	3.77
Cultivar							
Katalin	37297	298374	216089	82285	38.08	5.79	2.21
Kordia	42602	340818	216089	124729	57.72	5.07	2.93
Fertilization							
$N_0P_0K_0$	17513	140104	214679	-74575	-34.74	12.26	-4.26
$N_8P_8K_8$	42900	343200	214919	128281	59.69	5.01	2.99
$N_{16}P_{16}K_{16}$	49052	392416	215159	177257	82.38	4.39	3.61
$N_{24}P_{24}K_{24}$	50333	402664	215399	187265	86.94	4.28	3.72

Therefore, considering the unilateral effect of irrigation, there is a gradual increase in the economic efficiency of grafted tree production as the irrigation rate changes. This is associated with a 15% increase in the profit margin between the 10 mm and 20 mm rates, and a further 19% increase when irrigation is supplemented from 20 mm to 30 mm. Analysing the economic indicators for the two cultivars, there is a small variation in the cost price, ranging from 5.79 Ron/tree for the Katalin cultivar down to 5.07 Ron/tree for Kordia grafted trees. Correspondingly, the profit margin varies from 38.08% to 57.72%. As a result, the profit per grafted tree for the two cultivars was 2.21-2.93 Ron. Considering the unilateral effect of fertilization, the cost price ranged between 4.28 Ron/tree for the soil fertilized with 24% NPK and 12.26 Ron/tree for the unfertilized soil. Furthermore, the profit and profit margin associated with the three fertilization levels were proportional to the cost price, recording values of 2.99-3.72 Ron/tree and 59.69-86.94%, respectively. In the case of the non-irrigated variant, expenses were higher than revenues, resulting in a negative profit margin. The cultivar's effect on the economic efficiency of grafted tree production was higher for un-irrigated soil, with a profit variation from 16,716 Ron/ha for the Kordia cultivar to 108,140 Ron/ha for the Katalin cultivar (Table 2).

Table 2. Economic efficiency indicators for grafted cherry trees production of the two cultivars under different irrigation conditions

Experimental factors	Grafted trees/ha	Income (Ron/ha)	Costs (Ron/ha)	Profit (Ron/ha)	Profit rate/ha (%)
0 mm					
Katalin	22855	182840	211860	-29020	-13.70
Kordia	17046	136368	211860	-75492	-35.63
10 mm					
Katalin	38357	306856	216759	90097	41.57
Kordia	45690	365520	216759	148761	68.63
20 mm					
Katalin	39857	318856	217498	101358	46.60
Kordia	52603	420824	217498	203326	93.48
30 mm					
Katalin	48118	384944	218238	166706	76.39
Kordia	55070	440560	218238	222322	101.87

When using a 30 mm irrigation rate, the economic efficiency of grafted tree production showed no differences between the cultivars. The profit margin for non-irrigated conditions ranged from 7.89% for the Kordia cultivar to 51.04% for the Katalin cultivar. However, under the effect of the 30 mm irrigation rate, it reached a value of 109.47% for both cultivars. Irrigation had a greater influence on the economic efficiency of grafted tree production for the Kordia cultivar, leading to a 104% increase in profit margin with the 10 mm rate and a 129-147% increase with the 20-30 mm rates. For the Katalin cultivar, irrigation with 10-20 mm rates generated a 55-60% increase in profit margin, while the 30 mm irrigation rate led to a 90% increase in profit margin. Considering the economic efficiency indicators for grafted tree production (Table 3), it is observed that across the entire experiment, the cost per tree varied from 3.81 Ron for the variant fertilized with 24 % NPK on soil irrigated with a 20 mm rate, up to 18.94 Ron for the 10 mm irrigation rate on unfertilized soil. Consequently, the profit margin showed a variation ranging from -57.75% for the variant combining the 10 mm rate with $N_0P_0K_0$ (no fertilizer) to 109.84% for the $N_{24}P_{24}K_{24}$ variant irrigated with a 20 mm rate.

Table 3. Economic efficiency indicators for grafted cherry trees production under different irrigation and fertilization conditions

Experimental factors	Grafted trees/ha	Income (Ron/ha)	Costs (Ron/ha)	Profit (Ron/ha)	Profit margin/ ha (%)	Cost price (Ron/tree)	Profit (Ron/tree)
0 mm							
$N_0P_0K_0$	0	0	211500				
$N_8P_8K_8$	20072	160576	211740	-51164	-24.16	10.55	-2.55
$N_{16}P_{16}K_{16}$	28777	230216	211980	18236	8.60	7.37	0.63
$N_{24}P_{24}K_{24}$	30953	247624	212220	35404	16.68	6.86	1.14
10 mm							
$N_0P_0K_0$	11428	91424	216399	-124975	-57.75	18.94	-10.94
$N_8P_8K_8$	45428	363424	216639	146785	67.76	4.77	3.23
$N_{16}P_{16}K_{16}$	55143	441144	216879	224265	103.41	3.93	4.07
$N_{24}P_{24}K_{24}$	56094	448752	217119	231633	106.68	3.87	4.13
20 mm							
$N_0P_0K_0$	19492	155936	217138	-61202	-28.19	11.14	-3.14
$N_8P_8K_8$	52143	417144	217378	199766	91.90	4.17	3.83
$N_{16}P_{16}K_{16}$	56143	449144	217618	231526	106.39	3.88	4.12
$N_{24}P_{24}K_{24}$	57143	457144	217858	239286	109.84	3.81	4.19
30 mm							
$N_0P_0K_0$	39139	313112	217878	95234	43.71	5.57	2.43
$N_8P_8K_8$	53958	431664	218118	213546	97.90	4.04	3.96
$N_{16}P_{16}K_{16}$	56143	449144	218358	230786	105.69	3.89	4.11
$N_{24}P_{24}K_{24}$	57143	457144	218598	238546	109.13	3.83	4.17

In the absence of irrigation, fertilization led to an increase in the profit margin from 8.60% with the 16 % NPK level to 16.68% for the 24 % NPK level. This also translated to an increase in profit from 0.63 to 1.14 Ron/tree. For the unfertilized variant, the grafted trees did not reach the required vigour to be sold. With a 10 mm irrigation rate, the absence of fertilization resulted in a negative profit margin. However, under the effect of fertilization, the profit margin ranged from 67.76% to 106.68%, corresponding to a profit of 3.23-4.13 Ron/tree. When using a 20 mm irrigation rate, the profit margin varied from 91.90% for the variant fertilized with 8 % NPK to 109.84% for the 24 % NPK level, yielding profits of 3.83-4.19 Ron/tree. Under the effect of a 30 mm irrigation rate, fertilization had a lesser influence on the economic efficiency of grafted tree production compared to other irrigation rates. This is because the profit margin recorded a positive value of 43.71% on unfertilized soil. For the three NPK levels, the profit margin was 97.90-109.13%, and the profit was 3.96-4.17 Ron/tree. For unfertilized soil, only 30 mm of irrigation resulted in a positive profit margin, yielding a profit of 2.43 Ron/tree. When fertilizing with 8 % NPK, 10 mm of irrigation led to a significant increase in the profit margin from -24.16% to 67.76% and a 5.78 Ron increase in profit per tree. Increasing the irrigation rate from 10 mm to 20 mm resulted in a substantial 24.14% increase in the profit margin and a 0.6 Ron increase in profit per tree. However, changing the irrigation rate from 20 mm to 30 mm had a smaller impact on the economic efficiency of grafted tree production, evidenced by a 6% increase in the profit margin and a 0.16 Ron increase in net profit per tree. Under the effect of 16% NPK fertilization, a substantial increase in the profit margin of approximately 95% was observed with the 10 mm irrigation rate. Subsequently, increasing the irrigation rate led to a smaller positive variation in the profit margin of 2.5-3% and a 0.04-0.05 Ron increase in net profit per tree. With 24% NPK fertilization, irrigation significantly boosted profit by 90-93% and reduced the cost price by approximately 3 Ron/tree. Changing the irrigation rate from 10 mm to 20 mm resulted in a 3.2% increase in the profit margin and a 0.06 Ron increase in profit per tree. In contrast, increasing the irrigation rate from 20 mm to 30 mm had a very limited impact on the economic indicators of grafted tree production. When it comes to the interaction between cultivars and fertilization (Table 4), it can be observed that the cultivar's effect on the economic efficiency of grafted tree production was more pronounced in unfertilized soil. This was associated with a profit margin variation from -72.85% for the Katalin cultivar to 3.37% for the Kordia cultivar. The differences between cultivars in terms of economic efficiency indicators decrease as the applied fertilization level increases. Thus, under the effect of the 24 % NPK treatment, the profit margin for the two cultivars was between 86.67% and 87.20%. Fertilization had a greater influence on the economic efficiency of grafted tree production for the Katalin cultivar, leading to a 132.95% increase in profit margin with the 8 % NPK level and a 153.66-159.52% increase with the 16-24 % levels. For the Kordia cultivar, fertilization with 8 % NPK resulted in an approximate 56% increase in profit margin.

Table 4. Economic efficiency indicators for grafted cherry trees production of the two cultivars under different fertilization conditions

Experimental factors	Grafting trees/ha	Income (Ron/ha)	Costs (Ron/ha)	Profit (Ron/ha)	Profit rate/ha (%)
$N_0P_0K_0$					
Katalin	7286	58288	214679	-156391	-72.85
Kordia	27740	221920	214679	7241	3.37
$N_8P_8K_8$					
Katalin	43011	344088	214919	129169	60.10
Kordia	42790	342320	214919	127401	59.28
$N_{16}P_{16}K_{16}$					
Katalin	48629	389032	215159	173873	80.81
Kordia	49474	395792	215159	180633	83.95
$N_{24}P_{24}K_{24}$					
Katalin	50262	402096	215399	186697	86.67
Kordia	50404	403232	215399	187833	87.20

Supplementing fertilization from 8 to 16% NPK led to a significant 24.67% increase in the profit margin. However, changing the level from 16 to 24% NPK had a smaller impact, showing only a 3.25% influence on the economic efficiency of grafted tree production. Regarding the cost and profit for grafted trees of the two cultivars under different irrigation conditions (Figure 1), it can be observed that for the Katalin cultivar, the cost price ranged from 4.54 Ron with a 30 mm irrigation rate to 9.27 Ron under non-irrigated conditions.

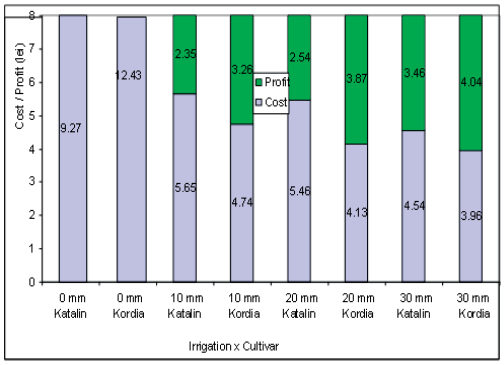


Figure 1. Cost and profit for grafted trees of the two cultivars under different irrigation conditions

This correlated with profits of 2.35-3.45 Ron/tree. For the Kordia cultivar, the cost price varied from 12.43 Ron in the absence of irrigation to 3.96 Ron with a 20 mm irrigation rate, corresponding to profits of 3.26-4.04 Ron/tree. Under non-irrigated conditions, the cost price for Katalin grafted trees was lower than for Kordia. However, with 10-30 mm of irrigation, the profit for the Kordia cultivar was higher by 0.91-1.33 Ron/tree, with the highest value occurring under 20 mm irrigation. Fertilization had a greater influence on the economic efficiency of grafted tree production for the Katalin cultivar, showing a significant 83% reduction in cost price compared to the unfertilized variant (Figure 2).

Supplementing fertilization from 8 to 16 % NPK led to a 0.58 Ron increase in profit per tree, while changing the level from 16 to 24 % NPK did not significantly affect the cost price per tree.

For the Kordia cultivar, a significant 35% reduction in cost price was observed when fertilized with 8% NPK, compared to the unfertilized variant.

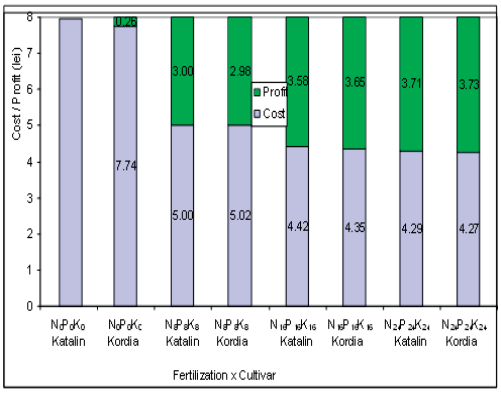


Figure 2. Cost and profit for grafted trees of the two cultivars under different fertilization conditions

Subsequently, increasing the level from 8 to 16% NPK generated a 25% increase in profit per tree, while supplementing fertilization from 16 to 24% NPK resulted in a smaller 2.2% variation. The cultivar's effect on the efficiency of grafted tree production was more pronounced in unfertilized soil. This was associated with a cost price variation from 29.46 Ron/tree for the Katalin cultivar down to 7.74 Ron/tree for the Kordia cultivar. The differences in profit per grafted tree between cultivars decreased with increased fertilization. Thus, under the effect of fertilization, profits varied from 2.98-3.0 Ron/tree for the 8% NPK level, up to 3.71-3.73 Ron/tree with the 24% NPK level. Considering the cost estimates for various fertilization treatments under non-irrigated conditions, it is observed that manual labour accounts for the largest share of total expenses, at 55.32-55.51%. This is followed by material costs at 41.32-41.52%, and mechanical operations at 3.16-3.17%. Under a 10 mm irrigation regime, material costs contributed 54.26-54.44% to total costs. This was associated with a 42.46-42.65% contribution from manual labour expenses and 3.9-3.10% from mechanical operations. With a 20 mm irrigation rate, applying different fertilization variants resulted in a cost structure composed of 54.07-54.25% for manual labour costs, 42.66-42.85% for material costs, and 3.08-3.09% for mechanical operations. Finally, with a 30 mm irrigation rate, material costs contributed 53.89-54.07% to total costs. This was associated with a 42.85-43.04% contribution from manual labour expenses and 3.07-3.08% from mechanical operations.

CONCLUSIONS

The profit margin for different irrigation rates ranged from 55.10% for trees irrigated with a 10 mm rate to 89.13% for trees grown under a 30 mm irrigation rate. This shows a gradual increase in the economic efficiency of grafted tree production as the irrigation rate changes, associated with a 15% increase in the profit margin between the 10 mm and 20 mm rates, and a further 19% increase when irrigation is supplemented from 20 mm to 30 mm. Considering the economic indicators for the two cultivars, there's a small variation in the cost price, from 5.79 Ron/tree for the Katalin cultivar down to 5.07 Ron/tree for Kordia grafted trees. Correspondingly, the profit margin varies from 38.08% to 57.72%. As a result, the profit for the two cultivars was 2.21-2.93 Ron/grafted tree. Regarding fertilization, the cost price ranged between 4.28 Ron/tree for soil fertilized with 24 % NPK and 12.26 Ron/tree for unfertilized soil. Furthermore, the profit and profit margin associated with the three fertilization levels were proportional to the cost price, recording values of 2.99-3.72 Ron/tree and 59.69-86.94%, respectively. In the case of the non-irrigated variant, expenses were higher than revenues, resulting in a negative profit margin. Irrigation had a greater influence on the economic efficiency of grafted tree production for the Kordia cultivar, leading to a 104% increase in the profit margin with the 10 mm rate and a 129-147% increase with the 20-30 mm rates. For the Katalin cultivar, irrigation with 10-20 mm rates generated a 55-60% increase in the profit margin, while the 30 mm irrigation rate led to a 90% increase in the profit margin. In the absence of irrigation, fertilization led to an increase in the profit margin from 8.60% when applying the 16% NPK level to 16.68% for the 24 % NPK level, corresponding to a profit increase from 0.63 to 1.14 Ron/tree. For the unfertilized variant, the grafted trees did not reach adequate vigour for commercialization. Fertilization had a greater influence on the economic efficiency of grafted tree production for the Katalin cultivar, causing a profit margin increase of 132.95% with the 8% NPK level, and 153.66-159.52% with the 16-24% levels. For the Kordia cultivar, fertilization with 8 % NPK resulted in an approximate 56% increase in the profit margin. Supplementing fertilization from 8 to 16% NPK generated a significant 24.67% increase in the profit margin, while changing the level from 16 to 24% NPK had a lesser influence of 3.25% on the economic efficiency of grafted tree production. From an economic efficiency standpoint, the recommended options are those involving fertilization with 24% NPK on soils irrigated with 20-30 mm norms. These conditions allowed for profit margins of 109.13-109.84%, yielding profits of 4.17-4.19 Ron per tree.

REFERENCES

Blind, S. (2020). *The Old Fruit Trees Cultivars*, Cologne, DE Du Mont Publishing House.

Fasani, E., Franceschi, C., Furini, A. *et al.* (2025). Effect of biostimulants combined with fertilization on yield and nutritional value of wheat crops. *BMC Plant Biol*, 25, 736 .

Ferrara, R.M., Bruno, M.R., Ruggieri, S. et al. (2025). Water use of an irrigated peach orchard treated with different plastic mulching films under Mediterranean climate. *Irrig Sci*, 43, 887–907.

Ghorbanian, M., Ojaghlou, H. & Ebrahimian, H. (2025). An approach to estimate optimal cut-off time under deficit irrigation. *Irrig Sci*, 43, 855–869.

Jäger, H. (2021). *Die Baumschule*, Paderborn, DE: Salzwasser Publishing House.

Khan, F.U., Qu, Y., Zaman, F. et al. (2025). Sustainable Crop Cultivation and Fertilization: Five-Year Effects on Soil Quality in the Loess Plateau. *J Soil Sci Plant Nutr*, 25, 3901–3915.

Mayer, J. (2019). *Mein kleiner Obstbaum*, Stuttgart, DE: Franckh Kosmos Publishing House.

Megh, R. G. (2021). *Micro Irrigation Scheduling and Practices*, Florida, U.S.A.: Apple Academic Press

Smith, P. (2022). *Trees: From Root to Leaf*, Chicago, USA: University of Chicago Press.

Thomas, C. G. (2021). *Irrigation and Water Management*, New Delhi, India: Ane Books Press.

Wallin, C. (2020) *Growing Trees for Profit*, Chicago, U.S.A.: Second Edition Publishing

Wheeler, J. (2023). *Growing Fruit Trees*, Chicago, U.S.A: University of Chicago Press.


EX·TERRA·AVRVM

VITICULTURE AND OENOLOGY


EX·TERRA·AVRVM

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

STUDIES ON THE EVALUATION OF THE PHENOLIC POTENTIAL OF GRAPE POMACE FOR RED WINES

Victoria ARTEM[1], Cristina CIOBANU[1], Arina Oana ANTOCE[2], Ancuța NECHITA[3], Georgeta TUDOR[4]

[1]Research Station for Viticulture and Oenology Murfatlar, 2 Calea Bucuresti, 905100, Murfatlar, Constanţa, Romania
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Horticulture, Department of Bioengineering of Horti-Viticultural Systems, 59 Mărăşti Blvd, District 1, 011464, Bucharest, Romania,
[3]Research Station for Viticulture and Oenology laşi, 48 Mihail Sadoveanu Alley, 700 490 laşi, Romania
[4]Research Institute for Viticulture and Oenology Valea Călugărească, 1 Valea Mantei, Valea Călugărească, Prahova, Romania

Corresponding author email: arina.antoce@horticultura-bucuresti.ro

Abstract

The valorisation of phenolic compounds from grape pomace continues to remain a challenge, but also an opportunity to promote sustainable practices in wine industry. The objective of this study was to evaluate the phenolic potential of the red wine fermented-pomace obtained during 2023 harvest from the Feteasca Neagră and Cabernet Sauvignon varieties from three renowned Romanian vineyards: Murfatlar, Dealu Mare and Iaşi. The extraction method used was the classical solid-liquid method, that is the maceration of the pomace at ambient temperature for 24 hours, with discontinuous stirring. As extraction solutions there were used mixtures of ethyl alcohol and water in different concentrations: 25%, 50%, and 75% v./v. The polyphenolic content of the resulting extracts was evaluated by UV-VIS spectrophotometric determinations: total polyphenols (g GAE/L), anthocyanins content (mg/100 g grape pomace, colour intensity and antioxidant activity (DPPH%). A series of phenolic indices, such as the total polyphenol index and the Folin-Ciocalteu index, were also calculated. The obtained results highlighted that many factors influence the extraction process. The highest values for extracted polyphenols were recorded for the 50% hydroalcoholic solution, but also significant differences were induced by the grape variety and the viticultural terroir. The values obtained for the total phenolic compounds content fluctuated in the range of 7.1-31.9 g GAE/L and between 253-798 mg/100 g in the case of anthocyanin pigments. All phenolic extracts presented high antioxidant activity, confirming that they can be used as cheap and easily available sources of bioactive compounds for the pharmaceutical, cosmetic and food industries.

Key words*: grape pomace, hydroalcoholic extract, total phenolic compounds, antioxidant activity, anthocyanins.*

INTRODUCTION

The grape pomace is one of the main secondary products that result after pressing from the winemaking process. It consists mainly of skins, seeds and remains of the stems, overall representing 20-25% of the mass of the processed grapes (Vukusik et al., 2023). The grape pomace is an attractive natural resource due to its high content of phenolic compounds, which are well known for their antioxidant activity and nutraceutical benefits (Iliyas et al., 2021). It is estimated that 60-70% of the phenolic compounds of grapes remain in the pomace after winemaking and make up 4.8-5.4% of the dry matter of the pomace (Taladrid et al., 2023). The polyphenols in pomace include flavonoids, tannins and stilbenes (Nurgel & Reicher, 2020) but also anthocyanins. The variability of phenolic compounds and the extraction yield depend on the grape variety, the terroir, climate, soil conditions and winemaking process technology (Caldas et al., 2018). The choice of extraction technique for the recovery of bioactive compounds is essential because it significantly influences both the yield and the recovery of these compounds. One of the most used and traditional extraction techniques is the solid-liquid extraction by maceration (Rodrighez et

al., 2022). Although this method is laborious and time-consuming, it does not involve the use of expensive equipment. It is a widely used conventional technique for extracting bioactive compounds from plant materials, including grapes and grape pomace. Due to the polar nature of polyphenols, organic solvents such as ethanol, methanol, acetone, ethyl acetate, and chloroform are commonly used (Jara-Palacios et al., 2020). The typical process for solid-liquid extraction involves maceration and mechanical agitation, and the main factors influencing the efficiency of this method are: the type of solvent, the solvent-sample ratio, the particle size, the pH, the extraction temperature and the duration of the extraction process (Castelanos, 2022). According to the literature, the choice of solvent is one of the most important operational parameters. Thus, considering the polar nature of polyphenols, better extractive yields can be obtained using polar protic solvents, which can form hydrogen bonds, such as hydroalcoholic solutions (Fontana et al., 2013). The objective of this study was to evaluate the content of phenolic compounds in the pomace obtained from the vinification process of red grapes from the Fetească Neagră and Cabernet Sauvignon varieties from three renowned vineyards using a classic solid-liquid extraction method, simple, efficient and accessible.

MATERIALS AND METHODS

The pomace used for extractions was obtained in 2023, following the winemaking process of two *Vitis vinifera* grape varieties for red wines, one autochthonous, Fetească Neagră, and one international variety, Cabernet Sauvignon, from three renowned wine regions in Romania: Murfatlar, Dealu Mare and Iaşi. The winemaking technology was classic with five-day maceration and fermentation, followed by pressing using a hydraulic press. The pomace was conditioned by drying in a thin layer at ambient temperature, being palletized every 24 hours to facilitate water evaporation and to prevent the development of bacteria and fungi in the wet layer. The extraction was carried out at ambient temperature, with discontinuous stirring, for 24 hours, the pomace/solvent ratio being 1/4. The extraction solution was a hydroalcoholic mixture of different concentrations, respectively 25%, 50% and 75%. The liquid extracts obtained were separated from the solid part by filtration (using filter paper) and then by centrifugation (1700 rpm for 15 minutes) and kept in a refrigerator at 4°C for approximately 1-2 days until the analyses were carried out by spectrophotometric determinations using the Helios Alpha UV-VIS spectrophotometer (Thermo Spectronic, Marea Britanie). ¸

The experimental extractions variants and procedure are chematically presented in Figure 1.

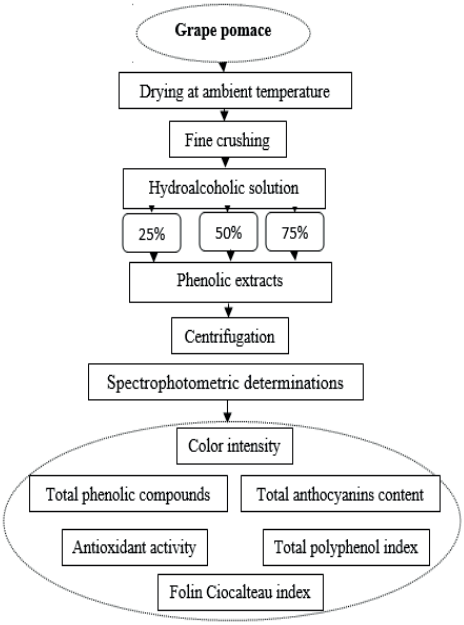


Figure 1. Experimental extraction scheme of pomace

The *total phenolic compounds*, expressed as (GAE g/L), were determined using the Folin-Ciocalteu method, by measuring the absorbance at 760 nm. The *anthocyanin content* (mg/100 g grape seed) was evaluated by the Ribèreau-Gayon method, based on the colour change of anthocyanins depending on pH; this consists of measuring the variation in absorbance at 520 nm of the colour of anthocyanins at pH 0.6 and 3.5 using distilled water as a control (Ribèreau-Gayon et al., 2017). The *antioxidant activity* (AA%) was analyzed according to the method proposed by Brand-Williams et al. (1995) using 0.1 ml of extract diluted 1:10. Thus 0.1 mL of each

sample was mixed with 3.9 mL of 4% DPPH diluted in 96% (v/v) ethanol and stirred. The homogenized mixture was incubated in the dark for 30 minutes at room temperature and subsequently, the absorbance was measured at 517 nm. The *colour intensity* was determined based on the sum of the absorbance values measured at 420 nm, 520 nm and 620 nm, expressed in absorbance units (Au). The *total polyphenol index* is based on the fact that the benzene rings, characteristic of phenolic compounds, absorb strongly the UV light, having an absorption maximum at a wavelength of 280 nm. The *Folin-Ciocalteu index* is based on the reaction of hydroxyl groups with a phospho-molybdenum reagent which is reduced to a mixture of blue tungsten oxide and molybdenum. The blue colour produced has a maximum absorption at 750 nm and is proportional to the total amount of phenolic compounds.

RESULTS AND DISCUSSION

The efficiency of the extraction process of bioactive compounds is influenced by a number of factors, including the type of solvent and its concentration (Schieber et al., 2017). Previous research has shown that milder extraction methods performed at ambient temperatures can reduce the degradation of bioactive compounds and improve extraction yield (Zhang et al., 2020).

The results regarding the recovery of phenolic compounds from the grape pomace collected in the three wine regions studied - Murfatlar, Dealu Mare and Iaşi - are highlighted in Table 1. The parameters evaluated include total phenolic compounds, anthocyanins, colour intensity, antioxidant activity, Folin-Ciocalteau index, and total polyphenol index. The values of total phenolic compounds were lower in the case of the Fetească Neagră variety (7.81-24.4 GAE g/L) compared to the Cabernet Sauvignon variety (16.67-31.89 GAE g/L), the differences being given by the source of pomace and concentration of the solvent mixture (ethanol/water). The highest efficiency in the recovery of phenolic compounds was observed with the 50% hydroalcoholic solution, recording the highest values of phenolic compounds (24.4 GAE g/L for Dealu Mare and 23.7 GAE g/L for Iaşi), values significantly higher compared to those obtained with solutions of 25% concentration. In the case of the Cabernet Sauvignon variety, the values of phenolic compounds were also higher in the case of a hydroalcoholic concentration of 50%, registering 31.89 GAE g/L in Murfatlar and 21.7 GAE g/L in Iaşi. These differences can be explained by the higher polyphenol content of the Cabernet Sauvignon variety compared to Fetească Neagră, as well as by the influence of terroir on the chemical composition of the pomace. Moderate concentrations of alcohol have been shown to be more effective in extracting phenolic compounds (González et al., 2010), fact also demonstrated by our results. Regarding the anthocyanin content of the analysed phenolic extracts, a significant variability of the values was observed, which were located in a fairly wide range, between 253-798 mg/100 g of grape pomace. The values were lower in the case of the 25% ethanol solvent for the Cabernet Sauvignon variety from Murfatlar. An increase in values was observed with the increase in the concentration of the hydroalcoholic solution, at 50% reaching a maximum of 588-646 mg/100 g of pomace in the case of the Fetească Neagră variety and 790 mg/100 g of pomace in the case of Cabernet Sauvignon. These results suggest that a medium concentration of ethanol is optimal for the efficient extraction of anthocyanins from the cellular structures of the grape pomace, without damaging them. In the case of the 75% hydroalcoholic solution, the anthocyanin content decreased slightly, on average by 9.2% for Fetească Neagră and 4.3% for Cabernet Sauvignon. Regarding the colour intensity of phenolic extracts, in the case of the Fetească Neagră variety, the value was 8.27 AU at 25% alcohol and increased significantly at concentrations of 50% and 75% alcohol, to 22.45 AU and 18.23 AU, respectively. In this case, the most efficient extraction was with the solution of 50%. In Cabernet Sauvignon, the colour intensity was highest at extractions with 75% alcohol (38.9 AU), followed by the extraction with alcoholic solution of 50% (20.23 AU), the lowest being at 25% alcohol (10.07 AU). More concentrated solvents can cause faster extraction of some compounds, but also an incomplete solubilization of others,

which can lead to the loss of important fractions of polyphenols, especially the most delicate or poorly soluble ones. The antioxidant activity, an essential indicator of the ability of grape pomace to neutralize free radicals, is a key factor in assessing its potential to protect the body against oxidative stress. The antioxidant activities of the extracts were relatively constant for both varieties extracted with solutions of 50% and 75% alcohol, exceeding 90%. These high antioxidant activities are mainly due to the content of the extracted flavonoid compounds, including catechins and anthocyanins, highlighting the potential of these extracts to combat oxidative stress (Nakamura & Okada, 2020). The research of Zhang et al. (2020) confirms that the antioxidant activity of grape pomace is directly correlated with the content of polyphenols and that the high alcohol concentrations are able to efficiently extract these compounds from the cellular structures of the grape pomace. The Folin-Ciocalteau index measures the total polyphenol content, being an essential tool in the analysis of bioactive compounds in phenolic extract (Kumar & Goel, 2019). In the case of the Fetească Neagră variety, the highest values were obtained with the 50% hydroalcoholic solution, with 22.78 AU in Dealu Mare and 17.89 AU in Iaşi. Similar results were recorded for the Cabernet Sauvignon variety with 15.42 AU in Iaşi and 26.16 AU in Murfatlar. These data suggest higher extraction efficiency at moderate alcohol concentrations. The total polyphenol index, also known as the 280 nm index, is based on the absorption characteristic of phenolic benzene rings present in (+)-catechin and its polymeric derivatives (tannins), the former being identified as the main monomer in grape skins (Riberau Gayon, 2000; Spigno et. al., 2007). For this reason, the index is correlated with the total phenolic compound values. Due to the particularities of the extraction procedure (extraction method, solid/solvent ratio, variety, temperature, time, hydroalcoholic solution), different from the models presented in the specialized literature, the data obtained could not be compared with those in the existing literature.

Tabelul 1. The phenolic potential of extracts obtained from the varieties Fetească Neagră and Cabernet Sauvignon in the wine regions of Dealu Mare, Iaşi and Murfatlar obtained with ethanolic solutions of 25, 50 and 75%

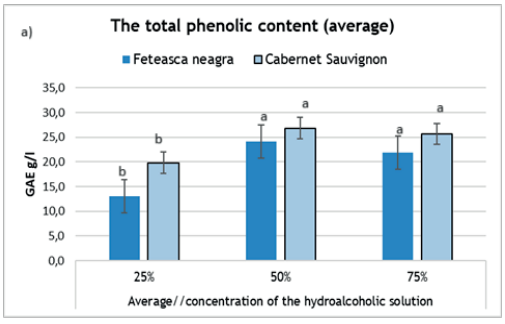
Measured parameters	Fetească Neagră					
	Dealu Mare			Iaşi		
	25%	50%	75%	25%	50%	75%
Total phenolic compounds g GAE /L	7.81±1.8b	24.4±9.6a	20,67±4.2a	18,27±2.1a	23,7±4.6a	23,01±3.7a
Anthocyanins (mg/100 g pomace)	331±22d	588±30ab	521±28c	537±40bc	646±36a	632±45a
Colour intensity (AU)	8.27±1.5c	22.45±3.2b	18.23±3.2b	21.61±2.0b	29.54±3.4a	22,3±2.6b
Antioxidant activity (%)	-	-	-	90,23±7.9a	91,83±10.6a	90,06±8.5a
Folin-Ciocalteu index, 750 nm (AU)	7.84±1.5d	22,78±2.4a	19.48±2.0ab	13,85±1.6c	17.89±1.9b	17,38±2.1b
Total polyphenols index, 280 nm (AU)	78.93±15.2c	233,24±25.4b	219.51±23.2b	206.21±20.1b	301.53±27.2a	290.96±22.4a
Measured parameters	Cabernet Sauvignon					
	Murfatlar			Iaşi		
	25%	50%	75%	25%	50%	75%
Total phenolic compounds g GAE /L	22.98±8.5a	31.89±10.2a	30.69±11.0a	16.67±5.9a	21.7±4.7a	20.6±4.5a
Anthocyanins (mg/100 g pomace)	253±18c	798±48a	790±41a	559±35b	790±49a	797±53a
Colour intensity (AU)	10.07±2.2d	20,23±2.5bc	18,35±2.1c	24.49±2.8b	36,77±3.0a	38.96±3.5a
Antioxidant activity (%)	88.21±3.9a	90.35±4.7a	91.02±4.3a	91.85±4.8a	92.53±5.0a	92.93±3.8a
Folin-Ciocalteu index, 750 nm (AU)	18.21±2.2b	26,16±2.8a	24.3±3.0a	12,82±1.8c	15.42±1.9bc	14.66±2.0bc
Total polyphenols index, 280 nm (AU)	199.1±11.2b	193.3±15.2b	278,9±17.9a	217.55±20.5b	284.7±24.3a	280.7±20.2a

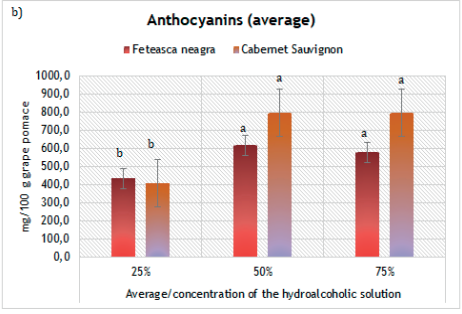
Average values ± standard errors (n = 3). The different letters show the statistical difference among results for $p<0.05$. For the same compound, a common letter for 2 or more variants means no significant difference among them.

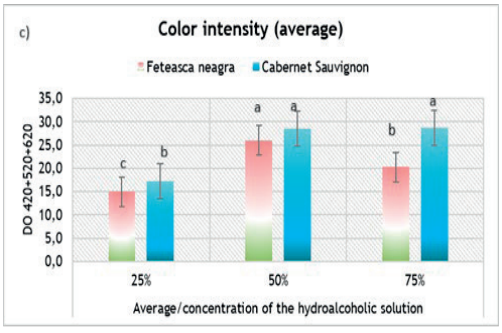
Figure 2 presents the averages obtained for each of the two varieties studied: Fetească Neagră and Cabernet Sauvignon, using three hydroalcoholic solutions: 25%, 50%, 75% for

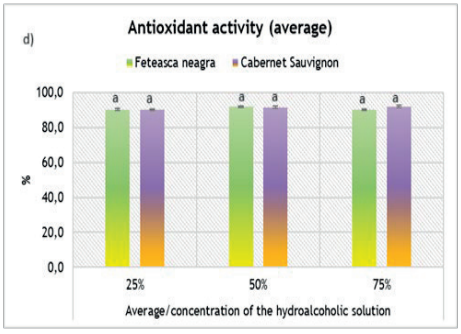
the following analysed parameters: a) total phenolic compounds, b) anthocyanins, c) colour intensity, d) antioxidant activity, e) total polyphenol index and f) Folin-Ciocalteu index. It is observed that the most effective hydroalcoholic extraction solutions are those of 50%, a result also supported by the specialized literature which mentions that at moderate alcohol concentrations (50%), there is a favourable balance between the solubility of phenolic compounds and the properties of the solvent (Liu et al., 2020). In the case of total phenolic compounds, the average obtained for the 50% hydroalcoholic solution was 24.1 GAE g/L for the Feteasc Neagră variety and 26.8 GAE g/L for Cabernet Sauvignon. A significant increase in values for both varieties compared to the 25% hydroalcoholic solution is noted, by 84.4% for Feteasc Neagră and by 35.2% for Cabernet Sauvignon. These results are consistent with the literature, which suggests a higher efficiency in the extraction of phenolic compounds from grape pomace at moderate alcohol concentrations (Sánchez & Rodríguez, 2020). Regarding the extraction of anthocyanins from the pomace, the highest values were obtained at a concentration of 50%, with an average value of 675 mg/100 g pomace for Feteasc Neagră and 794 mg/100 g pomace for Cabernet Sauvignon. The higher anthocyanin content obtained in the extract obtained from the 50% alcohol solution for the Cabernet Sauvignon variety correlates with a higher colour intensity of the phenolic concentrate (28.5 AU), this value being 8.7% higher compared to that obtained from the Feteasc Neagră variety. The antioxidant activity is slightly higher at 50% alcohol (91.83%) compared to the other solvent concentrations, and in the case of the Cabernet Sauvignon variety, the maximum value of 91.98% is also obtained at 50% alcohol.

The increase in the values obtained for the two analysed phenolic indices (total polyphenol index and Folin-Ciocalteu index) at a concentration of 50% alcohol suggests an efficient and complete extraction of a wide spectrum of phenolic compounds, highlighting the essential role of moderate alcohol concentrations in optimizing the polyphenol extraction process. These results emphasize the importance of the balance between solvent and solubility of phenolic compounds, which allows for their maximum extraction without compromising the integrity of the bioactive structures.









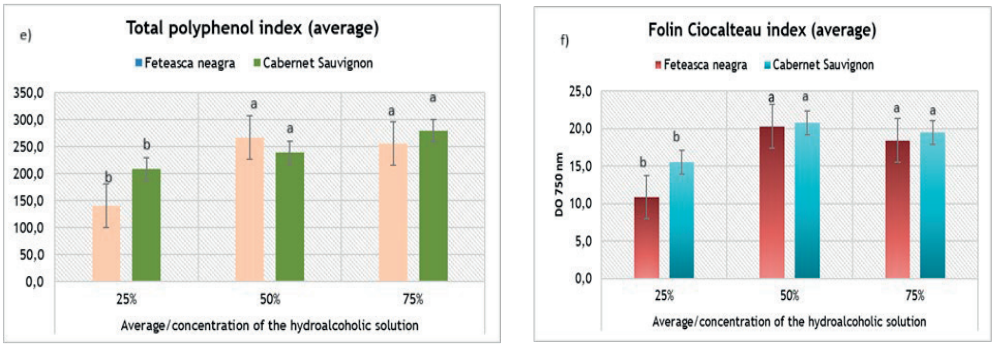


Figure 2. The average values obtained for: a) total phenolic compounds, b) anthocyanins, c) colour intensity, d) antioxidant activity, e) total polyphenol index, f) Folin-Ciocalteu index. Average values ± standard errors (n = 3). The letters show the statistical difference among results for $p<0.05$. For the same compound, a common letter for 2 or more variants shows no significant difference among them

CONCLUSIONS

The grape pomace represents a valuable source of bioactive compounds, having significant potential for use in various industries, especially in the food and cosmetic industries, due to its antioxidant and nutritional properties.

The classical solid-solvent extraction method, using hydroalcoholic solutions of different concentrations, has proven to be an effective technique for extracting phenolic compounds from grape pomace.

The results obtained showed that hydroalcoholic solutions with a concentration of 50% were the most effective in extracting bioactive compounds from both the Fetească Neagră and Cabernet Sauvignon varieties, indicating a favourable balance between the solubility of phenolic compounds and the properties of the solvent at this concentration.

Higher alcohol concentrations (75%) resulted in a slight decrease in extraction efficiency, possibly due to the reduced solubility of phenolic compounds in solutions with higher alcohol content, suggesting that a medium alcohol concentration is optimal for efficient extraction. Also, the differences between the studied varieties (Fetească Neagră and Cabernet Sauvignon) revealed that the thicker grape skin of the Cabernet Sauvignon variety allows for a more efficient extraction of phenolic compounds, which is reflected in the higher values of antioxidant activity and colour intensity.

ACKNOWLEDGEMENTS

This work was supported by the Ministry of Agriculture and Rural Development, Sectorial Plan, under ADER 6.3.21 Project.

REFERENCES

Brand-Williams, W., Cuvelier, M.E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *Food Science and Technology*, 28, 25-30.

Caldas, E. D., Ferreira, S. R., & Silva, T. M. (2018). Phenolic compounds and antioxidant activity of grape pomace from different grape varieties. *Food Science & Technology*, *38*(2), 300-306.

Castellanos-Gallo, L., Ballinas-Casarrubias, L., Espinoza-Hicks, J.C., Hernández-Ochoa, L.R., Munoz-Castellanos, L.N., Zermeno-Ortega, M.R., Borrego-Loya, A., Salas, E. (2022). Grape pomace valorization by extraction of phenolic polymeric pigments: A Review. *Processes*. 10, 469.

Fontana, A.R., Antoniolli, A., Bottini, R. (2013). Grape pomace as a sustainable source of bioactive compounds: Extraction, characterization, and biotechnological applications of phenolics. *Journal of Agricultural and Food Chemistry, 61*(38), 8987-9003.

González de Peredo, A., González-SanJosé, M. L., López de Cerain, A. (2010). Influence of ethanol concentration on the extraction of color and phenolic compounds from the skin and seeds of Tempranillo grapes at different stages of ripening. *Food Research International*, *43*(1), 217-224.

Ilyas, T., Chowdhary, P., Chaurasia, D., Gnansounou, E., Pandey, A., Chaturvedi, P. (2021). Sustainable green processing of grape pomace for the production of value-added products: An overview. *Environmental Technology & Innovation*, 23.

Jara-Palacios, M.J, Gonçalves, S., Heredia, FJ, Hernanz, D., Romano, A. (2020). Extraction of antioxidants from winemaking by products: Effect of the solvent on phenolic composition, antioxidant and anti-cholinesterase activities and electrochemical behavior. *Antioxidants*, 9, 675.

Kumar, S., & Goel, R. K. (2019). Extraction of phenolic compounds from grape pomace using green solvents: Optimization and kinetic modeling. *Journal of Food Science*, *84*(9), 2458-2466.

Liu, Z., Li, J., Zhang, W., Wang, J., & Wang, J. (2020). New insights of the application of water or ethanol-water plant extraction solvents. *Molecules*, *25*(16), 3793.

Nakamura, S., & Okada, S. (2020). Antioxidant and anti-inflammatory properties of anthocyanins and their potential in human health. *Antioxidants*, *9*(5), 452.

Nurgel, C., & Reicher, S. (2020). Extraction Methods of Polyphenols from Grape Pomace. *Food Chemistry*, 312, 126058.

Ogunlade, A. O., Oladele, S. O., Ogunjobi, O. A., & Oyedepo, O. O. (2020). Influence of temperature, solvent and pH on the selective extraction of phenolic compounds from tiger nut (*Cyperus esculentus* L.) by-products. *Antioxidants*, *9*(12), 1317.

Ribereau-Gayon, P., Glories, Y., Maujean, A., & Dubourdieu, D. (2017). Traité d'oenologie. Chimie du vin. *Stabilisation et traitements*, Tome 2 (7e édition). Paris: Dunod.

Ribéreau-Gayon, P. (2000). *Chimie du Vin - Stabilisation et Traitements*. Edition Dunod, Paris.

Rodrigues, R.P., Gando-Ferreira, L.M., Quina, M.J. (2022). Increasing value of winery residues through integrated biorefinery processes: A review. *Molecules*, *27*, 4709.

Sánchez-Alonso, I., & Rodríguez-Delgado, M. Á. (2020). Extraction of phenolic compounds from grape pomace: Optimization of the process by response surface methodology. *Antioxidants*, *9*(10), 1021

Schieber, A. (2017). Side streams of plant food processing as a source of valuable compounds: selected examples. *Annual Review of Food Science and Technology*, 8, 97-112.

Spigno, G., & De Faveri, D. M. (2007). Cell wall polysaccharides and phenolic compounds of grape pomace. *Food Research International*, *40*(3), 462-468.

Taladrid, D., Rebollo-Hernanz, M., Martin Cabrejas, M.A., Moreno-Arribas, M. V., Bartolomè, B., (2023). *Antioxidants* 12, 979.

Vukuši´c, L. J., Millenautzki, T., Reichert, L., Saaid, A.M., Muller, L., Clavijo, L., Hof, J., Mosche, M., Barbe, S. (2023). Conversion of problematic winery waste into valuable substrate for baker's yeast production and solid biofuel: A circular economy approach. *Food Technology. Biotechnology*, 61, 430-438.

Zhang, Y., Li, Y., & Li, X. (2019). Extraction of phenolic compounds from grape pomace by pressurized liquid extraction. *Food Science & Nutrition*, *7*(3), 1082-1090.

Zhang, Y., Li, X., & Wang, Z. (2020). Effect of extraction methods on bioactive compounds from medicinal plants. *Journal of Ethnopharmacology*, 254, 112718.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

INVESTIGATING THE IMPACT OF CLUSTER LOAD ON THE CHARACTERISTICS OF TWO WHITE WINE GRAPE VARIETIES ('KIRÁLYLEÁNYKA'/'FETEASCĂ REGALĂ' AND 'KERNER')

Géza BALLA, Zsolt SZEKELY-VARGA, Attila BANDI, Csaba MOLDOVAN, Endre KENTELKY

Department of Horticulture, Faculty of Technical and Human Sciences,
Sapientia Hungarian University of Transylvania, 2 Calea Sighisoarei, Târgu-Mureș, Romania

Corresponding author emails: zsolt.szekely-varga@ms.sapientia.ro, kentelky@ms.sapientia.ro

Abstract

Globally, grapes are cultivated across 8.5 million hectares of land, with 60% of these grapes originating from Europe, where France, Italy, and Spain are the leading producers of both grapes and wine. The Carpathian Basin has a rich viticulture history that spans 2,000 years. This experiment aims to explore the effects of different cluster loadings (30% and 50%) on both the quantity and quality of yields from two grape varieties, 'Kerner' and 'Fetească regală' ('Királyleánka'). The study was conducted in Mica village, Mureș County. Results indicated that the control group yielded the highest quantities for both grape varieties. The 30% cluster load resulted in the highest sugar content and the lowest acid content, while the values at the 50% load were comparable. For producing high-quality wine, the 50% load was found to be the most effective, whereas the 30% load was more favorable in terms of the quantity of grape berries produced for both varieties.

Key words*: cluster load, grape berries, viticulture, wine.*

INTRODUCTION

Grapes worldwide occupies the fifth place in fruit production sector between 2000 to 2022 (Fao, 2023). In an environment of increasing competition in global markets, it is crucial to strive for elevated quality standards in the vineyard (Matese & Filippo Di Gennaro, 2015). Viticulture and winemaking hold significant socioeconomic importance in numerous European regions, the climate plays a crucial role in the terroir of a specific wine region, as it effectively regulates the microclimate of the vine canopy, the growth of the vines, their physiology, the yield, and the composition of the berries. These factors collectively shape the characteristics and distinctiveness of the wine produced (Fraga, 2019; Santos et al., 2020). Singh et al., 2017 mentioned that grape is the third cultivated fruit all around the world after citrus and bananas. According to the data gathered by International Organization of Vine and Wine from twenty-nine countries, which make up 94% of the global production in 2022, the projected world wine production (excluding juices and musts) for 2023 falls within the range of 241.7 mhl to 246.6 mhl. The midpoint estimates stand at 244.1 mhl, this indicates a decline of 7% compared to the already below-average volume of 2022 (OIV, 2023).

In Romania the viticulture dates back for many centuries, with a long viticultural tradition (Patriche & Irimia, 2022; Chiurciu et al., 2020). According to report of the International Organization of Vine and Wine, Romania stands at the 10 places at the vineyard surface areas of major vine-growing countries in 2022 (OIV, 2022). Mălăescu et al., (2022) reported that Romania's climate and topography support the quality potential of grapes and wines, and production remains stable year after year.

According to Reeve et al., (2018) high quality growers of 'Pinot noir' reduce yield by cluster thinning to increase fruit quality, nevertheless, there are no clearly defined yield goals to reach optimal fruit composition. Thinning clusters throughout the growing season is a commonly employed technique in vineyard management with the goal of aligning crop yield with the vine's ability to properly mature the fruit (King et al., 2015). A study demonstrated that, crop thinning enhanced ripeness, reduced acidity, and heightened levels of anthocyanins and phenolics in Cabernet Sauvignon grapes grown in the

warm Riverland and Sunraysia areas of Australia (Petrie & Clingeleffer, 2006). In another research is mentioned that cluster thinning increase Brix and im-prove wine aroma, colour, and flavour (Reynolds et al., 1996).

Balanced pruning resulted in an optimized yield by limiting the number of nodes to 30 per 454 g of dormant pruning's, additionally, cluster thinning was implemented to limit the number of clusters to two per shoots, this approach not only maintained the fruit composition but also improved the cold hardiness of the primary buds, it achieved an ideal fruit weight-to-pruning weight ratio of 10.0 kg·kg^{-1} (Wilson et al., 2014). In a previous study it is determined that cluster thinning has not been consistently enhanced or refined the fruit chemistry (Smith & Centinari, 2019). However, in another study is also reported that cluster tip removal of inflorescences decreased cluster compactness, lowered titratable acidity, and increased total soluble solids in grape berries, however increased the levels of overall phenolics, anthocyanins, tannins, and flavonoids in the pedicel end of berry skins were found to be higher, along with increased concentrations of 12 phenolic compounds, additionally, the concentrations of total flavanols and anthocyanins in wines were also elevated (Liu et al., 2021).

Moreover, is also reported that grapevine's bud load is the quantity of buds remaining after trimming (Poni et al., 2016). Furthermore, the grapevine's optimal bud load is determined by the type of grape, the growth environment, and the intended yield (Dobrei et al., 2016).

The present study investigated the effect of cluster load of two white wine varieties 'Kerner' and 'Királyleánnyka'/'Feteasc ă regală' to increase the quality and quantity of grape berry, furthermore to achieve a higher quality wine.

MATERIALS AND METHODS

The experiment was conducted in Mica, Mureș County in a vineyard in 2020.

The average temperature was 10°C (Figure 1) and the highest precipitation was in July approximatively ~135 mm and the lowest August when 21 mm (Figure 2).

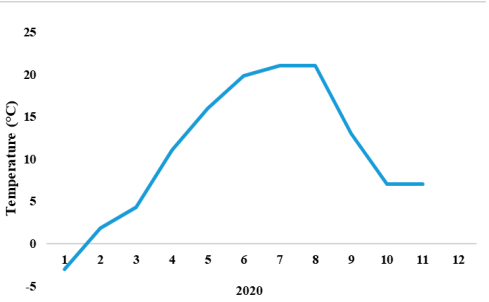


Figure 1. Temperature during the experiment

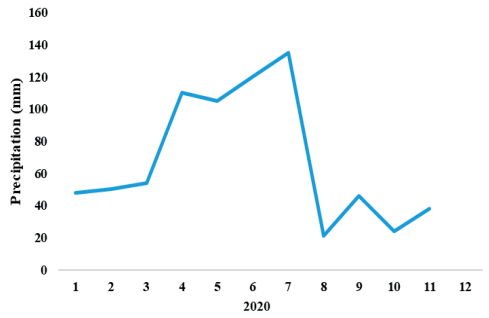


Figure 2. Precipitation during the experiment

The soil type was determined on the experimental site clay-washed, brown forest soil.

US 2. Clay-washed, brown forest soil (Table 1 and Table 2) (soils have moderate limitations that reduce the choice of plants or require moderate conservation practices).

Description:

Ap (0-10 cm) clayey – adobe, dark brown, granular structure, frequent root system, coprolitic, loose;

Ao (10-40 cm) clayey – adobe, dark brown, granular structure, frequent root system, coprolitic, loose;

Bt (40-68 cm) clay-washed, yellow-brown, prismatic structure;

Ck (68 cm) clay-washed, yellow, dense, lime concentration.

US 2. Luvisol

Description:

Ap (0-10 cm) clay-loam texture, dark brown, granular structure, frequent root presence, coprolitic, loose;

Ao (10-40 cm) clay-loam texture, dark brown, granular structure, frequent root presence, coprolitic, loose;

Bt (40-68 cm) leaching clay accumulation (illuviation), yellow-brown, prismatic structure; Ck (68 cm) leaching clay accumulation (illuviation), yellow, compact, lime con-centration.

Table 1. Chemical analysis of US 2. soil

Horizont	Ap	Ao	Bt	Ck
Depth (cm)	0-10	10-40	40-68	68
pH	5.34	5.30	5.90	8.14
Humus	6.50	4.08	1.03	0.59
N %	0,311	0.183	0.076	-
P ppm	7	3	2	-
K ppm	18.5	8.1	10.2	-

Table 2. Physical analysis of US 2. soil (grain size analysis)

Horizont	Ap	Ao	Bt	Ck
Depth (cm)	0-10	10-40	40-68	68
Coarse sand 2-0.2 mm	1.9	2.7	0.7	0.2
Fine sand 0.2-0.02 mm	32.7	33.3	18	16
Powder				
0.02-0.002 mm	28	31	29.4	40.1
Clay				

As plant material we have selected the 'Királyleánka'/'Fetească regală' variety which is native to Transylvania, Romania it is said that is a natural cross-breeding between 'Frâncuşe' and 'Fetească regală'. The 'Kerner' is native to Germany from Weinsberg and it is a cross-breeding between 'Schiava Grossa' and 'White Riesling'. The cultivation area for the 'Királyleánka'/'Fetească regală' was 3 m^2 (2.5 × 1.2 m) pruned with short-pin pruning and for the 'Kerner' with snip pruning 2.25 m^2 (2.5 × 0.9 m).

In the study two types of treatments and one untreated (control) was set up. The treatments were carried out in 4 repetitions on 12 plants per repetition, so the effects of each treatment were examined on a total of 48 plants, per variety. The cluster thinning was made after the flowering stage, when the 50 and 30% cluster loads were set up, moreover in the case of control clusters remained in the same way. At the 50% cluster thinning in every case the shoot first cluster was kept, and the rest of the clusters were removed. In the case of the 30% cluster load, on the grape's productive bases from the two kept shoots from one of the shoots every cluster was removed, and from the other shoot the second and the third clusters were removed.

The parameters calculated: grape berry weight (g), cluster weight (g), harvest quantity (t/ha), sugar con-tent (g/L), acid level (g/L), dry matter content (%), and the number of seeds were evaluated.

The following methods were applied to determine the acidity, sugar, and dry matter content:

Determination of dry matter content:

Measurement of volume using a pycnometer for wine and water, along with distillation and subsequent calculations.

Removal of carbon dioxide from the wine through stirring.

Thorough rinsing of the pycnometer with the wine to be analyzed, followed by insertion of the wine and thermometer.

Adjustment of the wine to 20°C.

Filling the pycnometer to the mark, drying it, and weighing it to four decimal places.

Calculation:

Density = (mass of wine - mass of empty pycnometer) / mass of water.

Extract = 1 + wine density - distillate density.

Result interpretation using a reference table.

Calculation of Non-reducing Dry Extract:

Non-reducing dry extract = Total dry extract – residual sugar.

Determination of sugar:

Utilization of the Rebelein method.

Preparation:

In a flask, combine 10 mL of copper sulphate (CuSO4), 5 mL of Seignette salt, a few grains of pumice stone, and 2 ml of the wine to be analysed.

Boil the solution for 2 minutes and allow it to cool.

Titration:

Add 10 mL of potassium iodide solution, 10 mL of sulfuric acid, and 10 mL of starch to the cooled solution.

Titrate the dark-colored solution with sodium thiosulfate until a yellowish-white color is achieved. Read the sugar content directly in g/L from the biuret. Dilution, if necessary, for sugar content above 28 g/L, dilute the wine with distilled water according to the specified factors.

Determination of total acidity:

Employment of the Schliessmann reagent/method.

Preparation:

In a flask, prepare 25 ml of the wine to be analysed, ensuring removal of carbon dioxide by heating and subsequent cooling.

Titration:
Titrate with BLAULAUGE 1/3 N solution until a dark green color is reached (indicating neutral pH, confirmed with pH paper).
The amount of sodium hydroxide solution used corresponds to the acidity of the wine.
The significance of the differences between the treatments was tested by applying one-way ANOVA, at a confidence level of 95%. When the ANOVA null hypothesis was rejected, Tukey's post hoc test was carried out to establish the statistically significant differences at $p < 0.05$ (Hammer et al., 2001).

RESULTS AND DISCUSSIONS

Comparing the cluster loads, could be clearly observed that significant differences were determined between the treatments at 'Kerner' variety (Figure 3). The greatest grape berry weight was recorded at the 30% cluster load at 'Kerner', with approximatively ~ 350 g. In the case of 'Királyleánnyka'/'Fetească regală' no significant differences were observed. Similar to our findings Fawzi et al. (2015) reported that increasing bud load on the vine significantly decreased berry weight and firmness.

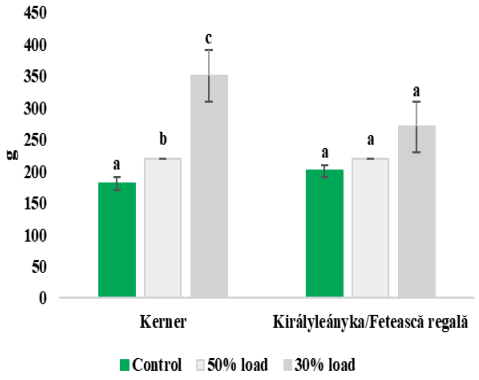


Figure 3. Grape berry weight under the effect of cluster load (50 and 30%). Bars represent the means ± SE (n = 48). Different letters above the bars indicate significant differences between cluster load ($p < 0.05$)

Regarding the cluster weight, again significant differences were recorded between the treatments at both white wine varieties (Figure 4). The cluster weight increases at 50% compared to control and it is followed by the 30% cluster load at 'Kerner'. Similar increase is observed also at the 'Királyleánnyka'/'Fetească regală'. The greatest bunch weight was determined at the lowest crop load level (Somkuwar et al., 2014), as was reported in the present experiment. However, in a study is reported that cluster thinning did not affect the fruit composition and berry size at 'Cabernet Sauvignon' (Calderon-Orellana et al., 2014).

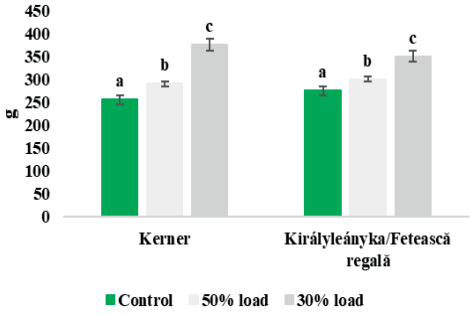


Figure 4. Cluster weight under the effect of cluster load (50 and 30%). Bars represent the means ± SE (n = 48). Different letters above the bars indicate significant differences between cluster load ($p < 0.05$)

As it was expected the highest quantity of grapes was recorded at the control plants, which is significantly different compared to the other treatments at both white wine varieties (Figure 5). No significant changes were observed between the two cluster load treatments (50% and 30%) at 'Kerner', on the other significant increase was reported at 50% cluster load compared to the 30% at 'Királyleánnyka'/'Fetească regală'.
In a study it was determined that the highest cluster weight was recorded by 8 buds/cane treatment (Khamis et al., 2017).

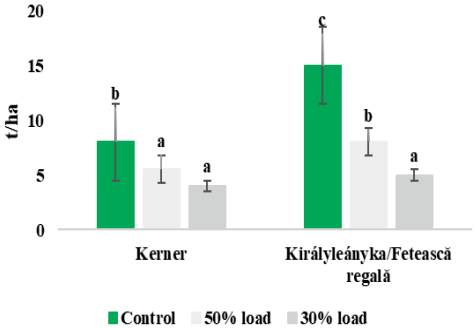


Figure 5. Harvest quantity under the effect of cluster load (50 and 30%). Bars represent the means ± SE (n = 48). Different letters above the bars indicate significant differences between cluster load ($p < 0.05$)

When comparing the sugar content of the two white wine varieties, no significant differences were determined (Figure 6). The sugar content at 'Kerner' was approximatively ~ 240 g/L and at 'Királyleánka'/'Fetească regală' ~ 220 g/L. Regarding the sugar content in some authors determined that cluster thinning increased the sugar content (Tardaguila et al., 2008; Wang et al., 2022). However, in our case the sugar content was higher than at control, yet no significant differences were reported.

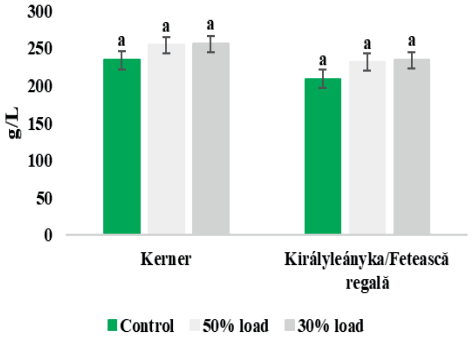


Figure 6. Sugar content under the effect of cluster load (50 and 30%). Bars represent the means ± SE (n = 48). Different letters above the bars indicate significant differences between cluster load ($p < 0.05$)

Here again, no significant changes were observed at the acid level (Figure 7). The cluster load did not influence the acid levels of the white wine varieties.

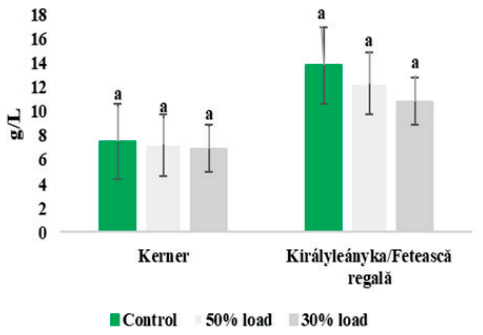


Figure 7. Acid level under the effect of cluster load (50 and 30%). Bars represent the means ± SE (n = 48). Different letters above the bars indicate significant differences between cluster load ($p < 0.05$)

In the case of 'Kerner' the acid level was approximatively ~ 7 g/L, and at 'Királyleánka'/'Fetească regală' ~ 11.5 g/L. Wang et al. (2022), reported that acid level was decreased by the effect of cluster thinning. Similar data could be observed also in our case when the acid level content was reduced by cluster thinning, however no significant differences were observed.

Regarding the dry matter content (Figure 8), could be determined that at 'Kerner' variety significant increase was observed at the two cluster loads (50 and 30%) com-pared to control. On the other no significant increase was recorded at the 'Királyleánka'/'Fetească regală' variety between the treatments.

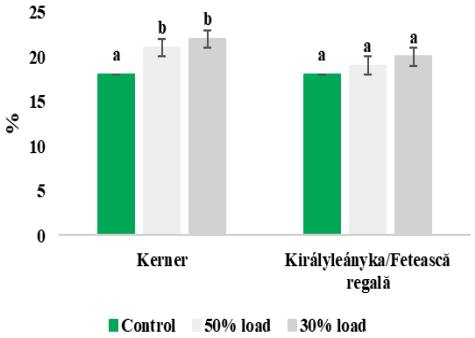


Figure 8. Dry matter content under the effect of cluster load (50 and 30%). Bars represent the means ± SE (n = 48). Different letters above the bars indicate significant differences between cluster load ($p < 0.05$)

At both cluster load treatments, the number of seeds increased at 1, 2, 3, and 4 seed/berry compared to control, and a decrease could be observed at the 0 seed/berry (Figure 9). Could be determined grape berry fertility increased, and at 30% cluster load was the highest.

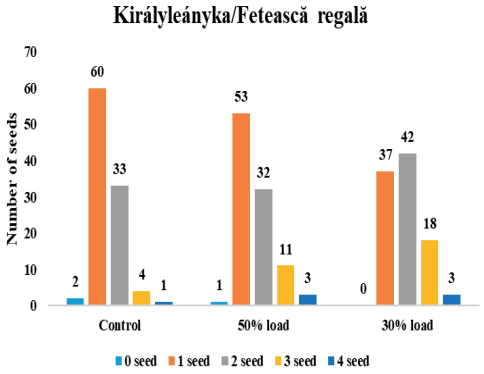


Figure 9. Number of seeds at 'Királyleánka'/'Fetească regală' under the effect of cluster load (50 and 30%)

Here once again, the number of seeds increased at 1, 2, 3, and 4 seed/berry, and decrease was observed at 0 seed/berry (Figure 10). From the results could be deter-mined that the grape berry fertility increased by the treatments, the highest seed number was reported at the 30% cluster load.

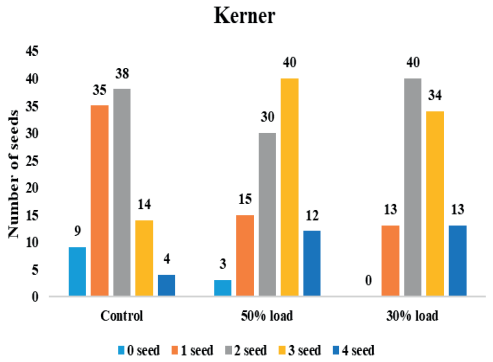


Figure 10. Number of seeds at 'Kerner' under the effect of cluster load (50 and 30%).

In some previous studies was reported that cluster thinning and its timing have little or no effect on shoot growth, leaf area, cutting weight, berry number, berry weight, and fruit composition (soluble solids, titratable acidity, pH, color) in the current and subsequent seasons. Differences in vegetative growth, yield development, and fruit composition within varieties are primarily due to season rather than yield or crop size (Ough & Nagaoka, 1984; Keller et al., 2005). However, other authors determined that grape composition more affected by cluster thinning severity than timing (VanderWeide et al., 2024). Increasing bud load up to the vine's capacity results in a greater distribution of carbohydrates in the fruit at the expense of vegetative tissue, which accounts for the majority of dry matter in the fruit (Miller & Howell, 1998). Furthermore, cluster thinning at an intensity of 35% produces wines with more terpenes, esters, higher alcohols, other alcohols, volatile phenolic compounds, lactones and other compounds compared to other treatments (Mucalo et al., 2022). Cluster thinning can also have influence on the metabolomic profile of the wine, to obtain the desired grape composition and wine quality (Škrab et al., 2021), and could also have a positive effect on the aroma and colour of the wine (Condurso et al., 2016).

CONCLUSIONS

The present experiment provides data on the comparison of cluster load effect on two white wine varieties ('Királyleánka'/'Fetească regală' and 'Kerner'). According to the obtained results, it can be concluded that the grape weight and cluster weight was significantly increased at 30% cluster load, on the other hand which was expected the highest quantity of grapes was observed at the control treatment. The sugar content and acid level were not affected by the cluster load treatments. Furthermore, the highest number of seeds at 'Királyleánka'/'Fetească regală' and 'Kerner' was determined at the 30% cluster load.

REFERENCES

Calderon-Orellana, A., Mercenaro, L., Shackel, K.A., Willits, N., & Matthews, M.A. (2014). Responses of fruit uniformity to deficit irrigation and cluster thinning in commercial winegrape production. *American Journal of Enology and Viticulture*, *65*(3), 354–362.

Condurso, C., Cincotta, F., Tripodi, G., Sparacio, A., Giglio, D.M.L., Sparla, S., & Verzera, A. (2016). Effects of cluster thinning on wine quality of Syrah cultivar (*Vitis vinifera* L.). *European Food Research and Technology*, *242*, 1719–1726.

Chiurciu, I.A., Zaharia, I., Soare, E. (2020). Production of wine grapes and cultural traditions related to vine in Romania. *Scientific Papers Series Management, Economic Engineering in Agriculture and Rural Development*, 20, 133–143.

Dobrei, A., Posta, G., Danci, M., Nistor, E., Camen, D., Mălăescu, M., Sala, F. (2016). Research concerning the correlation between crop load, leaf area and grape yield in few grapevine varieties. *Agriculture and Agricultural Science Procedia*, *10*, 222–232.

FAO. (2023). The State of Food and Agriculture 2023 – Revealing the true cost of food to transform agrifood systems. Rome. https://doi.org/10.4060/cc7724enFawzi, M.I.F., Laila, F.H., Shahin, M.F.M., Merwad, M.A., Genaidy, E.A.E. (2015). Effect of vine bud load on bud behavior, yield, fruit quality and wood ripening of superior grape cultivar. *Journal of Agricultural Technology*, *11*(5), 1275–1284.

Fraga, H. (2019). Viticulture and winemaking under climate change. *Agronomy*, *9*(12), 783.

Hammer, Ø., Harper, D.A., Ryan, P.D. (2001). Past: Paleontological statistics software package for education and data analysis. *Palaeont. Electr*., 4, 1–9.

International Organization of Vine and Wine (OIV) (2022). State of the World Vine and Wine Sector in 2022. https://www.oiv.int/sites/default/files/documents/OIV_State_of_the_world_Vine_and_Wine_sector_in_2022_2.pdf.

International Organization of Vine and Wine (OIV) (2023). https://www.oiv.int/press/2023-world-wine-production-expected-be-smallest-last-60-years.

Keller, M., Mills, L.J., Wample, R.L. and Spayd, S.E., 2005. Cluster thinning effects on three deficit-irrigated Vitis vinifera cultivars. *American Journal of Enology and Viticulture*, 56(2), 91–103.

Khamis, M.A., Atawia, A.A.R., El-Badawy, H.E.M., Abd El-Samea, A.A.M. (2017). Effect of buds load on growth, yield and fruit quality of superior grapevines. *Middle East Journal of Agriculture Research*, *6*(1), 152–160.

King, P.D., Smart, R.E., & McClellan, D.J. (2015). Timing of crop removal has limited effect on Merlot grape and wine composition. *Agricultural Sciences*, *6*(04), 456.

Liu, M., Song, Y., Liu, H., Tang, M., Yao, Y., Zhai, H., Gao, Z., Du, Y. (2021). Effects of flower cluster tip removal on phenolics and antioxidant activity of grape berries and wines. *American Journal of Enology and Viticulture*, *72*(4), 298–306.

Matese, A., & Filippo Di Gennaro, S. (2015). Technology in precision viticulture: A state of the art review. *International journal of wine research*, 69–81.

Mălăescu, M., Dobrei, A., Nistor, E., Velicevici, G., Dobrei, A. (2022). An overview on the evolution of viticulture in Romania and worldwide in the last two decades. *Journal of Horticulture, Forestry and Biotechnology*, 26(3), 46–55.

Miller, D.P., & Howell, G.S. (1998). Influence of vine capacity and crop load on canopy development, morphology, and dry matter partitioning in Concord grapevines. *American Journal of Enology and Viticulture*, *49*(2), 183–190.

Mucalo, A., Lukšić, K., Budić-Leto, I., & Zdunić, G. (2022). Cluster thinning improves aroma complexity of white Maraština (*Vitis vinifera* L.) wines compared to defoliation under Mediterranean climate. *Applied Sciences*, *12*(14), 7327.

Ough, C.S., Nagaoka, R. (1984). Effect of cluster thinning and vineyard yields on grape and wine composition and wine quality of Cabernet Sauvignon. *American journal of enology and viticulture*, *35*(1), 30–34.

Patriche, C.V. & Irimia, L.M. (2022). Mapping the impact of recent climate change on viticultural potential in Romania. *Theor. Appl. Climatol.*, 148(3), 1035–1056.

Petrie, P.R., & Clingeleffer, P.R. (2006). Crop thinning (hand versus mechanical), grape maturity and anthocyanin concentration: outcomes from irrigated Cabernet Sauvignon (*Vitis vinifera* L.) in a warm climate. *Australian Journal of Grape and Wine Research*, *12*(1), 21–29.

Poni, S., Tombesi, S., Palliotti, A., Ughini, V., & Gatti, M. (2016). Mechanical winter pruning of grapevine: Physiological bases and applications. *Scientia Horticulturae*, *204*, 88–98.

Reeve, A.L., Skinkis, P.A., Vance, A.J., McLaughlin, K.R., Tomasino, E., Lee, J., & Tarara, J.M. (2018). Vineyard floor management and cluster thinning inconsistently affect 'Pinot noir'crop load, berry composition, and wine quality. *HortScience*, *53*(3), 318–328.

Reynolds, A.G., Yerle, S., Watson, B., Price, S.F., Wardle, D.A. (1996). Fruit environment and crop level effects on Pinot noir. III. Composition and descriptive analysis of Oregon and British Columbia wines. *American Journal of Enology and Viticulture*, *47*(3), 329–339.

Santos, J.A., Fraga, H., Malheiro, A.C., Moutinho-Pereira, J., Dinis, L.T., Correia, C., Moriondo, M., Leolini, L., Dibari, C., Costafreda-Aumedes, S., Kartschall, T. (2020). A review of the potential climate change impacts and adaptation options for European viticulture. *Applied Sciences*, *10*(9), 3092.

Singh, S., Arora, N.K., Gill, M.I.S., Gill, K.S. (2017). Differential crop load and hormonal applications for enhancing fruit quality and yield attributes of grapes var. Flame Seedless. *Journal of Environmental Biology*, *38*(5), 713.

Škrab, D., Sivilotti, P., Comuzzo, P., Voce, S., Degano, F., Carlin, S., Arapitsas, P., Masuero, D., & Vrhovšek, U. (2021). Cluster thinning and vineyard site modulate the metabolomic profile of Ribolla Gialla base and sparkling wines. *Metabolites*, *11*(5), 331.

Smith, M.S., & Centinari, M. (2019). Impacts of early leaf removal and cluster thinning on grüner veltliner production, fruit composition, and vine health. *American Journal of Enology and Viticulture*, *70*(3), 308–317.

Somkuwar, R.G., Samarth, R.R., Itroutwar, P., & Navale, S. (2014). Effect of cluster thinning on bunch yield, berry quality and biochemical changes in local clone of table grape cv. Jumbo Seedless (Nana Purple). *Indian Journal of Horticulture*, *71*(2), 184–189.

Tardaguila, J., Petrie, P.R., Poni, S., Diago, M.P., & De Toda, F.M. (2008). Effects of mechanical thinning on yield and fruit composition of Tempranillo and Grenache grapes trained to a vertical shoot-positioned canopy. *American Journal of Enology and Viticulture*, *59*(4), 412–417.

VanderWeide, J., Nasrollahiazar, E., Schultze, S., Sabbatini, P., & Castellarin, S.D. (2024). Impact of Cluster Thinning on Wine Grape Yield and Fruit Composition: A Review and Meta-Analysis. *Australian Journal of Grape and Wine Research*, 2024(1), 2504396.

Wang, W., Liang, Y., Quan, G., Wang, X., & Xi, Z. (2022). Thinning of cluster improves berry composition and sugar accumulation in Syrah grapes. Scientia *Horticulturae*, *297*, 110966.

Wilson, P.E., Archbold, D.D., Masabni, J.G., & Kurtural, S.K. (2014). Cropload management of 'Vidal blanc'improves primary bud cold hardiness and maintains berry composition in the lower midwestern United States. *HortScience*, *49*(7), 874–880.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

ELICITORS IN VITICULTURE AND THEIR EFFECT IN WINEMAKING: A SUSTAINABLE APPROACH TO CLIMATE RESILIENCE

Traian Ștefan COSMA[1, 2], Aurora RANCA[1], Anamaria TĂNASE[1], Iulia Cristina CIOBANU[1], Sergiu Ayar ENE[1], Arina Oana ANTOCE[2]

[1]Research Station for Viticulture and Oenology Murfatlar,
2 Calea Bucuresti, Murfatlar, Constanţa, Romania
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest,
59 Mărăşti Blvd, District 1, Bucharest, Romania

Corresponding author email: arina.antoce@usamv.ro

Abstract

Climate change exerts an increasingly pronounced impact on the viticultural sector, requiring sustainable solutions for adaptation and maintaining production quality. The use of elicitors, natural or synthetic compounds that stimulate the grapevine`s defense mechanisms, represents an innovative approach to reducing pesticide dependency and optimizing the biochemical and organoleptic properties of grapes and wines. Elicitors such as chitosan and salicylic acid significantly enhance the grapevine`s resistance to pathogens, including to Plasmopara viticola and Botrytis cinerea, while promoting the accumulation of secondary metabolites with enological value, such as polyphenols. Their use in vine growin can influence fermentation during winemaking or can lead to more stable wines or with enhanced aromatic profile. This review synthesizes recent advancements in the use of elicitors, highlighting their mechanisms of action, practical applications, and impact on wine quality, while exploring opportunities for their integration into climate-smart agricultural systems to enhance the resilience and sustainability of viticulture and winemaking.

Key words*: chitosan, climate-smart viticulture, polyphenols, sustainability, grape oenology.*

INTRODUCTION

Modern viticulture faces significant challenges, primarily driven by climate change, which affects both crop productivity and final product quality. These challenges are amplified by abiotic and biotic stress, as well as the strict requirements for reducing chemical use in agriculture (Delaunois et al., 2014). In this context, adopting sustainable practices becomes imperative for ensuring the long-term viability of the viticulture sector. The use of elicitors, compounds that stimulate the vine's defence mechanisms, offers an innovative and effective solution (Pieterse et al., 2012). These elicitors, whether natural or synthetic, enhance plant resistance against pathogens and stress without negatively impacting the environment.

Elicitors like chitosan and salicylic acid have been extensively studied for their ability to induce physiological and biochemical responses in grapevines (Aziz et al., 2006; Iriti & Faoro, 2009). These responses mimic stress effects, but without compromising plant health. For example, chitosan has shown significant efficiency in enhancing grapevine resistance to infections caused by pathogens such as *Plasmopara viticola* and *Botrytis cinerea*, which can lead to substantial crop losses (Romanazzi & Mlikota Gabler, 2021; Verhagen et al., 2010). Moreover, the application of these elicitors stimulates the biosynthesis of valuable secondary metabolites, including polyphenols, essential for the organoleptic and nutritional quality of grapes and wine (Chitarrini et al., 2017; Garde-Cerdán & Pérez-Álvarez, 2022). Integrating elicitors into viticultural practices not only reduces pesticide dependency but also positively influences winemaking processes. Elicitor treatments can improve must fermentation, wine stability, and aromatic profile - critical aspects for consumer satisfaction and the commercial success of the final product (Gómez-Plaza & Martínez-Cutillas, 2022). This approach contributes to developing climate-smart viticultural systems capable of effectively addressing climate challenges while maintaining market-quality standards (Buonassisi et al., 2023).

The objective of this paper is to synthesize recent advances in the use of elicitors in viticulture and winemaking, analysing their

mechanisms of action, practical applicability, and impact on wine quality. Furthermore, the paper explores opportunities for integrating these compounds into sustainable agricultural systems, thereby contributing to the long-term resilience and viability of the viticulture sector. This approach becomes increasingly relevant given the complexity of current challenges and the necessity of adopting innovative solutions for sustainable viticulture.

ELICITORS: CLASSIFICATION, MECHANISMS OF ACTION, AND IMPACT ON PLANTS

Elicitors are substances or stimuli that induce physiological and biochemical responses in plants, which are fundamental in enhancing plant resilience to biotic and abiotic stressors. These plant responses can be classified into two main categories: biotic and abiotic elicitors. According to Bhatt & Mathpal (2022), biotic elicitors originate from living organisms and include compounds such as chitosan, which is derived from crustacean shells, polysaccharides, microorganisms, microbial proteins and extracts, while abiotic elicitors include molecules with hormonal effect (salicylic acid methyl jasmonate), chemical agents (heavy metals, mineral salts, gaseous toxins) and physical agents (UV radiation, osmotic stress, salinity, drought). Understanding the classification and functionality of these elicitors is crucial for leveraging them in agricultural practices to improve plant health and productivity (Kantayos et al., 2021).

Biotic elicitors, particularly chitosan, have attracted considerable attention for their role in stimulating the production of secondary metabolites, which are critical for plant defence (Kahromi & Khara, 2021). These metabolites include phenolic compounds and alkaloids, which play vital roles in protecting plants against pathogens and pests. Studies have shown that chitosan application can lead to the upregulation of various defence-related genes, ultimately enhancing the plant's intrinsic resistance mechanisms. When plants are exposed to chitin-derived compounds like chitosan, they activate specific signalling pathways that enhance their defence responses. Moreover, the interaction of biotic elicitors with environmental factors, such as nutrient availability and microbial symbiosis, appears to significantly influence the biosynthetic pathways that lead to secondary metabolite accumulation (Özkurt & Bektaş, 2022).

Abiotic elicitors also have profound effects on plant physiology, particularly under stress conditions. Salicylic acid is one of the most well-studied abiotic elicitors, known for its pivotal role in mediating plant defence mechanisms (Suksawat & Panichayupakaranant, 2024). It functions by activating defence pathways and promoting the accumulation of not just protein-based defences, but also secondary metabolite production, vital for stress tolerance. This compound has been documented to enhance tolerance to abiotic stresses such as drought, salinity, and extreme temperatures by regulating osmotic balance and improving nutrient uptake (Abdi et al., 2022). The mechanism by which salicylic acid functions includes the modulation of ion channels, which could be crucial for generating a physiological response that leads to increased stress tolerance (Nofal & Attia, 2023).

The interactions between biotic and abiotic elicitors further complicate our understanding of plant-environment interactions. For instance, exogenous application of salicylic acid specifically has been found to synergistically interact with other signalling molecules and elicitors to trigger more robust defence responses in plants (Deshmukh et al., 2023). A study highlighted that the co-application of salicylic acid with chitosan not only improved disease resistance, but also augmented the levels of bioactive compounds in treated plants, showing the necessity of a multi-faceted approach to elicitor application (Vishala et al., 2023). Additionally, distinct concentrations and application timings of these elicitors have been shown to modulate the efficacy of the stress response, illustrating the importance of precision in biotechnological applications (Miclea et al., 2020).

Recent advancements in elicitor research have elucidated the specific biochemical pathways involved in plant responses to biotic and abiotic stressors. For instance, oxidative stress mitigation through the accumulation of

antioxidants such as flavonoids and phenolics has been a significant focus area (Nazir et al., 2020). Chitosan and salicylic acid have both been recognized as potent initiators of these pathways, providing a dual benefit of stress tolerance and enhanced nutritional value for crops (Kantayos et al., 2021). Furthermore, salicylic acid's ability to influence the phenolic profile of fruits underscores the potential of elicitors in improving crop quality beyond mere stress resistance (Gačnik et al., 2021).

Another vital aspect of elicitors is their impact on the secondary metabolism of plants, which can influence flavour, colour, and the nutritional quality of produce (Alwakil et al., 2022). For example, the application of various biotic and abiotic elicitors has resulted in increased levels of bioactive compounds in plants like garlic and certain medicinal herbs, where both growth parameters and metabolic profiles were significantly enhanced (Andrade et al., 2020). This relationship emphasizes the commercial significance of elicitors, particularly in the production of nutraceuticals and functional foods.

Moreover, recent studies have indicated that the timing and frequency of elicitor application are crucial for maximizing their beneficial effects on plant resilience and secondary metabolite production. The integration of elicitation protocols with traditional agricultural techniques is gaining traction, as farmers assess the benefits of improved crop yields and quality (Ahmad et al., 2019). Understanding these interactions is crucial for the development of sustainable agricultural practices that rely on natural elicitors to boost plant defences and reduce reliance on chemical pesticides (Rusadi et al., 2023).

Emerging research also suggests that elicitors have the potential to enhance the efficacy of other agricultural inputs, like fertilizers and biocontrol agents, leading to synergistic effects that maximize growth and resistance (Gačnik et al., 2021).

APPLICATION OF ELICITORS IN VITICULTURE

The application of elicitors in viticulture has gained notable attention, primarily due to their effects on grapevine growth and their ability to confer resilience against abiotic stresses such as drought and extreme temperatures. Elicitors, such as salicylic acid and chitosan, have been studied for their role in enhancing grapevine physiological responses under adverse conditions, which has critical implications for maintaining grape quality and productivity in the face of environmental challenges. For instance, the use of salicylic acid in grapevines is associated with significant increases in antioxidant enzyme activity, which serves as a protective mechanism against oxidative stress that can arise during unfavourable environmental conditions (Alwakil et al., 2022; Abd-Elall, 2019). This increase in antioxidant capacity not only mitigates potential damage, but also supports overall plant health and growth during periods of stress (Oraei et al., 2019). Moreover, the enhancement in defence mechanisms elicited by these compounds translates not only to improved stress tolerance, but also promotes overall plant growth metrics, including increased biomass and root development. Such growth improvements are essential for ensuring that grapevines can effectively uptake water and nutrients, leading to healthier plants that produce better yields and higher quality grapes. Elicitors such as salicylic acid and chitosan have been shown to facilitate these physiological enhancements, including greater root biomass, which is crucial for absorption efficiency. Research indicates that the use of elicitors can potentiate the development of primary and secondary metabolites within the grapevine, thereby enhancing not just the plant's defensive capabilities, but also the qualitative aspects of the grapes produced (Martín et al., 2023). For example, salicylic acid has been found to promote the biosynthesis of anthocyanins, leading to grapes with more vibrant colours and potentially enhanced market value. Similarly, the increased levels of phenolic compounds due to elicitor treatment can contribute positively to the flavour profiles of wines made from these grapes, demonstrating the utility of elicitor application not only from a plant health perspective, but also from an economic standpoint (Oraei et al., 2019; García-Pastor et al., 2020).

Table 1. Elicitors and their mode of action in enhancing plant defence mechanisms

Elicitor type	Mode of action	Recommended dosage	References
Chitosan	Enhances disease resistance by triggering plant defense pathways and stimulating phenolic compound synthesis.	0.1-1% solution (foliar)	Salifu et al. (2022), Romanazzi et al. (2013)
Methyl Jasmonate (MeJA)	Induces synthesis of secondary metabolites (like phenolics) and boosts resistance to abiotic stress.	100-200 µM (foliar)	Garde-Cerdán et al. (2022); Martínez-Lapuente et al. (2023)
Salicylic acid	Modulates gene expression related to pathogen resistance, enhances systemic acquired resistance (SAR).	0.1-0.5% solution (foliar)	Adrian et al. (2017)

The interplay between elicitors and other environmental stressors is another area where significant findings have emerged. Elicitors such as salicylic acid enhance the grapevine's ability to cope with combined stress conditions, including heat and drought (Jalili et al., 2023; Zhao et al., 2023). Studies have elucidated how salicylic acid interacts with plant regulatory pathways, leading to enhanced expression of genes associated with stress tolerance and antioxidant defence mechanisms, allowing grapevines to manage oxidative challenges during intense sunlight and heat periods (Pálfi et al., 2021). The implications for vineyard management practices are thus profound, as strategic timing and application of elicitors could be pivotal in ensuring grapevine health and productivity in changing climates (Pálfi et al., 2021; Oraei et al., 2019).

The impact of elicitors extends beyond individual plant physiology; they can enhance the overall vineyard ecosystem resilience. For instance, the application of chitosan has been shown to reduce pathogen incidence while bolstering the plant's immune responses, which contributes to less reliance on chemical fungicides (Martín et al., 2023; Wang et al., 2023). This promotes a healthier vineyard environment and also aligns with increasing consumer demand for sustainably produced wines (García-Pastor et al., 2020; Zhang et al., 2019). By adopting these sustainable practices involving the use of elicitors, viticulturists can cultivate grapes that are resilient to stressors while also enhancing flavour profiles, opening avenues for premium wine production (Martín et al., 2023; García-Pastor et al., 2020). A summary of the main elicitors used in viticulture, their mode of action in enhancing plant defence mechanisms and recommended dosage is presented in Table 1.

The application of elicitors in viticulture significantly enhances grape quality, particularly regarding aroma, polyphenol content, and antioxidant levels. Research findings indicate that the use of elicitors such as methyl jasmonate (MeJA) elevates the concentrations of anthocyanins and other phenolic compounds in grapes, thereby improving both the organoleptic qualities of the wine produced and its nutritional profile. For instance, Paladines-Quezada et al. (2022) reported that MeJA application during the veraison stage led to a notable increase in anthocyanin levels in Monastrell grapes, significantly enhancing the sensory characteristics of the resulting wine .This study underscores the critical role of timing in elicitor application to maximize the phenolic profile of grapes (Paladines-Quezada et al., 2022 ; Qu et al., 2022).

Moreover, elicitors such as chitosan have been shown to promote higher production of polyphenols, which boast significant antioxidant properties. These polyphenols serve a dual role: they protect plants against biotic and abiotic stresses while also contributing to enhanced wine quality by improving flavor and health benefits. For example, studies have documented that chitosan-treated grapes exhibit increased polyphenol concentrations compared to untreated controls, highlighting the potential for using elicitors to elevate grape and wine quality (Miliordos et al., 2021; Nardello et al., 2021). In addition to their influences on phenolic accumulation, elicitors also play a crucial role in enhancing the aromatic profile of grapes. The biosynthesis of volatile compounds

responsible for wine aroma can be stimulated through elicitor application, making these compounds pivotal for consumer acceptance and preference (Paladines-Quezada et al., 2019; Garde-Cerdán et al., 2023). Specifically, MeJA application has been correlated with increased terpene concentrations in Muscat Hamburg grapes, resulting in a more intense and complex aromatic profile in the final wine product (Yue et al., 2020). This ability to modulate wine aroma emphasizes the multifaceted benefits of using elicitors in viticultural practices, reinforcing the notion that they serve as valuable tools for flavor enhancement.

Furthermore, certain studies emphasize how elicitor treatment can significantly alter the chemical composition of grape skins, which in turn affects the winemaking process. Research shows that MeJA can enhance the cell wall composition of Monastrell grape skins, potentially contributing to better extraction of phenolic compounds during winemaking (Giménez-Bañón et al., 2023). This interaction improves the organoleptic properties of the wine and underlines the potential of elicitors to influence the structure and quality of the grape itself.

The impact of elicitors is not limited to enhancing aroma and phenolic content; they also substantially influence the antioxidant properties of grapes. Elicitors such as MeJA promote the plant's natural defense mechanisms, leading to an accumulation of secondary metabolites with antioxidant properties (Miliordos et al., 2021; Gutiérrez-Gamboa et al., 2019). The increased antioxidant capacity of treated grapes can enhance the health benefits associated with wine consumption, further justifying the use of elicitors as a strategy to improve grape quality from multiple angles (Nardello et al., 2021; Yang et al., 2019).

Additionally, elicitors may have synergistic effects when applied in combination with other treatments, like pre-fermentation cold maceration, enhancing overall polyphenol extraction from grapes during winemaking (Pastore et al., 2019). Elicitors enhance grape composition directly and optimize the winemaking process itself, ensuring that more bioactive components are captured from the fruit.

As viticulturists seek to improve grape quality sustainably, the evidence supporting the use of elicitors continues to grow. Employing these natural compounds can help reduce reliance on synthetic chemicals while simultaneously enhancing grape and wine quality (Cunha et al., 2021; Shricharan et al., 2024). This aligns with current trends in agriculture towards more sustainable and environmentally friendly practices, making the exploration and application of elicitors in viticulture increasingly relevant.

INFLUENCE OF ELICITORS ON TASTE, AROMA, CLARITY, FERMENTATION AND WINE MATURATION

The sensory profile of wine encompasses essential attributes, including taste, aroma, and clarity, all of which can be significantly influenced by the application of elicitors. Elicitors, such as methyl jasmonate (MeJA) and chitosan, have been shown to enhance various organoleptic qualities of wine, making them critical tools in modern viticulture and winemaking practices (Sherman et al., 2020; Martínez-Lapuente et al., 2023).

Aroma is one of the most vital aspects of the sensory profile that can be profoundly affected by elicitor treatments. Elicitors induce the biosynthesis of aromatic compounds, leading to a richer and more complex aromatic profile in wines. For example, the application of MeJA has been linked to an increase in terpene concentrations in Muscat grapes, which resulted in wines exhibiting more pronounced floral aromas. This enhancement improves the sensory appeal of wines and contributes significantly to consumer satisfaction and market success (Martínez-Lapuente et al., 2023; Bilko et al., 2022). The complexity provided by these aromatic compounds is crucial for achieving a high-quality wine experience, where the integration of various volatile substances interacts with the olfactory system to produce distinct and pleasurable sensory perceptions (Sáenz-Navajas et al., 2021; Armstrong et al., 2020). Aside of the function of elicitor, it was demonstrated in a study on the use of chitosan in winemaking, that the application of 1 g/L of fungoid chitosan for fining an aromatic white wine lead to

significant improvements in wine stability and composition. Specifically, chitosan effectively removed chitinases while causing only a negligible depletion of thaumatins. Moreover, the heat stability of the treated wine samples was significantly enhanced. Additionally, reductions in tartaric and malic acids, potassium, and iron were observed, along with a decrease in free terpenols. However, the glycosylated terpenols and fermentative aroma compounds remained unaffected, preserving the wine's aromatic profile (Colangelo et al., 2023). The effects of elicitors extend beyond aroma to significantly influence the taste of wine. Elicitors can elevate sweetness perception and balance acidity and tannins in wine, aligning with consumer trends favoring sweeter varietals, especially in the context of climate change-induced fluctuations in grape sugar levels (Marín et al., 2020; Kazumyan et al., 2023). This modulation of taste is essential for winemakers aiming to adapt their products to evolving consumer preferences while ensuring consistency across varying environmental conditions. The interaction between taste and aroma is noteworthy, as sweetness can enhance the perception of aromatic compounds, illustrating the interdependent nature of these sensory attributes (González-Lázaro et al., 2020).

Table 2. Elicitors for improving wine quality and grapevine resistance

Elicitor type	Mode of action	Dosage	References
Chitosan	Induces the production of secondary metabolites (e.g., terpenes, resveratrol) and enhances resistance against pathogens; as fining agent improves wine quality characteristics.	0.1-1% (foliar)	Salifu et al., 2022; Bavaresco et al., 2023; Singh et al., 2022
Methyl Jasmonate (MeJA)	Stimulates phenolic and aromatic compound synthesis, enhancing flavor complexity and resistance to stress; effective in improving overall wine composition.	100-200 µM (foliar)	Martínez-Lapuente et al. (2023)
Salicylic acid	Promotes systemic acquired resistance (SAR) and enhances the biosynthesis of phenolic compounds, influencing wine flavor and stability.	0.1-0.5% (foliar)	Delaunois et al., 2013; Cataldo et al., 2022

Also, the characteristics of clarity are not solely dependent on microbiological stability but also on the molecular interactions facilitated by polysaccharides produced in response to elicitors. For example, MeJA treatments have been linked to changes in polysaccharide composition in wines, affecting mouthfeel and the overall sensory experience (Martínez-Lapuente et al., 2023; Bilko et al., 2022). This aspect is particularly important, as polysaccharides contribute to the structure and body of the wine, influencing both taste and texture.

Furthermore, the comprehensive evaluation of the sensory profile, utilizing advanced analytical methods, allows winemakers to predict and tailor wine characteristics based on elicitors' effects on grape composition (Phan et al., 2021; Wang et al., 2021). This precision in winemaking enhances the potential for producing high-quality wines consistently, ensuring that the sensory attributes align with consumer expectations while adapting to environmental changes and market trends. Recent research has delved into the effects of elicitors on fermentation and wine maturation processes, revealing their significant impact on the final quality of wines. Elicitors can influence enzymatic activity during fermentation, thereby enhancing fermentation efficiency and leading to an increased production of aromatic compounds essential for high-quality wine production. For instance, the application of elicitors can optimize the enzymatic reactions that occur during fermentation, resulting in a more effective transformation of grape must into wine while promoting the enhancement of desirable aromatic profiles (Paladines-Quezada et al., 2019).

The main elicitors used for improving wine quality and grapevine resistance are presented in Table 2.

CLIMATE CHANGE MITIGATION AND SUSTAINABLE VITICULTURE ADAPTATION: REDUCING WATER STRESS AND TEMPERATURE RESILIENCE

In the context of climate change, elicitors have emerged as essential tools to mitigate water stress and enhance grapevine resilience to extreme temperatures. The application of elicitors, such as salicylic acid and chitosan,

has shown promising results in improving plant tolerance to drought and high heat conditions (Tscholl & Vigl 2024 ; Alsafadi et al., 2023). Salicylic acid has been documented to increase antioxidant enzyme activity, thereby shielding plants from oxidative stress induced by adverse environmental factors like drought and elevated temperatures, which is crucial for maintaining grapevine health, particularly during periods of water deficiency, increasingly common in many viticultural regions as climate change progresses (Irimia et al., 2024).

Chitosan has demonstrated efficacy in enhancing water and nutrient uptake during drought stress. The ability of chitosan to improve physiological responses relating to hydration and nutrient acquisition is integral to sustaining vine vitality under challenging climatic conditions. Leveraging elicitors like chitosan supports crop health and also enhance fruit quality, a crucial consideration for sustainable wine production (Hall & Blackman, 2019; Lemaître-Guillier et al., 2021).

The integration of elicitors into precision viticulture practices can further optimize vineyard resilience to climate challenges. By applying elicitors alongside established water management practices, grape growers can develop effective strategies to address the impacts of climate change, thus ensuring long-term sustainability in viticulture. This approach involves a comprehensive understanding of the physiological responses induced by elicitors and the environmental conditions affecting grapevine performance (Monteiro et al., 2024).

Recent studies have highlighted significant spatial shifts in viticulture potential due to climate change, necessitating adaptation strategies that consider both bioclimatic indices and crop varieties (Irimia et al., 2024; Biasi et al., 2019). As traditional grape-growing regions may become unsuitable for certain cultivars, the importance of adapting management practices, including the use of elicitors, is underscored. These shifts in climate necessitate selecting more heat-tolerant grape varieties, alongside innovative agricultural strategies to foster resilience against changing weather patterns (Alsafadi et al., 2023; Naigeon et al., 2023).

Additionally, advancements in understanding the genetic and phenotypic responses of grapevines are essential. Considering local genetic variations and adopting indigenous cultivars can enhance grapevine adaptability to increasingly erratic climate conditions (Molitor & Junk, 2019). By integrating these genetic strategies with elicitor applications, wine producers can cultivate varieties that withstand environmental stressors while maintaining desirable quality traits essential for high-quality wine production (Blanco-Ward et al., 2019; Candiago et al., 2022).

Collaboration across scientific disciplines is also essential in developing adaptive strategies for viticulture. The use of data analytics to monitor climate impacts can inform growers about the optimal application of elicitors and other management practices tailored to local conditions. Recognizing the interplay between climate models and viticultural responses allows for more precise decision-making in vineyard management (Naigeon et al., 2023; Martins et al., 2021).

MITIGATION: REDUCING CHEMICAL INPUTS AND CARBON FOOTPRINT

Elicitors, such as chitosan and salicylic acid, are increasingly recognized for their potential to mitigate climate change impacts in viticulture by reducing chemical inputs and minimizing carbon footprints. By effectively controlling grapevine diseases like downy and powdery mildew, these natural alternatives to synthetic pesticides help enhance both environmental health and biodiversity within vineyard ecosystems (Naigeon et al. 2023 ; Nerva et al., 2019). The use of elicitors not only decreases chemical dependency but also aligns with sustainable practices that support cultivation methods aimed at preserving ecological balance (Héloir et al., 2019).

The use of elicitors can optimize production processes, thereby lowering carbon emissions associated with agricultural inputs. For instance, research has shown that elicitor applications enhance grapevine health and fruit quality, reducing the need for frequent pesticide and fertilizer applications (Candiago et al., 2022; Pérez-Álvarez et al., 2020). This reduction in input frequency lowers the overall carbon footprint of grape production and contributes to a more sustainable approach to

vineyard management, particularly as agriculture becomes a significant contributor to global greenhouse gas emissions (Thiollet-Scholtus et al., 2020). The interplay of improved plant resilience and reduced chemical application fosters environmentally friendly viticultural practices that benefit both producers and consumers.

Combining elicitors with advanced water management practices represents a promising avenue for developing climate-smart viticulture approaches. Integrating elicitors into precision viticulture systems empowers grape growers to formulate effective strategies that can handle the multitude of climate-related challenges presented by rising temperatures and water scarcity (Madouas et al., 2023). This synergy ensures the long-term viability of vineyards and promotes economic benefits while preserving environmental resources. Such holistic strategies encompass a wide range of practices aimed at enhancing both the resilience of grapevines and the sustainability of the broader agricultural system. In addition to their direct effects on plant health, elicitors are also associated with optimizing soil health and promoting sustainability. By encouraging beneficial microbial communities in the phyllosphere, elicitor applications can enhance the overall health of grapevines, facilitating nutrient exchanges and improving soil structure (Nerva et al., 2019). This focus on soil management is paramount in sustainable viticulture, as healthy soils contribute significantly to grape quality while playing a critical role in carbon sequestration efforts (Gaiotti et al., 2021).

Furthermore, the adoption of elicitors aligns with the growing body of research advocating for reduced chemical use in agriculture. The environmental and health risks associated with synthetic pesticides have led to increased scrutiny of traditional viticulture practices, making elicitor application an attractive alternative. By minimizing reliance on chemical inputs, grape growers can enhance their ecological footprint while meeting consumer demands for sustainably produced wines that resonate with modern environmental consciousness (Candiago et al., 2022; Héloir et al., 2019).

CONCLUSIONS

The application of elicitors in viticulture and winemaking has proven to be a transformative strategy, offering significant benefits to both plant health and wine quality. Elicitors such as salicylic acid, chitosan and methyl jasmonate have been directly linked to the enhancement of polyphenol and anthocyanin content within grapes, which are critical determinants of wine colour, flavour profile, and antioxidant capacity. By boosting these vital compounds, elicitors not only elevate the overall quality of wine but also contribute to the resilience of grapevines in the face of diseases and abiotic stressors like drought and high temperatures, thereby ensuring sustainable production practices. Also, the integration of elicitors throughout the winemaking process - including during fermentation and aging - has demonstrated positive effects on sensory characteristics, resulting in wines that are more complex and aligned with consumer preferences. This capability to enhance wine quality positions elicitors as essential components for sustainable viticulture, especially in the context of adapting to climate change by promoting resilience and reducing dependency on synthetic inputs.

For effective implementation, it is crucial that specialists foster the development of standardized protocols for the use of elicitors in viticulture. Such protocols should encompass optimal dosages, appropriate application timing, and the specific environmental conditions required to maximize the efficacy of elicitors.

Research in this field must continue to evolve, focusing on elucidating the mechanisms through which elicitors operate and their interactions with various environmental factors. Future studies should aim to explore how different types of elicitors influence grape composition, wine quality, and the broader vineyard ecosystem, ensuring that findings benefit practitioners within the industry. Additionally, conducting cost-benefit analyses that compare the economic viability of elicitor use versus conventional agricultural practices will yield valuable insights for growers, empowering them to make informed decisions regarding their implementation.

REFERENCES

Abd-Elall, E. (2019). Spraying of garlic extract, fructose and salicylic acid accelerates bud burst and improves productivity and fruit quality of superior grapevines. J*ournal of Plant Production, 10*(3), 257-263.

Abdi, N., Biljon, A., Steyn, C., & Labuschagne, M. (2022). Salicylic acid improves growth and physiological attributes and salt tolerance differentially in two bread wheat cultivars. *Plants, 11*(14), 1853.

Adebanjo, O., Setordjie, E., & Almat, A. (2022). The physiological response of salt and drought-stressed plant to exogenous application of salicylic acid. *bioRxiv 2022*.10.13.512118 (preprint).

Adrian, M., Lucio, M., Roullier-Gall, C., Héloir, M., Trouvelot, S., Daire, X., … & Schmitt-Kopplin, P. (2017). Metabolic fingerprint of ps3-induced resistance of grapevine leaves against *Plasmopara viticola* revealed differences in elicitor-triggered defenses. *Frontiers in Plant Science*, *08*.

Ahmad, B., Zaid, A., Sadiq, Y., Bashir, S., Wani, S.H. (2019). Role of selective exogenous elicitors in plant responses to abiotic stress tolerance. In: Hasanuzzaman, M., Hakeem, K., Nahar, K., Alharby, H. (eds*) Plant abiotic stress tolerance*. Springer, Cham.

Alsafadi, K., Bi, S., Bashir, B., Alsalman, A., & Srivastava, A. (2023). Future scenarios of bioclimatic viticulture indices in the eastern mediterranean: insights into sustainable vineyard management in a changing climate. *Sustainability, 15*, 11740.

Alwakil, N., Annuar, M., & Jalil, M. (2022). Synergistic effects of plant growth regulators and elicitors on α-humulene and zerumbone production in zingiber zerumbet smith adventitious root cultures. *Molecules, 27*(15), 4744.

Andrade, W., Melo, A., Melo, Y., Sá, F., Rocha, M., Oliveira, A., … & Fernandes-Júnior, P. (2020). *Bradyrhizobium* inoculation plus foliar application of salicylic acid mitigates water deficit effects on cowpea. *Journal of Plant Growth Regulation, 40*(2), 656-667.

Armstrong, C., Ristić, R., Boss, P., Pagay, V., & Jeffery, D. (2020). Effect of grape heterogeneity on wine chemical composition and sensory attributes for *Vitis vinifera* cv. Cabernet sauvignon. *Australian Journal of Grape and Wine Research, 27*(2), 206-218.

Aziz, A., Trotel-Aziz, P., Dhuicq, L., Jeandet, P., Couderchet, M., & Vernet, G. (2006). Chitosan stimulates defense reactions in grapevine leaves and inhibits development of *Botrytis cinerea*. *European Journal of Plant Pathology*, 114(4), 405-413.

Bavaresco, L., Belisomo, E., Furiosi, M., & Lucini, L. (2023). Role of chitosan against grapevine downy mildew. *Bio Web of Conferences, 68*, 01001.

Bhatt, B., & Mathpal, S. (2022). Elicitors: As Plant Defense Inducers. *International Journal of Botany Studies*, *7*(2), 12-18.

Biasi, R., Brunori, E., Ferrara, C., & Salvati, L. (2019). Assessing impacts of climate change on phenology and quality traits of *Vitis vinifera* L.: the contribution of local knowledge. *Plants, 8*(5), 121.

Bilko, M., Gunko, S., Babych, I., & Doboniy, I. (2022). Influence of tannins and mannoproteins products on the improvement of organoleptic characteristics of wines. *Eureka Life Sciences, 1*, 32-37.

Blanco-Ward, D., Monteiro, A., Lopes, M., Borrego, C., Silveira, C., Viceto, C., … & Miranda, A. (2019). Climate change impact on a wine-producing region using a dynamical downscaling approach: climate parameters, bioclimatic indices and extreme indices. *International Journal of Climatology, 39*(15), 5741-5760.

Buonassisi, D., Colombo, M., Migliaro, D., Peressotti, E., & Moser, C. (2023). Advances in the molecular mechanism of grapevine resistance to fungal pathogens. *Horticulture Research*, 10, 1-14.

Candiago, S., Winkler, K., Giombini, V., Giupponi, C., & Vigl, L. (2022). An ecosystem service approach to the study of vineyard landscapes in the context of climate change: a review. *Sustainability Science, 18*(2), 997-1013.

Chitarrini, G., Zulini, L., Masuero, D., Vrhovsek, U., & Mattivi, F. (2017). Effect of methyl jasmonate and benzothiadiazole on the secondary metabolite profile of Vitis vinifera cv. Merlot berries. *Journal of Agricultural and Food Chemistry*, 65(30), 6362-6371.

Colangelo, D., Torchio, F., De Faveri, D. M., & Lambri, M. (2018). The use of chitosan as alternative to bentonite for wine fining: Effects on heat-stability, proteins, organic acids, colour, and volatile compounds in an aromatic white wine. *Food Chemistry, 275*, 1-9.

Cunha, J., Caldeira, I., & Canas, S. (2021). New insights into viticulture, enology and vitivinicultural economy: *Ciência e Técnica Vitivinícola, 36*(2), 173-175.

Delaunois, B., Farace, G., Jeandet, P., Clément, C., Baillieul, F., Dorey, S., … & Cordelier, S. (2013). Elicitors as alternative strategy to pesticides in grapevine? current knowledge on their mode of action from controlled conditions to vineyard. *Environmental Science and Pollution Research, 21*(7), 4837-4846.

Deshmukh, V., Deshpande, J., & Wani, M. (2023). Elicitation based enhancement of solasodine production in in-vitro cultures of different solanum species. *Journal of Environmental Biology, 44*(2), 167-174.

Gačnik, S., Veberič, R., Hudina, M., Marinovic, S., Halbwirth, H., & Mikulič-Petkovšek, M. (2021). Salicylic and methyl salicylic acid affect quality and phenolic profile of apple fruits three weeks before the harvest. *Plants, 10*(9), 1807.

Gaiotti, F., Lucchetta, M., Rodegher, G., Lorenzoni, D., Longo, E., Boselli, E., … & Pii, Y. (2021). Urea-doped calcium phosphate nanoparticles as sustainable nitrogen nanofertilizers for viticulture: implications on yield and quality of Pinot gris grapevines. *Agronomy, 11*(6), 1026.

García-Pastor, M., Zapata, P., Castillo, S., Martínez-Romero, D., Guillén, F., Valero, D., … & Serrano, M. (2020). The effects of salicylic acid and its derivatives on increasing pomegranate fruit quality

and bioactive compounds at harvest and during storage. *Frontiers in Plant Science*, 11.

Garde-Cerdán, T., & Pérez-Álvarez, E. P. (2022). A review of the use of biostimulants in the vineyard for improved grape and wine quality. *Plants*, *11*(8), 1000.

Garde-Cerdán, T., González-Lázaro, M., Román, S., Urturi, I., Murillo-Peña, R., Rubio-Bretón, P., … & Pérez-Álvarez, E. (2023). Could foliar applications of methyl jasmonate and methyl jasmonate + urea improve must grape aroma composition? *Journal of the Science of Food and Agriculture*, *103*(10), 4813-4825.

Garde-Cerdán, T., Pérez-Álvarez, E., Fernández-Fernández, J., & Gil-Muñoz, R. (2022). Influence of elicitors application to Monastrell and Tempranillo vineyards on grape nitrogen composition over two vintages. *Oeno One, 56*(1).

Giménez-Bañón, M., Paladines-Quezada, D., Moreno-Olivares, J., Bleda-Sánchez, J., Fernández-Fernández, J., Parra-Torrejón, B., … & Gil-Muñoz, R. (2023). Methyl jasmonate and nanoparticles doped with methyl jasmonate affect the cell wall composition of Monastrell grape skins. *Molecules*, *28*(3), 1478.

Gómez-Plaza, E., & Martínez-Cutillas, A. (2022). The use of elicitors in viticulture: A tool to obtain highly colored wines. *OENO One*, 56(1), 1-14.

Gutiérrez-Gamboa, G., Garde-Cerdán, T., Martínez-Lapuente, L., Costa, B., Rubio-Bretón, P., & Pérez-Álvarez, E. (2019). Phenolic composition of Tempranillo blanco (*Vitis vinifera* L.) grapes and wines after biostimulation via a foliar seaweed application. *Journal of the Science of Food and Agriculture, 100*(2), 825-835.

Hall, A. and Blackman, J. (2019). Modelling within-region spatiotemporal variability in grapevine phenology with high resolution temperature data. *Oeno One, 53*(2).

Héloir, M., Adrian, M., Brulé, D., Claverie, J., Cordelier, S., Daire, X., … & Poinssot, B. (2019). Recognition of elicitors in grapevine: from mamp and damp perception to induced resistance. *Frontiers in Plant Science,* 10.

Irimia, L., Patriche, C., Petitjean, T., Tissot, C., Santesteban, L., Neethling, É., … & Quénol, H. (2024). Structural and spatial shifts in the viticulture potential of main European wine regions as an effect of climate change. *Horticulturae, 10*(4), 413.

Iriti, M., & Faoro, F. (2009). Chitosan as a MAMP, searching for a PRR. *Plant Signaling & Behavior*, 4(1), 66-68.

Jalili, I., Ebadi, A., Askari, A.M., Kalatehjari, S., & Aazami, M. (2023). Foliar application of putrescine, salicylic acid, and ascorbic acid mitigates frost stress damage in *Vitis vinifera* cv. 'Giziluzum'. B*MC Plant Biology, 23*(1).

Kahromi, S. and Khara, J. (2021). Chitosan stimulates secondary metabolite production and nutrient uptake in medicinal plant *Dracocephalum kotschyi*. *Journal of the Science of Food and Agriculture, 101*(9), 3898-3907.

Kantayos, V., Kim, J., & Baek, S. (2021). Enhancing resveratrol bioproduction and anti-melanogenic activities through elicitation in dj526 cell suspension. *Plants, 10*(8), 1653.

Kazumyan, K., Mikayelyan, M., Gevorkyan, E., & Jraghatspanyan, A. (2023). Investigating the impact of yeasts additives on the quality of the red wines: autolyzes of yeast and yeast derivatives introduced during alcoholic fermentation. *Agriscience and Technology*, 193-197.

Lemaître-Guillier, C., Dufresne, C., Chartier, A., Cluzet, S., Valls, J., Jacquens, L., … & Daire, X. (2021). Vocs are relevant biomarkers of elicitor-induced defences in grapevine. *Molecules, 26*(14), 4258.

Madouas, M., Henaux, M., Delrieu, V., Jaugey, C., Teillet, E., Perrin, M., … & Masson, J. (2023). Learning, reflexivity, decision-making, and behavioral change for sustainable viticulture associated with participatory action research. *Humanities and Social Sciences Communications, 10*(1).

Martín, L., Millán, A., Castaño, F., & Fontaine, F. (2023). Deciphering chitosan–host plant interaction in esca disease. *Agronomy*, *13*(5), 1290.

Martínez-Lapuente, L., Garde-Cerdán, T., Pérez-Álvarez, E., Guadalupe, Z., Parra-Torrejón, B., & Ayestarán, B. (2023). Foliar applications to vines of methyl jasmonate and nanoparticles doped with methyl jasmonate: impact on grape and wine polysaccharide composition. *Oeno One*, *57*(1), 1-14.

Martins, J., Fraga, H., Fonseca, A., & Santos, J. (2021). Climate projections for precipitation and temperature indicators in the Douro wine region: the importance of bias correction. *Agronomy*, *11*(5), 990.

Mejía-Teniente, L., Torres-Pacheco, I., González-Chavira, M. M., Ocampo-Velazquez, R. V., Herrera-Ruiz, G., Chapa-Oliver, A. M., & Guevara-González, R. G. (2010). Use of elicitors as an approach for sustainable agriculture. *African Journal of Biotechnology, 9*(54), 9155-9162.

Miclea, I., Suhani, A., Zăhan, M., & Bunea, A. (2020). Effect of jasmonic acid and salicylic acid on growth and biochemical composition of in-vitro-propagated lavandula angustifolia mill. *Agronomy*, *10*(11), 1722.

Miliordos, D., Tsiknia, M., Kontoudakis, N., Dimopoulou, M., Bouyioukos, C., & Kotseridis, Y. (2021). Impact of application of abscisic acid, benzothiadiazole and chitosan on berry quality characteristics and plant associated microbial communities of *Vitis vinifera* L. var. Mouhtaro plants. *Sustainability, 13*(11), 5802.

Molitor, D. and Junk, J. (2019). Climate change is implicating a two-fold impact on air temperature increase in the ripening period under the conditions of the Luxembourgish grape growing region. *Oeno One, 53*(3).

Monteiro, T., Patanita, M., Félix, M., Albuquerque, A., Ribeiro, J., Santos, F., … & Campos, M. (2024). Organic vs. integrated-production agriculture farming: which grapevine stress-responsive genes are affected by the application of resistance inducers and elicitors? *Agronomy, 14*(5), 892.

Naigeon, N., Picardat, S., & Auguste, P. (2023). Data for decision-making in viticulture in the face of climate

change: looking beyond production issues. *Bio Web of Conferences, 68*, 01040.

Nardello, I., Bender, A., Caliari, V., Souza, A., & Malgarim, M. (2021). Quality of juices from different grape cultivars obtained by distinct extraction systems. *Ciência E Técnica Vitivinícola, 36*(2), 163-172.

Nazir, R., Gupta, S., Kumar, V., Gupta, A., Dwivedi, P., Dey, A., … & Pandey, D. (2020). Enhanced in vitro production of diosgenin in shoot cultures of *Dioscorea deltoidea* by elicitation and precursor feeding. *Research Square*, preprint v1.

Nerva, L., Pagliarani, C., Pugliese, M., Monchiero, M., Gonthier, S., Gullino, M., … & Chitarra, W. (2019). Grapevine phyllosphere community analysis in response to elicitor application against powdery mildew. *Microorganisms, 7*(12), 662.

Nofal, m. and Attia, m. (2023). Management of root-knot nematode (*Meloidogyne incognita*) and improve defensive ability of cucumber plant using *Serratia marcescens,* salicylic acid and fosthiazate (Melotherin®) through adaptive processes and antioxidant capacity. *Menoufia Journal of Plant Protection*, *8*(3), 31-45.

Oraei, M., Panahirad, S., Zaare-Nahandi, F., & Gohari, G. (2019). Pre-véraison treatment of salicylic acid to enhance anthocyanin content of grape (*Vitis vinifera* L.) berries. *Journal of the Science of Food and Agriculture, 99*(13), 5946-5952.

Özkurt, N. and Bektaş, Y. (2022). Comparative evaluation of salicylic acid (sa) and 2,4-dichloro-6-{(e)-[(3methoxyphenyl)imino]methyl} phenol (dpmp) on growth and salt stress tolerance in forage pea (*Pisum sativum* L. *arvense*). *Black Sea Journal of Agriculture, 5*(3), 329-335.

Paladines-Quezada, D., Fernández-Fernández, J., Moreno-Olivares, J., Bleda-Sánchez, J., Gómez-Martínez, J., Martínez-Jiménez, J., … & Gil-Muñoz, R. (2021). Application of elicitors in two ripening periods of *Vitis vinifera* L. cv Monastrell: influence on anthocyanin concentration of grapes and wines. *Molecules, 26*(6), 1689.

Paladines-Quezada, D., Moreno-Olivares, J., Fernández-Fernández, J., Bleda-Sánchez, J., & Gil-Muñoz, R. (2022). Different response of proanthocyanidins from *Vitis vinifera* cv. Monastrell depending on time of elicitor application. *Journal of the Science of Food and Agriculture, 103*(1), 143-151.

Paladines-Quezada, D., Moreno-Olivares, J., Fernández-Fernández, J., Bleda-Sánchez, J., Martínez-Moreno, A., & Gil-Muñoz, R. (2019). Elicitors and pre-fermentative cold maceration: effects on polyphenol concentration in Monastrell grapes and wines. *Biomolecules, 9*(11), 671.

Pálfi, X., Lovas, M., Zsófi, Z., Kátai, J., Karácsony, Z., & Váczy, K. (2021). Paraffin oil induces resistance against powdery mildew in grapevine through salicylic acid signaling. *Pest Management Science, 77*(10), 4539-4544.

Pastore, C., Allegro, G., Valentini, G., Colucci, E., Battista, F., & Filippetti, I. (2019). Biochemical and molecular effects of yeast extract applications on anthocyanin accumulation in cv. Sangiovese. *Bio Web of Conferences, 13,* 03005.

Pérez-Álvarez, E., Ramírez-Rodríguez, G., Carmona, F., Martínez-Vidaurre, J., Masciocchi, N., Guagliardi, A., … & Delgado-López, J. (2020). Towards a more sustainable viticulture: foliar application of n-doped calcium phosphate nanoparticles on Tempranillo grapes. *Journal of the Science of Food and Agriculture, 101*(4), 1307-1313.

Phan, Q., Hoffman, S., & Tomasino, E. (2021). Contribution of lipids to taste and mouthfeel perception in a model wine solution. *Acs Food Science & Technology, 1*(9), 1561-1566.

Pieterse, C. M. J., Van der Does, D., Zamioudis, C., Leon-Reyes, A., & Van Wees, S. C. M. (2012). Hormonal modulation of plant immunity. *Annual Review of Cell and Developmental Biology*, 28, 489-521.

Qu, J., Liu, F., Pan, X., Liao, C., Li, T., Zhang, H., … & Yang, M. (2022). Coordinative changes in metabolites in grape cells exposed to endophytic fungi and their extracts. *Molecules, 27*(17), 5566.

Romanazzi, G., & Mlikota Gabler, F. (2021). Chitosan and other edible coatings to control postharvest gray mold of table grapes: A review of recent research. *Postharvest Biology and Technology*, 181, 111621.

Romanazzi, G., Murolo, S., & Feliziani, E. (2013). Effects of an innovative strategy to contain grapevine bois noir: field treatment with resistance inducers. *Phytopathology®, 103*(8), 785-791.

Rusadi, I., Phonpoem, W., & Hamim, H. (2023). Chitosan extracted from cricket (*Gryllus bimaculatus*) induced bioactive compound and pigment content in centella asiatica. *Iop Conference Series Earth and Environmental Science, 1271*(1), 012069.

Sáenz-Navajas, M., Arias-Pérez, I., Ferrero-del-Teso, S., Escudero, A., Ferreira, V., Fernández-Zurbano, P., … & Valentin, D. (2021). Access to wine experts' long-term memory to decipher an ill-defined sensory concept: the case of green red wine. *Oeno One, 55*(1), 69-79.

Salifu, R., Chen, C., Sam, F., & Jiang, Y. (2022). Application of elicitors in grapevine defense: impact on volatile compounds. *Horticulturae*, 8(5), 451.

Salifu, R., Chen, C., Sam, F., & Jiang, Y. (2022). Application of elicitors in grapevine defense: impact on volatile compounds. *Horticulturae*, *8*(5), 451.

Sherman, E., Coe, M., Grose, C., Martin, D., & Greenwood, D. (2020). Metabolomics approach to assess the relative contributions of the volatile and non-volatile composition to expert quality ratings of Pinot noir wine quality. *Journal of Agricultural and Food Chemistry, 68*(47), 13380-13396.

Shricharan, S., Hemanth, K., Bhattacharjee, A., Muniputtaiah, H., & Deore, R. (2024). *A novel calcium and biostimulant elicitors based preharvest treatments reduces postharvest berry rotting and maintains the quality of grapes cv. Thompson seedless*. Springer Science and Business Media LLC.

Singh, R., Ruíz-May, E., Rajput, V., Minkina, T., Gómez-Peraza, R., Verma, K., … & Quiroz-Figueroa, F. (2022). Viewpoint of chitosan

application in grapevine for abiotic stress/disease management towards more resilient viticulture practices. *Agriculture, 12*(9), 1369.

Suksawat, T. and Panichayupakaranant, P. (2024). Enhanced rhinacanthin production in *Rhinacanthus nasutus* roots using a hydroponics and elicitation system. *Journal of Young Pharmacists, 16*(2), 216-222.

Thiollet-Scholtus, M., Arceño, M., Valduga, M., & Sarrazin, F. (2020). Typing winegrower profiles to ease agroecological change in viticulture practices in the Loire valley, France. *Journal of Wine Research, 31*(4), 265-282.

Tscholl, S. and Vigl, L. (2024). The changing geography of wine climates and its implications on adaptation in the italian alps. *Climate Resilience and Sustainability, 3*(4).

Verhagen, B. W. M., Trotel-Aziz, P., Couderchet, M., Höfte, M., & Aziz, A. (2010). *Pseudomonas* spp.-induced systemic resistance to *Botrytis cinerea* is associated with induction and priming of defense responses in grapevine. *Journal of Experimental Botany*, *61*(1), 249-260.

Vishala, E., Kumar, V., Ruksana, F., Poornima, D., Anuradha, C., Prathap, H., … & Manjunatha, T. (2023). Hemolytic saponins product enhancement in *Gnidia glauca* (fresen.) gilg cell suspension cultures. *Biomedicine, 43*(01), 291-298.

Wang, Y., Huang, X., Aheto, J., Ren, Y., Zhang, X., & Wang, L. (2021). Novel colorimetric sensor array for Chinese rice wine evaluation based on color reactions of flavor compounds. *Journal of Food Process Engineering, 44*(12).

Wang, Y., Wang, X., Fang, J., Yin, W., Yan, X., Tu, M., … & Wang, X. (2023). Vqwrky56 interacts with vqbzipc22 in grapevine to promote proanthocyanidin biosynthesis and increase resistance to powdery mildew. *New Phytologist, 237*(5), 1856-1875.

Yang, Y., Wang, X., Tan, F., Fu, M., & Sun, F. (2019). Burdock fructooligosaccharides inhibits postharvest colour development in grape skins by altering phenylpropanoid fluxes from anthocyanin to the resistance-related phenolic substances. *Australian Journal of Grape and Wine Research, 25*(3), 307-315

Yue, X., Shi, P., Tang, Y., Zhang, H., Ma, X., Ju, Y., … & Zhang, Z. (2020). Effects of methyl jasmonate on the monoterpenes of Muscat hamburg grapes and wine. *Journal of the Science of Food and Agriculture, 101*(9), 3665-3675.

Zhang, Y., Yao, J., Feng, H., Jiang, J., Fan, X., Jia, Y., … & Liu, C. (2019). Identification of the defense-related gene vdwrky53 from the wild grapevine *Vitis davidii* using rna sequencing and ectopic expression analysis in arabidopsis. *Hereditas, 156*(1).

Zhao, M., Li, J., Shi, X., Malik, M., Quan, Y., Guo, D., … & Wang, S. (2023). Effects of exogenous plant regulators on growth and development of “Kyoho” grape under salt alkali stress. *Frontiers in Plant Science*, 14.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

RESEARCH ON THE REACTION OF THE CABERNET SAUVIGNON GRAPEVINE VARIETY TO THE VARIABILITY OF CLIMATIC CONDITIONS

Dorin Constantin COSTEA, Daniela Doloris CICHI, Constantin BĂDUCĂ-CÎMPEANU

University of Craiova, Faculty of Horticulture, 13 A.I. Cuza Street, Craiova, Dolj, Romania

Corresponding author email: dorin.costea@edu.ucv.ro

Abstract

Climate change are indisputable, and projections predict an intensification of the effects felt. Viticultural technologies must adapt quickly to ensure the unaltered quality of viticultural production. As a result, studies on the effect of climate change on the intensity of physiological and biochemical processes in the plant, on the quantity and quality of production have represented a priority for many research teams. The present study, carried out at the "Banu Mărăcine" Didactic Station of the University of Craiova, in a wine-growing area located in southern Romania, characterized in recent years by a high incidence of warm temperatures and unevenly distributed precipitation during the growing season, falls within this context. The research highlighted the reaction of the Cabernet Sauvignon variety in 2 years with different climatic profiles that influenced the rate of photosynthesis and transpiration, modified the length of the growing season and the dynamics of the accumulation of synthesized substances, and determined the quality and quantity of production. The results obtained can constitute reference points for establishing technological measures in the case of vineyard plantations affected by climate change.

Key words*: grapevine, climate changes, physiological and biochemical processes, quantity and quality of production.*

INTRODUCTION

The plasticity of the grapevine (*Vitis vinifera* L.) is known, its great capacity to adapt to varied environmental conditions, which has allowed the expansion of grapevine cultivation over large areas, on all continents, in different climatic conditions (Pastore et al., 2022). However, the greater intensity and frequency of extreme weather phenomena attributed to climate change change as a result of global warming, clearly also affects the sustainable cultivation of grapevines (Dejeu et al., 2008; Mărăcineanu et al., 2021).

Climate decisively influences the growth and ripening processes, as it requires appropriate temperatures, durations and intensities of radiation, specific levels of water availability throughout the vegetation cycle, thus influencing the yield, biomass, quantity and quality of production, the specific characteristics of the berries that will ultimately be found in the composition and aroma of the wine (Schultz & Jones, 2010).

In the projections made by the IPCC (The Intergovernmental Panel on Climate Change) on the effects of climate change for the coming years, it is predicted that all major components of the climate system will be affected, each region facing multiple and simultaneous changes (IPCC, 2023).

These climate changes are causing new challenges for viticulture because the quality and quantity of grape production, the timing of ripening, the chemical composition of berries and ultimately the quality of the wine obtained depend essentially on the main climatic factors (Battaglini et al., 2009; Cardell et al., 2019).

The availability and seasonal distribution of water from precipitation, hygroscopicity, solar radiation, temperature and concentration of greenhouse gases (CO_2), the incidence of extreme climatic phenomena are factors with a decisive impact on the quantity and quality of the production obtained (Ruml et al., 2016; Van Leuven et al., 2019; Căpruciu at al., 2022; Cichi et al., 2024).

All climate change projections for the coming period assume an impact on viticulture. In some regions climate change could have beneficial effects on viticulture (for example, extending the boundary of areas favorable for viticulture to

the east and north and into areas with higher altitudes, where temperatures for vines are currently too low), while the impact could be detrimental in other regions due to high temperatures during the grape ripening period that could negatively influence grape quality and wine production (Schultz & Jones, 2010; Bucur & Dejeu, 2016; Arias et al., 2022; Costea et al., 2023).

Each wine-growing region is characterized by a specific natural environment in which climatic conditions, soil characteristics, socio-economic requirements and the skill of the winegrower are the main factors that determine the choice of cultivated varieties and viticultural and oenological practices, thus providing the distinctive character, characteristic for the products of this area, imprinting the character of the terroir (Carbonneau, 2003; Santos et al., 2020).

The ecological conditions specific to each vineyard play a decisive role in determining the varieties cultivated to obtain a production that capitalizes on the specific characteristics of the respective area (Costea, 2006; Ubalde et al., 2010; Dobrei et al., 2018).

Studying the phenology of cultivated varieties can help winegrowers adapt applied technology to climate change, since since the progression of phenophases is extremely closely correlated with temperature (Jones, 2013; Ramos et al., 2020). Changing the duration and advancing the calendar date of the phenological stages as a result of increasing global temperatures can negatively affect the composition and quality of the grapes and therefore the wine obtained (Xenophon et al., 2020; Pastore et al., 2022; Dobrei et al., 2023).

Knowing and monitoring the variability of climatic conditions is particularly important because they cause different reactions, specific to each cultivated grape variety, to which winegrowers must and can react by adapting cultivation technology (Battaglini et al., 2009; Neethling et al., 2017).

MATERIALS AND METHODS

The results of the study presented in this paper were made at the vineyard farm within the "Banu Mărăcine" Didactic Station of the University of Craiova, located in the area delimited for the production of wines with Controlled Designation of Origin (DOC) "Banu Mărăcine". According to the objectives of the research topic, observations and determinations was focused on monitoring the main climatic factors and studying their influence on physiological and bioproductive parameters on the Cabernet Sauvignon variety grafted on the SO4 rootstock (Berlandieri x Riparia Selection Oppenheim 4), semi-tall growth, double cord training methods, spur pruning, 21 buds/plant.

The data for monitoring weather conditions in the 2023 and 2024 years of study and evaluating the effect of climatic conditions come from the weather station located in the vineyard as well as data provided by World Weather Online.

The result of the influence of the variation of climatic resources on the studied variety was evaluated by analysing physiological indicators and bioproductive parameters.

The determination of physiological indicators: the intensity of photosynthesis (A) ($\mu mols/m^2/s$), the intensity of transpiration (E) ($mmols/m^2/s$) was made with Lci-pro equipment during the vegetation period in annual dynamics, in representative phenophases: full flowering, berries pea-sized, beginning of ripening and softening of berries. Other observations and determinations made are specific to the field of study.

The effect of varied climatic resources was evaluated by analysing biological indicators (viability of buds, growth and maturation of shoots) and bioproductive parameters evolution of berry weight (g) and volume (ml), evolution of acidity (g/L H_2SO_4) and sugars of berries during ripening (g/L).

RESULTS AND DISCUSSIONS

Achieving the objectives set for this study could not have been achieved without careful monitoring of the main climatic factors. In order to correctly assess the variation of the recorded values and their effect on the growth and development of the studied variety, the values of the climatic factors from the experimental period are presented in parallel with the multiannual average for the period 2010-2022.

Comparing the average temperatures for the period 2010-2022 (Figure 1) with those from the experimental period, we note that the average

temperature values recorded are higher for both study years. The comparative analysis of the average monthly temperatures recorded in the 2 years of study (Figure 2) reveals that in 2024 the average monthly values recorded were higher than in 2023 by approximately 1°C, a trend observed throughout the vegetation period. If we refer to the month with the warmest temperatures, considered one of the reference values for viticulture, we note the existence of even greater differences between the 2 years of study, especially in terms of the average maximum temperatures: 36°C for 2024 compared to 33°C for 2023.

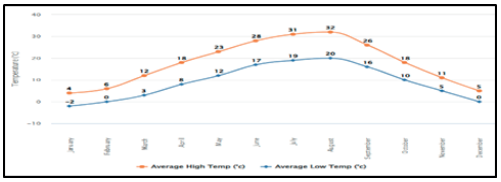


Figure 1. Average monthly temperature - multiannual values (2010-2022)

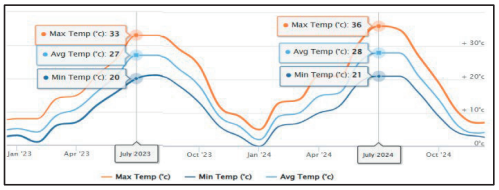


Figure 2. Maximum, average and minimum monthly temperatures from the experimental period

The values of insolation determine the values of temperatures and light intensity and provide information about the favorability of the crop year for vines. Comparing the insolation values, we observe small differences both between the study years and the multiannual values (Figure 3) and between the 2 study years (Figure 4), except for the beginning of the vegetation period (May 2024) when the recorded values were higher than in the same period in 2023.

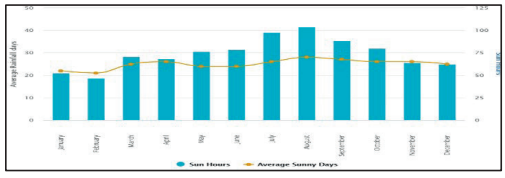


Figure 3. Average Sun hours and Sunny days (2010-2022)

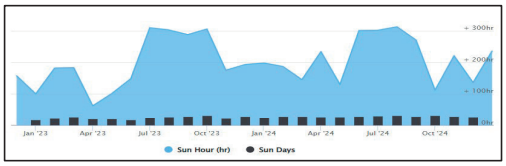


Figure 4. Sun hours and Sun days during the experimental period

Throughout the period we are referring to, the insolation values can be considered very good for the grapevine growth and fruiting processes, being recorded during the vegetation period (April 1 - September 30) 1510 hours in 2023 and 1615 hours in 2024.

To characterize the climatic favorability of the study year, the determination and analysis of data on ecoclimatic indices of precipitation and air hygroscopicity are of particular importance, given their role in the processes of growth, maturation, accumulation of chemical compounds and determination of the quantity and quality of the harvest.

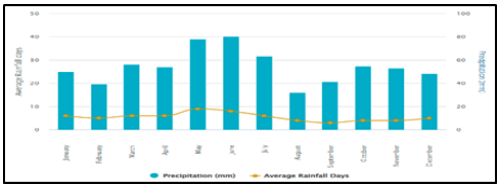


Figure 5. Average rainfall monthly amount and rainy days (2010-2022)

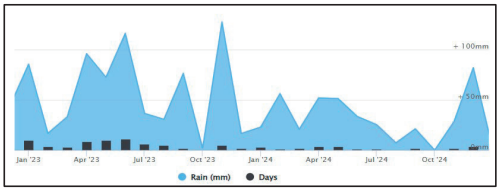


Figure 6. Rainfall amount and rainy days during the experimentation period

The distribution of precipitation in 2023 is similar to the multiannual pattern (Figure 5) with the mention that the amount of precipitation is lower than the multiannual average.

Comparing the volume and distribution of precipitation, the differences between the two study years can be easily highlighted (Figure 6). Overall, in 2023 the amount of precipitation was higher than in 2024, with bigger differences being recorded in May - June, in the phenophases of leaf development, intense shoot growth, flowering, fruit set, and in the late

August - September in the phenophases of ripening of berries (Figure 6).
Therefore, the year 2024 can be described as having a prolonged water deficit between July and September, which is reflected in the reaction of the vines (Figure 6).
The values of atmospheric humidity during the experimental period followed the precipitation distribution curve in the same period (Figure 7).

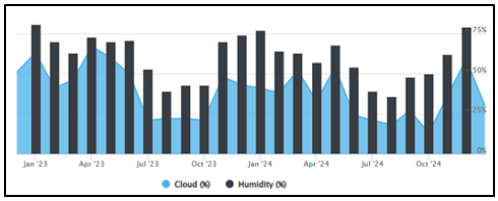


Figure 7. Average monthly atmospheric humidity and nebulosity during the experimentation period

The different climatic profile of the 2 years of study determined a different response of the Cabernet Sauvignon grapevine plants studied.
Determinations regarding the intensity of photosynthesis and transpiration carried out in representative phenophases for the identification of which the BBCH: full flowering (BBCH-65), berries pea-sized (BBCH -75), beginning of ripening: berries begin to develop variety-specific colour (BBCH-81) and softening of berries (BBCH-85), provide provides important information regarding the diurnal and seasonal dynamics of these physiological processes and about the influence of the specific climatic conditions of the year.

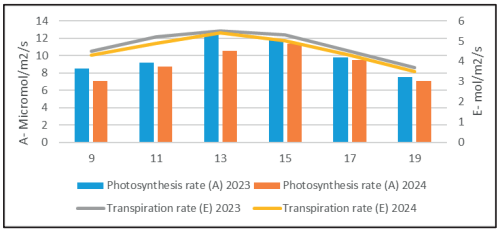


Figure 8. Photosynthesis and transpiration rate at full flowering (in 2023 and 2024)

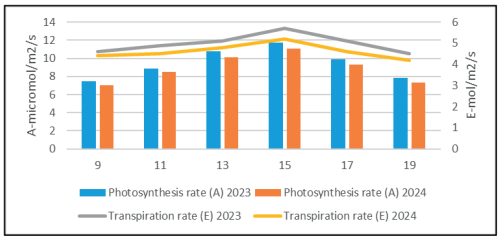


Figure 9. Photosynthesis and transpiration rate at berries pea-sized (in 2023 and 2024)

In the first 2 phenophases in which determinations were made: BBCH 65 - ull flowering and BBCH 75 - berries pea-sized, an almost perfect parallelism between the photosynthesis and transpiration rates in the two years is noted with the observation that in 2024 the determined values were lower compared to 2023, but the differences are not significant (Figures 8 and 9).
The diurnal dynamics of the 2 physiological processes has a diurnal dynamic with a maximum at 1 pm at full flowering (Figure 8) and at 3 pm at the berries pea-sized phenophase (Figure 9). Referring to the seasonal dynamics, the values determined at BBCH 65 and BBCH 75 are close; maximum values of the photosynthesis rate at full flowering at 13:00 were recorded (12.2 µmols/m^2/s) and transpiration rate of 5.6 mmols/m^2/s were recorded compared to BBCH 75 when the determination values were 11.7 µmols/m^2/s and 5.2 mmols/m^2/s, respectively. The parallelism and close values of the rate of physiological processes can be explained by the climatic conditions (regarding precipitation, temperature, insolation) with close values in the 2 years of study.

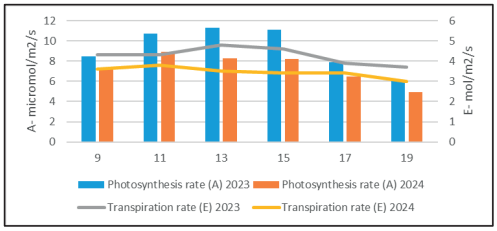


Figure 10. Photosynthesis and transpiration rate at beginning of ripening (in 2023 and 2024)

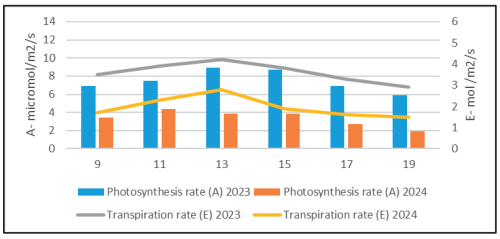


Figure 11. Photosynthesis and transpiration rate at softening of berries (in 2023 and 2024)

In the determinations made at the phenophases beginning of ripening (BBCH-81) and softening of berries (BBCH-85) the differences between the determinations made in the 2 years are higher (Figures 10 and 11), which is due to the completely different climatic profiles in which the respective phenophases took place.

In 2023, the values determined in both the diurnal and seasonal dynamics fall within the normal curve of the two physiological processes, but in 2024, the water deficit as a result of the lack of useful precipitation in August and the deficit of precipitation in September associated with the high temperatures specific to those months negatively influenced the rate of photosynthesis and transpiration. The values determined being only 3.8 µmols/m^2/s and 2.7 µmols/m^2/s (Figures 10 and 11).

Different climatic conditions, specific to the harvest year, determined the different dynamics of berry growth, of the accumulation of substances that determine the quantity and quality of production, highlighted the close dependence between the moment of reaching the maturity stage and environmental conditions, observations demonstrated by the values and dynamics of the bioproductive indices determined, in the period between the beginning of berry coloring and harvest, graphically represented in the Figures12 and 13.

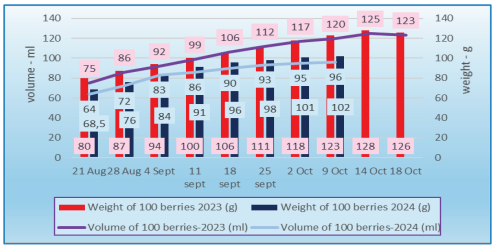


Figure 12. The dynamic evolution of the weight and volume of the berries during maturation

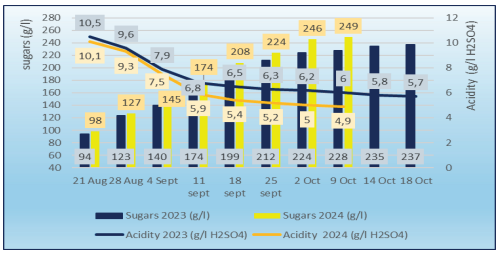


Figure 13. The dynamic of sugar content and acidity of berries during maturation

A first element that is easy to observe by analysing the 2 figures is the different time of completion of the determinations: as a result of the drought during the ripening period and the high temperatures in 2024, the grapes reached maturity and the harvest was carried out about 10 days earlier than in 2023 (on October 9 in 2024, compared to October 18 in 2023).

Another observation derived from the analysis of the results obtained is the influence of climatic conditions on the quantitative characteristics of the grapes; as a result of the water deficit, the berries had a lower weight and volume in 2024 than in 2023 (Figure 12). For example, the weight of 100 berries at the time of harvest was 126 g in 2023 and 102 g in 2024, which leads us to the conclusion that the production obtained in 2024 was significantly lower in quantity than in 2023.

Following the evolution in the dynamics of the qualitative indices, the determinations highlighted the more intense, faster accumulation of sugars in 2024, under the influence of high temperature conditions, lack of precipitation, reduced quantitative production and the reduced duration of the period between veraison and maturation by about 10 days. Under these conditions, at harvest, 249 g/L of sugars and an acidity of 4.9 g/L H_2SO_4 were recorded, while in 2023, under the specific conditions of that year, 237 g/L of sugars and 5.7 g/L of H_2SO_4 were recorded (Figure 13).

The Cabernet Sauvignon variety cultivated at "Banu Mărăcine" demonstrated a high potential for sugar accumulation, the values of this qualitative parameter increasing in 2024 by 151 g/L during the period between veraison and maturation, while in 2023 the sugar content increased by 143 g/L (Figure 13).

CONCLUSIONS

The study demonstrates the determining role of climate on the growth and development of the vine in general and of the Cabernet Sauvignon variety in particular.

The study highlights important results of the variation of climatic conditions, including the modification of phenology and the duration of the vegetation period, influencing the intensity of the development of physiological processes, modifying the dynamics of the accumulation of substances synthesized during the ripening period, all of which ultimately act on the quality and quantity of production.

The Cabernet Sauvignon variety interacted with environmental conditions, expressing its adaptation potential in a specific way.

Knowledge and modeling of these interactions can be a useful tool for planning and applying viticultural practices in current conditions characterized by a high level of variability of climatic conditions from one year to another and even during the same vegetation period.

ACKNOWLEDGEMENTS

The authors thank the staff from the Experimental Didactic Station "Banu Mărăcine" on the University of Craiova, for their collaboration, support and availability.

REFERENCES

Arias, L.A., Berli, F., Fontana, A., Bottini, R, & Piccoli, P. (2022). Climate change. Effects on grapevine physiology and biochemistry: benefits and challenges of high altitude as an adaptation strategy, *Frontiers in Plant Science*, *13,* 1-14.

Battaglini, A., Barbeau, G., Bindi, M. & Badeck, F.W. (2009). European winegrowers' perceptions of climate change impact and options for adaptation, *Regional Environmental Change*, *9*, 61-73.

Bucur, M.G. & Dejeu, L.C. (2016). Researches on situation and trends in climate change in south part of Romania and their effects on grapevine, *Scientific Papers. Series B, Horticulture, LXI*, 2017, 243-247.

Carbonneau, A. (2003). Ecophysiologie de la vigne et terroir. *Terroir, Zonazione, Viticoltura. Trattato Internazionale*; Phytoline: Centreville, USA, 61-102.

Cardell, M. F., Amengual, A. & Romero, R. (2019). Future effects of climate change on the suitability of wine grape production across Europe, *Regional Environmental Change*, *19,* 2299-2310.

Căpruciu, R., Cichi, D.D., Mărăcineanu, L.C. & Costea, D.C. (2022). The resveratrol content in black grapes skins at different development stages - *Scientific Papers. Series B, Horticulture*, *LXVI*(1), 245-252.

Cichi, D.D, Căpruciu, R., Stoica, F., Cichi, M., Costea, D.C. & Mărăcineanu, L.C. (2024) Ampelographic and bioproductive characteristics of Cabernet sauvignon clones in Oltenia winegrowing region, South-West Romania, *Scientific Papers. Series B, Horticulture, LXVII*(2), 262-269.

Costea, D.C, Căpruciu, R., Cichi, D.D. & Băducă Cîmpeanu, C. (2023). Research concerning the reaction of vines to variable climatic conditions *Scientific Papers. Series B, Horticulture*, *LXVII*(2), 222-227.

Costea, D.C. (2006). *Regimul hidric al viței-de-vie.* Craiova, RO: Universitaria Publishing House.

Dejeu, L., Patic, M., Mereanu, D., Bucur, G.M. & Gutue C. (2008). Impact of climat change on grapevine culture durability, *31st World Congress of Vine and Wine Proceeding*, Verona, Italia.

Dobrei, A., Dobrei, A.G., Nistor, E., Poșta, Gh., Mălăescu, M. & Balint M. (2018). Characterization of grape and wine quality influenced by terroir in different ecosystems from Romania cultivated with Feteasca neagră. *Scientific Papers. Series B, Horticulture., LXII,* 247-253.

Dobrei, A.G., Nistor, E., Scedei, D., Borca, F., Constantinescu, D.G. & Dobrei A.I. (2023). Improving some steps of grapevine growing technologies to reduce production costs, *Scientific papers. Series B, Horticulture, LXVII*(1), 277-284.

IPCC- The Intergovernmental Panel on Climate Change - IPCC, Climate Change (2023), Synthesis Report. Contribution of Working Groups I, II and III to the Sixth Assessment. *Report of the Inter-governmental Panel on Climate Change*. *Geneva, Switzerland,* 35-115.

Jones, G.V. (2013). Winegrape phenology, *Phenology: An Integrative Environmental Science.* M.D. Schwartz, Berlin, 563-584.

Mărăcineanu, L.C., Giugea, N., Mărăcineanu, E. & Căpruciu, R. (2021). Climate trends in Oltenia. Case study: Craiova - "Banu Mărăcine". *Scientific Papers. Series B, Horticulture, LXV*(1), 762-766.

Neethling, E., Petitjean,T., Quénol, H., & Barbeau, G. (2017). Assessing local climate vulnerability and winegrowers adaptive processes in the context of climate change*, Mitigation and adaptation strategies for Global change, 22*, 77-803.

Pastore, C., Frioni, T. & Diago, M. (2022). Resilience of grapevine to climate change: From plant physiology to adaptation strategies, *Frontiers in Plant Scienc*e, *13*, 1-5.

Ramos, M.C. & de Toda, F.M. (2020). Variability in the potential effects of climate change on phenology and on grape composition of Tempranillo in three zones of the Rioja DOCa (Spain), *European Journal of Agronomy, 115*, 1-40.

Ruml, M., Korać, N., Vujadinović, M., Vuković, A. & Ivanišević, D. (2016). Response of grapevine phenology to recent temperature change and variability in the wine-producing area of Sremski Karlovci, Serbia. *The Journal of Agricultural Science*, *154*, 186-206.

Santos, J., Fraga H., Malheiro, A., Moutinho-Pereira, J., Dinis, L.T., Correia, C., Moriondo, M., Leolini, L., Dibari, C., Costafreda-Aumedes, S., Kartschall, T., Menz, C., Molitor, D., Junk, J., Beyer, M. & Schultz, H. (2020). A review of the potential climate change impacts and adaptation options for European viticulture, *Applied Sciences*, *10*, 3092.

Schultz, H.R. & Jones, G.V. (2010). Climate induced historic and future changes in viticulture. *Journal of Wine Research*, *21*, 137-145.

Ubalde, J.M., Sort, X., Zayas, A. & Poch, R.M. (2010). Effects of soil and climatic conditions on grape ripening and wine quality of Cabernet Sauvignon. *Journal of Wine Resesearch*, *21*(1), 1-17.

Van Leeuwen, C., Destrac-Irvine, A., Dubernet, M., Duchêne, E., Gowdy, M., Marguerit, E., Pieri, P., Parker, A., De Rességuier, L. & Ollat, N. (2019). An update on the impact of climate change in viticulture and potential adaptations, *Agronomy, 9*(514), 1-20.

Xenophon, V., Korkas, E., Nisiotou, A. &, Georgios, G. (2020). Grapevine responses to heat stress and global warming, *Plants*, *9*(12), 17-54.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

EVALUATION OF SOME WHITE WINES' DYNAMIC VISCOSITY AND CORRELATION WITH OTHER PHYSICAL PARAMETERS

Iuliana CREȚESCU[1], Giancarla VELICEVICI[2], Mihaela MĂLĂESCU[2], Oana MUNTEANU[1], Raluca HORHAT[1]

[1]"Victor Babeș" University of Medicine and Pharmacy,
Faculty of Medicine, Department of Functional Sciences,
2 Eftimie Murgu Square, 300041, Timișoara, Romania
[2]University of Life Sciences "King Mihai I" from Timișoara,
Faculty of Engineering and Applied Technologies, Department of Genetic Engineering,
119 Calea Aradului Street, Timișoara, Romania

Corresponding author emails: giancarlavelicevici@usvt.ro, munteanu.oana@umft.ro

Abstract

The study sought to evaluate the dynamic viscosity of Chardonnay and Sauvignon Blanc dry and semi dry wines and to correlate it with other physical parameters. The samples originated from vineyards situated in different geographical regions of Romania (Banat, Transylvania, Oltenia, Muntenia, Moldavia and Dobrudja). A supplementary sample was introduced, a blending variety originating from the Cotesti vineyard (Moldavia region). Based on previous studies in the literature, the wine samples were considered to possess Newtonian fluid characteristics. Therefore, the wines' dynamic viscosity was computed using a mathematical formula, using an indirect method. The dynamic viscosity of the dry Chardonnay wine at 20ºC ranged between 1.5446 mPa·s (ISSA-Transylvania Hills) and 1.6602 mPa·s (Tarnave Jidvei region - Transylvania), while the one of the semidry Chardonnay variated from 1.6756 mPa·s (Tarnave Jidvei region - Transylvania) to 1.5344 mPa·s (Ovidiu-Dobrudja). The blending variety's dynamic viscosity had a mean value of 1.5627 mPa·s. The pH of the samples from the two varieties ranged between 2.97 (Dobrudja) and 3.46 (Banat), while for the blending variety we obtained the lowest value, with the exception of the dry Chardonnay originating in Dobrudja.

Key words: *˚Brix, colour, pH, viscosity, white wine.*

INTRODUCTION

Vine cultivation on Romanian territories is associated with a strong tradition, which gets lost in the mist of time. Recent tendencies of consumer preferences are more likely oriented towards white wine. Some of these varieties are rich in aromatic compounds, offering to the wines an intense and specific flavour and high gustatory and olfactory qualities. An example of such a variety is the Sauvignon Blanc. On the other hand, there are white varieties with a lower content of aromatic compounds, but in which, the chemical composition of the skin and core offers during the maturation original and interesting organoleptic properties. Such an example is the Chardonnay, a variety well appreciated by select consumers.

Dry and semidry varieties are characterized by an increased content of vitamins and minerals, natural antioxidants and anti-inflammatory substances. Usually, they are moderately alcoholised and much flavoured.

The Sauvignon Blanc is renowned for its fresh citric, melon and gooseberries flavours. The Chardonnay, considered, a sovereign among the white wines, is recognized by it's a cheerful and bright yellow-gold colour. It is a versatile variety, with green apple, peach and citric flavours.

Originating from Burgundy, Chardonnay is the most popular vine variety in the world. Its cultivation area is spread form New Zeeland to South Africa and serves to the preparation of many sparkling wines, including the renowned Champagne. It is a vigorous, equilibrated and malleable variety, that very easily takes over the characteristics of the terroirs and of the barrels in which it is kept. Therefore, it is considered an "author wine", with the ability to reflect the personality of the producer. The variety is rich in an important number of chemical substances,

such as alcohols, organic acids, esters, aldehydes, acetals and phenolic compounds. Previous studies have identified the concentrations of the most important non-volatile organic acids: tartric acid (2-6 g/l), malic acid (0.1-5 g/l), citric acid (0.1-0.8 g/l), gluconic acid (0.1-2 g/l), galacturonic acid (0.5-2 g/l), succinic acid (0.5-1.5 g/l) and lactic acid (0.1-0.5 g/l). The number and variety of these acids are responsible of the freshness and fruity character of the Chardonnay wine. They also serve as the main preservers of the wine's flavour (Țârdea et al., 2000).

The pH is an important biophysical factor, closely connected to the wine's sensorial properties and colour. Along with the acidity, it plays an important role in the preservation of the wine's flavour, most particularly in its sourness and astringency.

It has been found that climatic changes affect the anthocyanins, substances with an important impact on wine's colour. Temperatures above 30ºC are associated with lower concentrations of these compounds. The pH can affect the equilibrium between the different types of anthocyanins and lead to polymerization or condensation reactions of the pigments (Claire Payan et al., 2023).

The blend between the Sauvignon Blanc and the Chardonnay is an excellent dry, white wine, with a golden colour with green reflexions, perfectly complemented by rich floral and fruity flavours (elderflower, citrus, pineapple and grapefruit). This wine combines the characteristics of two special varieties: the queen and king of the white wines.

At 5ºC, the wines behave as a non-Newtonian fluid (Trávníček et al., 2016), whereas at temperatures above 10ºC they have the characteristics of a Newtonian fluid - with a linear relationship between the shear stress and deformation (Košmerl et al., 2000; Neto et al.; 2015; Yanniotis et al., 2005).

The viscosity is the property of the fluids to oppose resistance to flow, as result of the mechanical interaction between the constituent particles. For the fluids flowing in laminar regimen, the friction force (F) between the sheets of liquid is proportional with the contact surface between the sheets (S) and the speed gradient dv/dx. The expression of the friction force is provided by Newton's formula:

$$F = \eta \cdot S \cdot \frac{dv}{dx},$$

where: η = the dynamic viscosity coefficient.

If it remains constant and independent of the speed gradient dv/dx, the fluid is called newtonian (non-associated liquids); otherwise the liquid is considered non-newtonian (Neagu et al., 2024).

There is a limited number of studies in the literature to examine the dynamic viscosity of the dry wines. The viscosity of a wine tends to increase with its content of sugar, polysacharides and aminoacids (Burns & Noble, 1986; Gawel et al., 2016; Jones et al., 2008; Vidal et al., 2004).

The aim of the present study was to investigate the viscosity of dry and semidry varieties of Sauvignon Blanc and Chardonnay, as well as of a blend sample of the two and to evaluate its correlation with other physical and chemical parameters, such as the density, electrical conductivity, pH, ºBrix, colour intensity, chromatics, coloration, etc.

MATERIALS AND METHODS

White Wine Samples

There were studied a total of 22 white wine samples, as follows: 9 samples of Chardonnay dry white wine, 5 samples of Chardonnay semidry white wine, 7 samples of Sauvignon Blanc semidry white wine and one sample of Sauvignon Blanc Chardonnay blend white wine. The samples originated from different vineyards in the Banat, Transylvania, Muntenia, Moldavia and Dobruja regions. The blend sample included in the study originated from the Cotesti vineyard (Moldavia).

The wine bottles were bought from local stores and kept at room temperature.

All the samples consisted of wines produced out of grapes harvested in 2023. For each wine sample there were determined the viscosity. The density, the pH, the electrical conductivity and ºBrix were measured within 4-5 hours after opening the wine bottle.

Table 1 depicts the geographical region of origin and the alcohol content of the investigated white wines.

Table 1. The list of the investigated white wine samples, specifying the geographical region originating wine cellar, as well as the alcohol content corresponding to each sample

Group	Geographical Region	Sample Details	Wine cellar	EtOH Vol (%)
Dry CHARDONNAY	Transylvania	S1	ISSA-	12.5
	Transylvania	S2	VILLA VINEA	13
	Transylvania	S3	JIDVEI	12.5
	Oltenia	S4	SIMBURESTI	13
	Moldavia	S5	BECIUL DOMNESC	13
	Muntenia	S6	PRINCIAR TOHANI	13
	Banat	S7	RECAS SOLE	12.5
	Dobrudja	S8	ANA MARIA	12.5
	Muntenia	S9	PRAHOVA VALLEY	13
Semidry CHARDONNAY	Transylvania	S10	JIDVEI	13
	Oltenia	S11	SIMBUREL DE OLT	13
	Muntenia	S12	ALAI	12.5
	Dobrudja	S13	OVIDIU	13.5
	Moldavia	S14	HERMEZIU- RĂVASE	12
Semidry SAUVIGNON BLANC	Transylvania	S15	JIDVEI	13.5
	Moldavia	S16	BECIUL DOMNESC	13
	Muntenia	S17	TOHANI	12.5
	Muntenia	S18	BUDUREASCA	13.5
	Oltenia	S19	CASTEL VINARTE	12
	Dobrudja	S20	DOMENIILE OSTROV	12.5
	Banat	S21	CASTEL HUNIADE-RECAS	12.5
CHARDONNAY-SAUVIGNON BLANC blend	Moldavia	S22	TATA si FIUL	-

Physical and chemical parameters measurement

The physical and chemical parameters were measured according to the standardized and OIV methods recommended by the Technical Regulation "Analysis methods in wine production" (HG RM no 708 of 20.09.2011). the research was conducted in the Biophysics laboratories within the University of Medicine and Pharmacy "Victor Babes", Timisoara.

The determination of the absolute viscosity coefficient is associated with practical difficulties, therefore it is accepted the use of the relative viscosity coefficients (η_{rel}). The relative viscosity is defined as the ratio between the viscosity of the liquid to be determined and the viscosity coefficient of the reference liquid.

The formula enabling the computation of the dynamic viscosity coefficient is:

$$\eta = \eta_0 \cdot \frac{t \cdot \rho}{t_0 \cdot \rho_0}$$

where, t = the flowing time for the liquid to be determined; ρ = the density of the liquid to be determined at a given temperature; t_0 = the flowing time of the distilled water in seconds; ρ_0 = distilled water density at a given temperature; η_0 = water viscosity at given density and temperature. ρ_0 and η_0 are provided by STAS tables.

The relative viscosity was determined with the Ubbelohde viscometer at 20ºC.

The Brix degree was measured with a portable refractometer (VWR) allowing measurements within an interval between 0-54%.

The density of the wine samples was determined by the pycnometric standardized method (Neagu et al., 2024). There were used 10 ml pycnometers and an analytical balance with a 0.001 g accuracy (Sartorius TEI53 S).

The pH measurements- an important feature for the vinification process- were carried out using the analytical methods recommended by the International Organisation of Vine and Wine (OIV) (OIV, 2012).

The pH, as well as the electrical conductivity, were determined with the CONSORT 3010 multiparametric analyser. The measurements were preceded by an initial calibration. The conductance of the distilled water was checked, with an upper limit of 50 µS.

A wine's colour is completely defined by 3 parameters: brightness, cromatic (defined as the dominant wavelenght) and purity. The intensity of the colour (I) is given, by convention, by the following expression: $I = A_{420}+A_{520}+A_{620}$, using 3 decimals. The color (N) is given, by convention, by the following expression: $N = A_{420}/A_{520}$, where A_{420}, A_{520} and A_{620} represent the absorbance of the wine measured by spectrophotometry at the following wavelenghts: 420 nm, 520 nm and 620 nm. The spectrophotometry measurements were carried

out with a VIS Metertech spectrophotometer. (Neagu et al., 2024).

For an improved accuracy, all measurements were performed three times and the standard deviation (SD) was computed for each wine sample. The analysis of variance (ANOVA) was used in order to test for the differences between the samples. The statistical significance was set at $p<0.05$-.

RESULTS AND DISCUSSIONS

The measurements results for the dry and semidry Chardonnay are presented in Table 2 and respectively in Table 3. The values for the semidry Sauvignon Blanc and for the Chardonnay-Sauvignon Blanc blend can be found in Table 4, respectively in Table 5.

Table 2. Physical and chemical parameters of Chardonnay dry white wines varieties (mean value ± SD)

Sample no	Areas	pH	Brix degree	Viscosity (mPa·s)	Density at 20˚C	Electrical conductivity (mS)/cm)	Wine color (A420nm)	I	Color (N)
S1	Transylvania (ISSA)	3.31	8.3	1.5446±0.002	0.9915	1.76±0.041	0.096	0.11	8.72
S2	Transylvania (VILLA VINEA)	3.24	9.5	1.6367±0.014	0.9963	1.63±0.049	0.108	0.126	6.75
S3	Transylvania (JIDVEI)	3.42	9.9	1.6602±0.003	0.9952	1.67±0.02	0.096	0.106	9.6
S4	Oltenia (SIMBURESTI)	3.2	9.3	1.6261±0.008	0.9962	1.67±0.01	0.087	0.107	7.9
S5	Moldavia (BECIUL DOMNESC)	3.16	8.6	1.6235±0.003	0.9947	1.56±0.02	0.09	0.118	8.5
S6	Muntenia (PRINCIAR TOHANI)	3.26	10.5	1.6345±0.009	0.9968	1.57±0.015	0.123	0.174	4.55
S7	Banat (RECAS SOLE)	3.46	7.3	1.5460±0.006	0.9931	1.91±0.07	0.118	0.14	5.61
S8	Dobrudja (ANA MARIA)	2.97	7.2	1.6101±0.002	0.9905	1.32±0.04	0.114	0.129	8.14
S9	Muntenia (PRAHOVA VALLEY)	3.35	7.6	1.6305±0.001	0.9925	1.55±0.01	0.25	0.28	11.36

Table 3. Physical and chemical parameters of Chardonnay semi dry white wines varieties (mean value ± SD)

Sample no	Areas	pH	Brix degree	Viscosity (mPa·s)	Density at 20˚C	Electrical conductivity (mS)/cm)	Wine color (A420nm)	I	Color (N)
S10	Transylvania (JIDVEI)	3.43	8	1.6756±0.001	0.9932	1.67±0.0321	0.101	0.119	6.31
S11	Oltenia (SIMBUREL DE OLT)	3.18	7.5	1.6230±0.001	0.9933	1.36±0.0009	0.101	0.135	4.04
S12	Muntenia (ALAI)	3.37	7.6	1.6197±0.007	0.9954	1.82±0.0009	0.099	0.15	3.66
S13	Dobruja (OVIDIU)	3.4	7.8	1.5344±0.003	0.9031	1.47±0.0014	0.089	0.091	8.9
S14	Moldavia (HERMEZIU-RAVASE)	3.56	7.9	1.6229±0.021	0.9997	1.96±0.0550	0.11	0.14	5

Table 4. Physical and chemical parameters of Sauvignon semi dry white wines varieties (mean value ± SD)

Sample no	Areas	pH	Brix degree	Viscosity (mPa·s)	Density at 20˚C	Electrical conductivity (mS)/cm)	Wine color (A420nm)	I	Color (N)
S15	Transylvania (JIDVEI)	3.28	7.8	1.6237±0.002	0.9899	1.41±0.0818	0.068	0.079	17
S16	Moldavia (BECIUL DOMNESC)	3.26	7.7	1.6496±0.004	0.9919	1.5±0.017	0.054	0.066	5.4
S17	Muntenia (TOHANI)	3.52	7.9	1.62581±0.004	0.9938	1.85±0.058	0.112	0.128	9.33
S18	Muntenia (BUDUREASCA)	2.88	8	1.6607±0.004	0.9921	1.08±0.028	0.055	0.064	18.3
S19	Oltenia (CASTEL VINARTE)	3.02	7.5	1.6157±0.005	0.9918	1.37±0.032	0.052	0.059	26
S20	Dobrudja (DOMENIILE OSTROV)	3.17	7.8	1.6027±0.002	0.993416	1.47±0.032	0.115	0.13	9.58
S21	Banat (CASTEL HUNIADE-RECAS)	3.18	7.3	1.5612±0.013	0.997009	1.32±0.011	0.07	0.087	5.38

Table 5. Physical and chemical parameters of the blend Sauvignon-Chardonnay white wines variety (mean value ± SD)

Sample no	Areas	pH	Brix degree	Viscosity (mPa·s)	Density at 20°C	Electrical conductivity (mS)/cm)	Wine color (A420nm)	I	Color (N)
S22	Moldavia (TATA si FIUL)	3.01	7.4	1.5627±0.006	0.9737	1.47±0.025	0.12	0.15	5.45

The density of a solid, liquid or gas is defined as the ration between its mass and volume, therefore it represents the mass of the volume unit. Since the volume of any given object variates with its temperature, the density will change accordingly. Hence, when determining the density, the temperature must be taken into account.

It is aknowlodged that the density of the wine is directly influenced by the sugar content in the sample and that the alcohol has a lower density by comparison to the water. Therefore, the specific density of the wine is 0.8 times lower- or with 20% lower. As the sugar in the grape must is consummed, transforming it into alcohol, the density decreases. The fermentation completed, the specific weight of the wine should be smaller or approximatelly equal to 1 g/cm^3 (Pickering et al., 1998, Cretescu et al., 2024).

Among the dry Chardonnay, the smallest density- 0.9905 g/cm^3- was recorded in the wine originating form the Ana Maria cellar (Dobrudja) and the highest- 0.9963 g/cm^3- in the wine from Villa Vinea wine cellar (Transylvania). The lowest density in the semidry Chardonnay was 0.9031 g/cm^3, in a wine originating from Ovidiu cellar (Dobrudja), while the highest, 0.9997 g/cm^3, was found in a wine from Hermeziu-Ravase (Moldavia).

The wine originating from Jidvei wine cellar (Transylvania) had the lowest density (0.9899 g/cm^3) among the semidry Sauvignon Blanc. In the same cathegory the highest density was obtained for a wine originating from Recas cellar (Banat), reaching a value of 0.9970 g/cm^3. In a previous study, our reasearch team obtained the highest density also for a wine originating from the Recas cellar (Banat), a dry Sauvignon Blanc (Cretescu et al., 2024).

For the blend Chardonnay-Sauvignon Blanc, developped by the Tata si Fiul cellar (Moldavia) it was found a 0.9737 g/cm^{3}density.

Usually, the acidic or basic character of any solution is described in terms of hydronium ions concentration, converted in the pH scale. The pH of the wine influences not only the final flavour of the product, but also colour, the oxidation and its chemical stability. Therefore, the determination of the pH becomes essential and must be considered a key parameter in appreciating the quality of a given wine.

There are several studies finding for the pH of the wine rather large limits, between 2.8 and 4. However, most of the producers try to maintain the pH of the white wines between 3 and 3.5 and the one of the red wines between 3.3 and 3.8. The pH of a wine is correlated with its content of acids. A higher concentration of acids is associated with a smaller pH value and vice versa. A pH between 2.97 and 3.56 is considered ideal for a wine (Jacobson, 2006; Trávníček et al., 2016).

When monitoring a wine for longer periods of time, extreme values of pH are associated with a deterioration of its qualities. Higher pH values affect a wine's stability, are associated with a loss of its complexity and sometimes with a murine odour. On another hand, lower values of the pH can affect the colour of the red wine, as well as the fullness of the flavour. (Schneider, 2004)

In the dry Chardonnay cathegory the pH variates from 2.97, in the wine originating from the Ana Maria cellar (Dobrudja) up to 3.46 in the one from Recas cellar (Banat). For the semidry Chardonnay the pH ranged from 3.18 for the wine originating in Oltenia to 3.56 for the one developpped at Hermeziu cellar (Moldavia). The Sauvignon Blanc wines had the pH between 2.88 and 3.55.

The Chardonnay-Sauvignon Blanc blend wine, from the Tata si Fiul cellar (Moldavia) had a pH of 3.01. When comparing the pH values of the semidry Sauvignon Blanc wines originating from different regions in the country, the differences are not statistically significant ($p = 0.6762$). On the contrary, the differences between the dry Chardonnay varieties are very significant ($p<0.001$).

The alcohol content is influenced by several factors, such as the maturity of the grapes at

harvest, the processing technology or the fermentation technology (Kaltzin, 2012).

Previous research has found a connection between the sugar content of the grapes at harvest and the quality of the wine (Burg et al., 2013). A wine is considered strong when the alcohol content ranges between 12% and 14%. A high carbohydrates content, offered ofrered by quality grapes, ensures a ferementation without temperature regulation, with a high alcohol content, usually above 13% (Trávníček et al., 2016).

The dynamic viscosity for commercial Chardonnay dry wines at 20°C ranged from 1.5446 mPa·s to 1.6602 mPa·s and from 1.5344 mPa·s to 1.6756 mPa·s for the Chardonnay semidry wines and from 1.5612 mPa·s to 1.6607 mPa·s for the Sauvignon semidry white wines.

When analysing viscosity for the dry Chardonnay, the differences are significant between the samples originating from different geographical regions ($p = 0.0342$), as well as between samples originating from different cellars in the same region. Such an example is offered by the wines developed in Transylvania ($p<0.001$). A similar situation is present for the semidry Chardonnay and Sauvignon Blanc, with significant differences in the dynamic viscosity between the wines originating from from different geographic regions ($p<0.001$).

Previous research has found a viscosity of approximately 1.4 mPa·s in a commercial wine with 12% alcohol content at 25ºC (Pickering et al., 1998).

Danner et al. (2019) reported a dynamic viscosity between 1.448 mPa·s and 1.529 mPa·s for a dry Chardonnay wine at 20ºC. Christelle Abou Nader et al., 2017, reported a mean dynamic viscosity of 1.225 mPa·s for a dry Sauvignon Blanc with 6.95ºBrix and a 13.75% alcohol content, at 25ºC.

The present study complements previous research suggesting that viscosity is an important feature for tasters. The variation across the different varieties and geographical regions could likely become a criterion in the discriminatory process, as well as in the evaluation of the quality.

Table 6 showcases the Pearson correlation coefficients between viscosity, Brix degrees, pH and the alcohol content for the dry Chardonnay samples.

Table 6. Pearson correlation coefficient matrix between values of, viscosity, Brix degree, pH and alcohol content measurements for all wine samples of Chardonnay dry wine

	EtOH (%)	**pH**	**°Brix**	**Viscosity**
EtOH (%)	1			
pH	0.36425	1		
°Brix	-0.1141	0.132992	1	
Viscosity	-0.20444	-0.19208	0.593691	1

The Pearson correlation coefficients between the physical parameters for the semidry Chardonnay analyzed in the present study are presented in Table 7.

Table 7. Pearson correlation coefficient matrix between values of, viscosity, Brix degree, pH and alcohol content measurements for all wine samples of Chardonnay semidry wine

	EtOH (%)	**pH**	**°Brix**	**Viscosity**
EtOH (%)	1			
pH	-0.50253	1		
°Brix	-0.08459	0.806063	1	
Viscosity	-0.43595	0.043109	0.227937	1

Table 8 illustrates the Pearson correlation coefficients between the viscosity, Brix degrees, pH and alcohol content for semidry Sauvignon Blanc samples.

Table 8. Pearson correlation coefficient matrix between values of, viscosity, Brix degree, pH and alcohol content measurements for all the semidry Sauvignon Blanc samples

	EtOH (%)	**pH**	**°Brix**	**Viscosity**
EtOH (%)	1			
pH	-0.13651	1		
°Brix	0.574979	0.062275	1	
Viscosity	0.555702	-0.16752	0.773302	1

There can be observed a correlation between the alcohol content, the viscosity and the Brix degrees. It can be explained by the connection between the viscosity and the sugar content and the and the composition of the wine, illustrated by the Brix degrees. It is also justified by the common production techniques used for the commercial wines.

Table 9 shows the mean values of the pH, viscosity and Brix degrees for the dry Chardonnay, the semidry Chardonnay, the

semidry Sauvignon Blanc and the blend Chardonnay-Sauvignon Blanc wines originating form Moldavia. There can be observed significant differences between all the 4 categories of wines regarding the viscosity, the pH and the Brix degrees ($p<0.001$).

Table 9. Mean values for the pH, viscosity and Brix degrees for the wines originating from the Moldavia geographical region

Wine biophysical parameter	Dry Chardonay	Semidry Chardonay	Semidry Sauvignon Blanc	Chardonnay -Sauvignon Blanc blend
pH	3.16	3.56	3.26	3.01
Viscosity	1.6235	1.6229	1.6496	1.5627
°Brix	8.6	7.9	7.7	7.4

Regarding the electrical conductivity of the dry Chardonnay, the highest value was obtained for the S7 sample originating from Banat, 1.91 mS. For the semidry Chardonnay, the largest value was present in the S14 sample, from Moldavia, reaching a value of 1.96 mS. Lower values were recorded in the semidry Sauvignon Blanc, with a maximum of 1.85 mS for the S17 sample, originating from Muntenia. The blend Chardonnay-Sauvignon Blanc from Moldavia had a mean electrical conductivity of 1.47 mS.

The electrical conductivity of the dry and semidry Chardonnay samples showed significant differences according to the geographical region ($p<0.001$). By the contrary, the differences were didn't reach the statistical significance for the semidry Sauvignon Blanc ($p = 0.9409$). Each cellar can use different wine production techniques, resulting in individual characteristics of their wines. However, a common feature of the Chardonnay, as well as of the Sauvignon Blanc is their light yellow colour -extending to a darker yellow, when the wine is kept in oak barrels, the intense aromatic concentration and the increased acidity (Lund et al., 2009; Peirano-Bolelli et al., 2022; Cretescu et al., 2024).

The colour is one of the most important visual features of a wine, offering a large amount of relevant information. It is completely defined by the brightness, the chromatic and the purity. The chromatic is given by the dominant wavelength (which characterizes the colour) and by the purity of the colour. The dominant wavelength for the chromatic of the wine is 420 nm.

It is known that the Sauvignon Blanc has the highest intensity of the colour, while the Chardonnay has the most profound one. In addition, their colours are different lemon shades, from green lemon in the Sauvignon Blanc to average lemon to bright golden in the Chardonnay.

For the dry Chardonnay the highest intensity was obtained in the wine sample originating from the Princiar Tohani cellar (Moldavia) – 0.174. For the semidry Chardonnay the maximum was recorded in the sample from the Hermeziu-Ravase cellar (Moldavia), 0.140. smaller values were obtained in the semidry Sauvignon Blanc samples, with a maximum of 0.130 in the sample originating from the Domeniile Ostrov cellar (Dobrudja). The blend Chardonnay-Sauvignon Blanc had an intensity of 0.150.

CONCLUSIONS

The present study has found significant differences between the dry Chardonnay, semidry Chardonnay, semidry semidry Sauvignon Blanc and Chardonnay-Sauvignon Blanc samples regarding a number of biophysical parameters, such as the viscosity, the pH, the ºBrix, etc. these differences illustrate the variation in their chemical composition.

The dynamic viscosity for commercial Chardonnay dry wines at 20°C ranged from 1.5446 mPa·s to 1.6602mPa·s and from 1.5344 mPa·s to 1.6756 mPa·s for the Chardonnay semidry wines and from 1.5612 mPa·s to 1.6607 mPa·s for the Sauvignon semidry white wines. Regarding the dynamic viscosity, there are very significant differences between the samples originating from various geographical regions ($p<0.0001$).

The alcohol content, the ºBrix and the viscosity are correlated, illustrating the dependence to the chemical composition and the similar production techniques of the commercial wines.

REFERENCES

Burg, P., Vítěz, T., & Michálek, M. (2013). The evaluation of vine leaves development dynamic (in Czech), *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis, 61(1)*, 17-23.

Burns, D.J.W., & Noble, A, C. (1986). Evaluation of the separate contributions of viscosity and sweetness of sucrose to perceived viscosity, sweetness and bitterness of vermouth. *Journal of Texture Studies, 16*, 365-381.

Christelle, A. N., Hadi, L., Fabrice, P., Bernard, Le J. L., Guy Le B., Roger L. & Marie A. (2017). Assessing White Wine Viscosity Using Polarized laser Speckle: A promising Alternative to Wine Sensory. *Analiysis Sensors, 17(10)*, 2340-2352.

Claire, P., Anne-Laure, G., Michael, J., Monika C. & Pierre-Louis Teissedre. (2023). Wine acidification methods: a review. *OENO One, 57(3*),

Cretescu, I., Horhat, R., Velicevici, G., Mălăescu, M., & Munteanu, O. (2024). Physical and chemical parameters of Sauvignon Blanc dry white wines from different areas in Romania. *Journal of Horticulture, Forestry and Biotechnology, 28(2)*, 164-169.

Gawel, R., Smith, P. A., & Waters, E.J. (2016). Influence of polysaccharides on the taste and mouthfeel of white wine. *Australian Journal of Grape and Wine Research, 22*, 350-357.

Jacobson, J.L. (2006). *Introduction to Wine Laboratory Practices and Procedures*. New York: Springer Science, USA.

Jones, P, Gawel, R, Francis, I, & Waters, E. (2008). The influence of interactions between major white wine components on the aroma, flavour and texture of model white wine. *Food Quality and Preference, 19*, 596-607.

Kaltzin W. (2012). Natural wines als Trend. *Der Winzer, 10(4),* 85-87.

Košmerl, T., Abramovič, H., & Klofutar, C. (2000). The rheological properties of Slovenian wines. *Journal of Food Engineering, 46(3)*, 165-171.

Danner, L., Niimi,J., Wang,Y., Kustos, M., Muhlack, R.A., Bastian, S.E.P. (2019). Dynamic viscosity levels of dry red and white wines and determination of perceived viscosity difference thresholds. *American Journal of Enology and Viticulture 70,* 205-211.

Lund, C., Thompson, M., Benkwitz, F., Wohler, M., Triggs, C.R., Heymann, H. & Nicolau, L. (2009). *New Zealand Sauvignon Blanc distinct flavour characteristics: Sensory, chemical, and consumer aspects. American Journal of Enollogy and Viticulture, 60*, pp.1-12.

Neagu, M., Munteanu, O., Nagy, I., Neagu, A. (2024). *Lucrari practice de biofizica*. Timisoara, Editura Eurobit.

Neto, F.S.P.P., De Castilhos, M.B.M., Telis, V.R.N., & Telis-Romero, J. (2015). Effect of ethanol, dry extract and reducing sugars on density and viscosity of Brazilian red wines. *Journal of the Science of Food and Agriculture, 95*, 1421-1427.

OIV (2012). *Compendium of International Methods of Wine and Must Analysis*. Paris, France.

Peirano-Bolelli, P., Heller-Fuenzalida, F., Cuneo, I.F., Pena-Neira, A. & Cáceres-Mella, A. (2022). Changes in the composition of flavonols and organic acids during ripening for three cv. Sauvignon Blanc clones grown in a cool-climate valley. *Agronomy, 12*, 1357.

Trávníček, P., Burg, P., Krakowiak - Bal, A., Junga1 P., Vítěz, T., & Ziemiańczyk, U. (2016). Study of rheological behaviour of wines, *International Agrophysics,.30*, 509-518.

Pickering, G.J., Heatherbell, D.A., Vanhanen, L.P. & Barnes, M.F. (1998). The effect of ethanol concentration on the temporal perception of viscosity and density in white wine. *American Journal of Enology and Viticulture, 49*, .306-318.

Schneider V. (2004). Der pH-Wert und seine Interpretation. *Der Winzer, 50(5)*, 141-143.

Țârdea, C., Sârbu, G., & Țârdea, A. (2000). *Tratat de vinificație*, Iași, Editura Ion Ionescu de la Brad.

Vidal, S., Courcoux, P., Francis, L., Kwiatkowski, M., Gawel, R., Williams, P., Waters, E., & Cheynier, V. (2004). Use of an experimental design approach for evaluation of key wine components on mouth-feel perception. *Food Qual Prefer, 15*, 209-217.

Yanniotis, S., Kotseridis, G., Orfanidou, A., & Petraki, A. (2007). Effect of ethanol, dry extract and glycerol on the viscosity of wine. Journal of Food Engineering, 81, 399-403.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

THE INFLUENCE OF CLIMATE VARIABILITY ON GRAPE RIPENING AND QUALITY IN A VINEYARD LOCATED IN MINIŞ-MĂDERAT AREA

Alina Georgeta DOBREI[1], Eleonora NISTOR[1], Daniela DOBROMIR[2], Simion ALDA[1], Marcel DANCI[1], Daniela SCEDEI[1], George Dragoş CONSTANTINESCU[1, 3], Alin DOBREI[1]

[1]University of Life Sciences "King Mihai I" from Timisoara,
119 Calea Aradului Street, Timişoara, Romania
[2]Research and Development Station for Viticulture and Oenology Miniş,
1 Ghioroc, 317135, Miniş, Arad County, Romania
[3]National Office of Vine and Wine Products (ONVPV), 49 Iancului Road,
District 2, Bucharest, Romania

Corresponding author emails: alin1969tmro@yahoo.com, nisnora@yahoo.com

Abstract

Climate variability has necessitated adjustments in varietal selection and growing practices for many wine grape varieties. Simultaneously, rising temperatures and increased solar insolation have led to the accumulation of higher sugar levels in grapes, with important implications for the winemaking process. This study, conducted in a vineyard from Miniş-Măderat area, examined the influence of climate variability on the ripening time and berry sugars accumulation of several wine grape varieties. Climate data from recent years were compared to those from 2001, chosen as a benchmark year. Climate resources were analyzed based on the vine's requirements, with key climate indices calculated. Additionally, the evolution of grape ripening and the optimal harvest time were monitored, and differences were compared with the reference year. The results revealed that the majority of varieties ripened 20–30 days earlier, depending on the year and variety. A significant increase in must sugar content was also observed. These findings highlight the need for adaptive strategies in viticulture to address the challenges posed by climate variability.

Key words: *climate indices, grape, quality, ripening.*

INTRODUCTION

Climate plays a crucial role in shaping grape cultivation, influencing the geographic distribution of vineyards, varietal assortments, and the specific techniques employed in grape production (Jones et al., 2005; van Leeuwen & Darriet, 2016). Over recent years, significant climatic disturbances have increasingly impacted viticulture, affecting not only cultivation practices but also grape quality, winemaking processes, and the overall typology of wines (Hannah et al., 2013). These climatic changes present both challenges and opportunities for wine producers as they adapt to a shifting environmental landscape (Dobrei et al., 2023). One of the most notable consequences of changing climatic conditions is the rise in useful temperatures and real insolation, which has led to a substantial increase in sugar accumulation across all cultivated grape varieties (Sadras & Moran, 2012). This enhanced sugar content directly affects the ripening process, resulting in technological maturity occurring much earlier than in previous years (Palliotti et al., 2014). While this acceleration of grape maturity may seem advantageous, it poses significant challenges for winemakers (Bucur & Dejeu 2024). Increased sugar levels translate into higher potential alcohol content, necessitating earlier harvests to maintain the balance and quality of the resulting wines (De Orduña, 2010). This adjustment, however, influences other critical must and wine parameters, requiring modifications to both vineyard management and winemaking practices (Poni et al., 2018). The impact of these climatic shifts extends beyond sugar accumulation and harvest timing (Costea et al., 2023). Aromatic grape varieties are particularly vulnerable to elevated temperatures during the harvest period, which

can lead to the degradation or loss of volatile aromatic compounds (Stroe & Budescu, 2023). In response, winegrowers are adopting innovative strategies to preserve aromatic profiles, such as harvesting at night or relocating vineyards to cooler, more northern regions (Filimon et al., 2023). Additionally, many wineries are revising their varietal assortments to better align with the new climatic realities, selecting grape varieties that are more resilient to increased temperatures and earlier ripening (van Leeuwen & Destrac-Irvine, 2017; Mustea et al., 2011).

Given these profound and ongoing changes, understanding the relationship between climatic factors and grape quality is essential for future-proofing viticultural practices. This study aims to investigate how environmental variables—particularly temperature and insolation—affect key grape ripening parameters such as sugar accumulation and technological maturity across multiple grape varieties. The findings will contribute to a broader understanding of the adaptive strategies required to maintain grape and wine quality in the face of a rapidly changing climate.

MATERIALS AND METHODS

Study Area and Experimental Design

The research was conducted in the Miniș-Măderat Vineyard (Romania) over four consecutive years, from 2021 to 2024, focusing on three wine grape varieties: 'Fetească Regală', 'Pinot Noir', and 'Cabernet Sauvignon'. This vineyard is characterized by temperate-continental climatic conditions, making it suitable for studying the impact of climate variability on grape ripening and quality. The climatic conditions during the study period were compared with those of the year 2001, which was used as a reference year to assess long-term climatic changes and their effects on grape development.

Climatic Data Collection and Analysis

Meteorological data were collected throughout each growing season (April to October) from the local weather station situated within the vineyard. The climatic parameters recorded included air temperature, precipitation, and sunlight duration. These data were used to calculate key viticultural climatic indices that characterize the heliothermal and water resources critical to grapevine development. The indices calculated were: global heat balance (Σt°g) - (total sum of daily temperatures above 0°C during the growing season); active heat balance (Σt°a) - (sum of daily temperatures exceeding 10°C during the growing season); useful heat balance (Σt°u) - (sum of daily temperatures above 10°C, excluding non-vegetative periods); hydrothermal coefficient (CH) - (reflects the ratio between precipitation and temperature, indicating drought stress); Martonne aridity index (I-ar-DM) - (evaluates aridity based on temperature and precipitation levels); real heliothermal index (IHr) - (measures actual solar heat availability for vine development); viticultural bioclimatic index (Ibcv) - (integrates thermal and hydric variables to assess vine suitability); oenoclimatic aptitude index (IAOe) - (describes the suitability of the climatic conditions for wine production) and Huglin heliothermal index (IH) - (indicates the cumulative effect of temperature on grapevine phenology). These indices were calculated following established methodologies (Huglin, 1978; Tonietto & Carbonneau, 2004) to evaluate the interaction of biotope factors and their collective impact on grapevine growth and fruit composition.

Grape ripening monitoring

Grape ripening was monitored throughout each growing season to assess the dynamics of sugar accumulation and acidity levels. The technological maturity of grapes was determined by measuring the following parameters at regular intervals from veraison until harvest: must sugar (g/L) - determined using a refractometer (expressed as g/L); total acidity (g/L tartaric acid) - measured through titration with sodium hydroxide and expressed in g/L of tartaric acid; berry weight (g) - calculated as the mean weight of 100 randomly selected berries collected from multiple points across the vineyard. The date of technological maturity was established when the sugar content and acidity levels reached optimal values for each variety based on standard viticultural practices. All measurements were conducted in triplicate to ensure the accuracy and reproducibility of results.

Statistical Analysis

Statistical analyses were performed using Microsoft Excel (Microsoft Corporation, Redmond, WA, USA) and XLSTAT Cloud (Addinsoft, Paris, France). Descriptive statistics were calculated to summarize the climatic indices (Σt°g, Σt°a, Σt°u, CH, I-ar-DM, IHr, Ibcv, IAOe, and IH) and grape ripening parameters (sugar content, total acidity, and berry weight). Comparative analyses between the study years (2021-2024) and the reference year (2001) were conducted using one-way ANOVA, followed by Tukey's HSD post-hoc test to identify significant differences ($p < 0.05$). Pearson's correlation coefficients were calculated to assess relationships between climatic indices and grape ripening parameters. Additionally, Principal Component Analysis (PCA) was performed to identify the most influential climatic factors affecting grape maturity. All analyses were performed in triplicate for reproducibility, and statistical significance was set at $\alpha = 0.05$. Outputs from Microsoft Excel and XLSTAT Cloud were cross-validated to ensure consistency.

RESULTS AND DISCUSSIONS

Climatic resources play a pivotal role in determining varietal assortment, cultivation techniques, grape quality, and the economic performance of vineyards. In our study, significant changes were observed in all analyzed climatic elements during the research years (2021-2024) compared to 2001 and the 1991-2000 averages (Table 1). Throughout the study period, both average and maximum air temperatures exceeded those recorded in 2001. Notably, 2024 experienced the highest average temperature, surpassing 2021 by over 6°C. This substantial increase aligns with global observations, where viticultural regions have reported growing season mean temperature rises of approximately 1.3°C between 1950 and 1999, and 1.7°C from 1950 to 2004 (Santos et al., 2020). Elevated temperatures can significantly impact grapevine physiology, affecting phenology, berry composition, and overall wine quality. Studies have shown that higher temperatures can lead to advanced phenological stages, reduced acidity, and altered aromatic profiles in grapes (van Leeuwen & Darriet, 2016). Additionally, increased temperatures may exacerbate water deficits, further influencing grape development and vineyard management practices.

Similar trends have been observed in other wine-producing regions. For instance, research indicates that warmer growing seasons have led to challenges in achieving balance in fruit composition, resulting in higher alcohol levels and lower acidity in wines (Dobrei et al., 2021). Furthermore, studies have documented that rising temperatures and decreased water availability have affected grapevine physiology and biochemistry, impacting berry quality (Arias-Pérez et al., 2021).

Table 1. The main climatic parameters in the Miniș-Măderat vineyard from 2021 to 2024 period

Year	The mean air temperature (°C)			The mean of soil temperature (°C)	Precipitations (mm)	Hygroscopicity (%)	Insolation (hours)
	Mean	Maximum	Minimum				
2021	12.4	40.0	-12.0	13.6	970.5	74.0	2440
2022	13.4	41.4	-12.0	13.9	560.3	73.0	2130
2023	14.62	38,6	-7.8	14.7	924.9	76.3	2380
2024	15.24	41.7	-8.4	15.3	619.2	72.6	2562
Mean 2021-2024	13.95	40.4	-10	14,3	768.7	73,9	2378
Mean 1991-2000	11.2	36.5	-13.5	12.3	670.0	79.3	1834.9
2001 (C)	9.2	37.9	-13.3	10.7	826.0	81	1913.7

In terms of minimum temperatures recorded across the research period, all years exhibited higher minimum values compared to those recorded in 2021. With regard to the average soil temperature, significant changes are evident when compared to the reference year of 2001. In all four research years, the average soil temperature was 3-6°C higher than in 2001.

The average annual precipitation, however, displayed a contrasting pattern. The years 2022 and 2024 experienced lower precipitation

levels than the reference year (2001), while the years 2021 and 2023 recorded significantly higher values. In terms of hygroscopicity, all research years had values lower than those of the reference year. Conversely, all four research years recorded higher values for sunshine hours, with 2024 surpassing the 2001 values by more than 600 hours.

Since the precipitation regime is particularly significant for viticulture, both in terms of production quality and cultivation technology, it is well established that precipitation levels influence the intensity of disease and pest outbreaks, thereby affecting the phytosanitary treatment schedule (see Table 2). For this reason, a detailed analysis of the precipitation regime according to the Hellmann Criterion was deemed necessary. The research years varied considerably, both in terms of total precipitation amounts and seasonal distribution. Applying the Hellmann Criterion, the years ranged from extremely rainy in 2021 to normal in 2024. Although no research year was considered overall as deficient in precipitation, all four research years recorded lower precipitation levels during the grapevine growing season when compared to the reference year, 2001. Notably, the year 2024, classified as normal according to the Hellmann Criterion in terms of total annual precipitation, recorded the lowest precipitation during the grapevine growing season.

The climatic changes observed in the Miniș-Măderat vineyard align with broader trends in viticulture, particularly regarding rising temperatures and variable precipitation. Studies by Costa et al. (2023) and Duchêne et al. (2010) confirm increased soil and minimum temperatures, influencing vine development. Variations in precipitation, as noted by Williamson et al. (2024), highlight how rainfall patterns impact vine health and yield, with lower rainfall during critical periods potentially increasing pest and disease risks. Additionally, higher sunshine hours, as seen in 2024, may improve grape quality, compensating for water deficits. These findings emphasize the need for adaptive viticulture strategies to maintain productivity under changing climatic conditions.

Table 2. Precipitation trends and characterisation of the years 2021-2024 in the Miniș-Măderat vineyard

Year	Precipitation, L/m^2		The rainfall rating of the year according to the Hellmann criterion	Mean deviation (%)
	Total/year	Growing season		
2021	970.5	431.0	Extremely rainy	55,75
2022	560.3	461.2	Normal	-10,08
2023	924,95	415,7	Very rainy	48,44
2024	619,2	391,9	Normal	-0,63
2001 (C)	826.0	569.9	Slightly rainy	17.8

The rainfall classification of the year according to the Hellmann criterion/Deviation from the multi-year mean (%) (Extremely dry < -50%; Very dry -31% to -50%; Dry -21% to -30%; Slightly dry -11% to -20%; Normal -10% to 10%; Slightly rainy 11% to 20%; Rainy 21% to 30%; Very rainy 31% to 50%; Extremely rainy > 50%).

To more accurately assess the suitability of the Miniș-Măderat wine-growing area for viticulture based on its climatic resources, several climatic indices were calculated. Regarding temperature resources, it was observed that in all four years of the research, both the global heat balance values and the active and useful heat balance values exhibited a consistent increase. This trend clearly reflects the global warming phenomenon.

The warmest year in the entire research cycle was 2024, with its heat balance values showing significant positive deviations when compared to both the reference year (2001) and the multiannual average. Furthermore, the average values of the global active and useful heat balance over the four research years were notably higher than those of the reference year and the multiannual average. These findings suggest that in the Miniș-Măderat area, there has been a slight excess of temperature in recent years, which has considerable implications for the accumulation of sugars and the timing of the grape harvest.

The rise in temperature resources in the Miniș-Măderat region aligns with global warming

trends affecting viticulture. Studies by Jones et al. (2024) and Dominguez et al. (2022) highlight increased temperatures, earlier grape harvests, and higher sugar content, impacting grape composition and wine quality. Similarly, Fraga et al. (2023) note that rising heat balances can alter grape acidity and flavour. These trends are consistent across Mediterranean and European regions, including the Miniș-Măderat area, suggesting climate change's significant impact on viticulture. The warming influences sugar accumulation, harvest timing, and potentially wine characteristics.

Table 3. The main bioclimatic indicators values in the Miniș-Măderat vineyard from 2021 to 2024

Year	Σ t°g	Σ t°a	Σ t°u	CH	I-ar-DM	Ihr	Ibcv	IAOe	IH
2021	4586.0	3917.0	1913.5	1.2	43.3	2.9	6.1	4911.0	2247.9
2022	4943.7	4423.4	2152.4	1.3	23.9	2.6	4.9	4762.5	2507.4
2023	5276.7	4621.2	2353.5	2.0	37.57	5.6	7.4	6835.5	2523.5
2024	5739.6	5077.1	2577.2	1.2	24.53	6.6	10.9	5874.1	2937.2
Mean 2021-2024	5136.5	4509.6	2249.1	1.4	32.32	4.4	7.32	5595.7	2554.0
Mean 1991-2000	4361.5	3831.4	1756.1	1.2	31.8	2.1	6.1	4470.1	2142.0
2001(C)	4141.4	3636.4	1536.4	1.9	43.0	1.7	3.2	3972.7	1701.9
Mean 2001-2020	4724.6	4214.1	1971.7	0.9	26.2	2.6	9.6	4952.1	2362.8

Global heat balance (Σt°g); active heat balance (Σt°a); useful heat balance (Σt°u); hydrothermal coefficient (CH); Martonne aridity index (I-ar-DM); real heliothermal index (IHr); viticultural bioclimatic index (Ibcv); oenoclimatic aptitude index (IAOe); Huglin heliothermal index (IH); C-control.

The hydrothermal coefficient (CH) is a key indicator that reflects the water resources of a wine-growing area in relation to its thermal resources. The values of this coefficient in the Miniș-Măderat area throughout the four years of research indicate that the region offers very favourable conditions for producing quantitatively and qualitatively balanced yields. It is generally accepted that the most favourable values for grapevine cultivation fall between 1.1 and 2, and based on this range, the Miniș-Măderat region can be considered stable for viticulture (Table 3).

The real heliothermal index and the Huglin heliothermal index jointly describe the light and temperature resources available to a wine-growing area. The real heliothermal index in the Miniș-Măderat vineyard showed an upward trend compared to the reference year (2001). Throughout the four years of research, the heliothermal resources of the area proved to be favourable for grapevine cultivation, with the 2024 values suggesting a slight excess in light and heat, approaching the upper limit of favourability. While 2021 and 2022 values exceeded those of the reference year, they remained close to the multiannual average for the region. The Huglin heliothermal index (IH) demonstrated a similar dynamic to the real heliothermal index (IHr). Based on this index, 2024 again stands out as a year with a slight excess of light and temperature, approaching the maximum threshold for optimal grapevine growth. The bioclimatic viticultural index (Ibcv), which integrates the effects of temperature, light, and insolation, reflects the overall conditions for grapevine cultivation. Values between 5 and 15 are considered favourable, and in all four years of research, the values of this index were higher than those of the reference year, which was notably rainy. These values were also close to the multiannual average, indicating stable conditions for viticulture without significant imbalances in temperature, light, and humidity.

The only exception was the reference year of 2001, which, due to being one of the rainiest years in recent history, saw values of the bioclimatic viticultural index fall outside the optimal range for grapevine cultivation. Given that the Miniș-Măderat Vineyard is renowned for its red wines, the oenoclimatic aptitude index (OAI) was calculated to assess the region's potential for producing red wines. A vineyard is considered to have medium favourability for red wine production when OAI values exceed 4300, and high favourability when the values exceed 4600. In all four years of the study, the OAI values for Miniș-Măderat were well above 4300, demonstrating

that the region is highly favourable for red wine production.

These values were consistently superior to both the reference year of 2001 and the multiannual average. These findings emphasize the importance of heliothermal indices in predicting vine performance. The hydrothermal coefficient, as discussed by Fraga et al. (2023), also highlights favourable conditions for grapevine cultivation in regions with a coefficient between 1.1 and 2. The results from Miniș-Măderat suggest a stable and high-quality grape production environment, particularly for red wines. These findings are consistent with those from other wine-producing regions.

Table 4. The evolution of grape maturation in the 'Fetească Regală' variety in the Miniș-Măderat vineyard from 2021 to 2024

Year	Variables	Date of observations												
		July	August						September					
		25-31	1-5	6-10	11-15	16-21	22-26	27-31	1-5	6-10	11-15	16-21	22-26	27-30
2021	Sugar (g/l)				131	135	139		157	189	200			
	Total acidity (g/l Tartaric acid)				18.2	12.2	10		9.3	7.5	6.2			
	100 berry weight (g)				83	87	110		135	155	162			
2022	Sugar (g/l)			136	137		140	164	196	204				
	Total acidity (g/l Tartaric acid)			15.4	14.9		11.1	10.9	8.8	6.9				
	100 berry weight (g)			100	118		148	154	164	170				
2023	Sugar (g/l)			158		175	189	196		204	207			
	Total acidity (g/l Tartaric acid)			15.8		13.2	12.1	9.7		8.6	6.2			
	100 berry weight (g)			122		138	144	156		165	172			
2024	Sugar (g/l)	149	161		173	187	204		212					
	Total acidity (g/l Tartaric acid)	14.8	13.5		13.1	9.5	8.4		6.1					
	100 berry weight (g)	128	129		131	138	139		142					
2001 (Mt)	Sugar (g/l)							128	132		144	179	187	192
	Total acidity (g/l Tartaric acid)							19.3	14.9		10.1	8.7	7.4	6.9
	100 berry weight (g)							116	129		141	147	159	165

Regarding the evolution of grape ripening in the 'Fetească Regală' variety, it was observed that in all four years of research, the grapes reached maturity and accumulated significantly higher amounts of sugar compared to the reference year 2001.

The year 2024 was particularly notable, as the ripening process began at the end of July, with the grapes reaching technological maturity in the first days of September (Table 4). For the 'Pinot Noir' variety (Table 5), the evolution of grape ripening throughout the research cycle followed a similar pattern to that observed in the 'Fetească Regală' variety. For this variety as well, 2024 was the year with the earliest grape ripening and the highest sugar accumulation, while 2021 had the latest ripening and the lowest sugar levels. Compared to 2001, grape ripening in the 'Pinot Noir' variety occurred earlier, and sugar accumulation was higher in all four research years. Similar findings have been reported in other wine-growing regions. Jones et al. (2024) observed that rising temperatures across European vineyards have led to earlier grape ripening and increased sugar accumulation, particularly in early-ripening varieties like 'Pinot Noir'. Additionally, a study by van Leeuwen and Darriet (2016) in the Bordeaux region confirmed that warmer growing seasons accelerate phenological stages, leading to earlier harvests and higher sugar content, which directly influences wine quality and alcohol levels.

Table 5. The evolution of grape maturation in the 'Pinot noir' variety in the Miniș-Măderat vineyard from 2021 to 2024

Year	Variables	Date of observations												
		July	August						September					
		25-31	1-5	6-10	11-15	16-21	22-26	27-31	1-5	6-10	11-15	16-21	22-26	27-30
2021	Sugar (g/l)				94	108	125		153	185	202			
	Total acidity (g/l Tartaric acid)				17.8	15.3	11.1		8.9	6.7	5.9			
	100 berry weight (g)				72	83	113		124	138	140			
2022	Sugar (g/l)			108	125		153	185	192		210			
	Total acidity (g/l Tartaric acid)			15.2	14.9		13.3	9	6.4		5.9			
	100 berry weight (g)			107	125		127	130	132		138			
2023	Sugar (g/l)			172		180	196	212		220	238			
	Total acidity (g/l Tartaric acid)			16.3		14.7	13.2	11.4		8.3	6.1			
	100 berry weight (g)			107		116	125	132		139	142			
2024	Sugar (g/l)	140	163		172	197	212		230					
	Total acidity (g/l Tartaric acid)	18,3	16,2		13.9	12.9	8.1		6.3					
	100 berry weight (g)	112	115		120	127	140		147					
2001 (C)	Sugar (g/l)							89	105	124	173	184	191	196
	Total acidity (g/l Tartaric acid)							18.9	16.3	14.7	10.2	8.4	7.8	6.9
	100 berry weight (g)							110	116	121	129	136	139	141

These studies align with the results from the Miniș-Măderat vineyard, where the increased heat accumulation in recent years has advanced the ripening process and enhanced sugar levels in both 'Pinot Noir' and 'Fetească Regală' varieties.

For the 'Cabernet Sauvignon' variety (Table 6), it was observed that in all four years of research, the gap in reaching maturity between this variety and the other two was significantly smaller compared to 2001. Additionally, the date of technological maturity for the 'Cabernet Sauvignon' variety occurred earlier in all four research years than in the control year 2001. The highest sugar accumulations were recorded in 2024 and 2023, with all four research years showing superior sugar levels compared to the control year.

Table 6. The evolution of grape maturation in the 'Cabernet Sauvignon' variety in the Miniș-Măderat vineyard from 2021 to 2024

Year	Variables	Date of observations													
		July	August						September						October
		25-31	1-5	6-10	11-15	16-21	22-26	27-31	1-5	6-10	11-15	16-21	22-26	27-30	1-5
2021	Sugar (g/l)					71	83		110	155		180	185		
	Total acidity (g/l Tartaric acid)					19.1	16.7		14.8	11.5		9.6	7.3		
	100 berry weight (g)					61	85		100	107		120	129		
2022	Sugar (g/l)			96	115		144	157	169		183	190			
	Total acidity (g/l Tartaric acid)			21.8	20.4		18.3	12.7	9.5		7.2	7.0			
	100 berry weight (g)			96	102		116	119	125		129	131			
2023	Sugar (g/l)			155	170		182	199		207	210	214			
	Total acidity (g/l Tartaric acid)			19.7	17.5		13.2	10.9		8.6	7.4	6.9			
	100 berry weight (g)			90	105		114	121		129	132	133			
2024	Sugar (g/l)	121	156		160	189	210	221	223						
	Total acidity (g/l Tartaric acid)	21	17.9		13.7	11.8	7.8	6.9	6.6						
	100 berry weight (g)	87	91		94	95	102	109	112						
2001 (Mt)	Sugar (g/l)								75	87	118	145	169	180	183
	Total acidity (g/l Tartaric acid)								19.7	18.5	16.9	10.6	9.7	7.9	7.6
	100 berry weight (g)								75	83	89	102	114	123	126

Similar results have been reported in other wine-growing regions. According to Duchêne et al. (2010), rising temperatures in Bordeaux have led to earlier ripening and increased sugar accumulation in late-ripening varieties like 'Cabernet Sauvignon'. Likewise, a study by

Webb et al. (2023) in Australian vineyards found that climate warming shortens the ripening period and advances harvest dates across multiple grape varieties. These findings align with the results from the Miniș-Măderat vineyard, where the 'Cabernet Sauvignon' variety reached technological maturity earlier and exhibited higher sugar accumulation in all four research years compared to the control year. From the summary table (Table 7), it was observed that all varieties recorded higher sugar content values at technological maturity during the research years compared to the reference year. The year with the highest sugar accumulation potential was 2024 for the 'Fetească Regală' and 'Cabernet Sauvignon' varieties, and 2023 for the 'Pinot Noir' variety. The differences recorded relative to the control were significant, approximately 40 grams of sugar per liter, corresponding to an increase in the must's alcoholic potential of over 2.0-2.5% vol. alc. Among the all varieties, the lowest sugar accumulation values compared to the control year were recorded in 2021, which was characterized by exceptionally high rainfall. Regarding the achievement of technological maturity, substantial differences between the research years and the reference year (2001) were evident for all varieties. While technological
maturity was reached at the end of September and the beginning of October in the reference year, in 2024, it occurred in the first days of September. This indicates an advancement of 26-29 days compared to the control, depending on the variety. The results indicate a clear trend of increased sugar accumulation in all studied varieties during the research years compared to the reference year. Similar studies have shown that climatic variations, particularly temperature increases and reduced rainfall during the ripening period, contribute to higher sugar concentrations in grapes (Costea et al., 2023). The significant differences observed, particularly in 2024 for 'Feteasca Regală' and 'Cabernet Sauvignon' and in 2023 for 'Pinot Noir', align with previous research highlighting the influence of warmer growing seasons on sugar accumulation and earlier maturation (Cichi et al., 2024). The advancement of technological maturity by 26-29 days compared to the control year reflects the impact of climate change on grapevine phenology, as supported by similar findings in other viticultural regions (Bucur & Dejeu, 2022). The lower sugar accumulation recorded in 2021, an exceptionally rainy year, confirms the negative effect of excessive precipitation on grape ripening. These findings emphasize the increasing variability in grape development and the potential need for adaptive vineyard management strategies to maintain grape quality in the face of changing climatic conditions (Stroe, 2021).

Table 7. Results on must sugar concentration and the achievement of technological maturity in the varieties from the Miniș-Măderat vineyard during the 2021-2024 period

Year	Variety	Sugar concentration of the must at technological maturity (g/l)	Difference to control (C)	Date of reaching technological maturity	Difference compared to C, for technological maturity (days)
2021	'Feteasca regala' (FR21)	200	8	16.09	-12
	'Pinot noir' (PN21)	202	6	17.09	-12
	'Cabernet Sauvignon' (CS21)	185	2	23.09	-11
2022	'Feteasca regala' (FR22)	204	12	12.09	-16
	'Pinot noir' (PN22)	210	14	13.09	-16
	'Cabernet Sauvignon' (CS22)	190	7	17.09	-17
2023	'Feteasca regala' (FR23)	207	15	14.09	-14
	'Pinot noir' (PN23)	238	42	15.09	-14
	'Cabernet Sauvignon' (CS23)	214	31	16.9	-18
2024	'Feteasca regala' (FR24)	212	20	02.09	-26
	'Pinot noir' (PN24)	230	34	02.09	-27
	'Cabernet Sauvignon' (CS24)	223	40	05.09	-29
Mean 2021-2024	Feteasca regala (FR-M)	205.7	13.7	-	-
	Pinot noir' (PN-M)	220	24	-	-
	Cabernet Sauvignon' (CS-M)	203	20	-	-
2001 (C)	Feteasca regala' (FR-C)	192	-	28.09	-
	Pinot noir' (PN-C)	196	-	29.09	
	Cabernet Sauvignon' (CS-C)	183	-	04.10	

Principal Component Analysis (PCA) revealed that the first principal component (F1) accounted for 67.72% of the total variance, while the second principal component (F2) explained 14.45% (Figure 1). The position of the 'Cabernet Sauvignon' control sample (CS-C) indicates delayed technological maturity and lower sugar accumulation under control treatment. The samples from 2021 (CS21) and 2022 (CS22) demonstrated a moderate response to environmental factors, although sugar accumulation remained below the levels observed in more favourable years. In contrast, the 2023 (CS23) and 2024 (CS24) samples suggest a substantial increase in sugar accumulation, coupled with higher levels of sunlight exposure and elevated temperatures. This positioning indicates that 'Cabernet Sauvignon' exhibited a strong positive response to favourable environmental conditions during these years. The position of the 'Cabernet Sauvignon' (CS-M) across the 2021-2024 period reflects an average response across all growing seasons and experimental conditions.

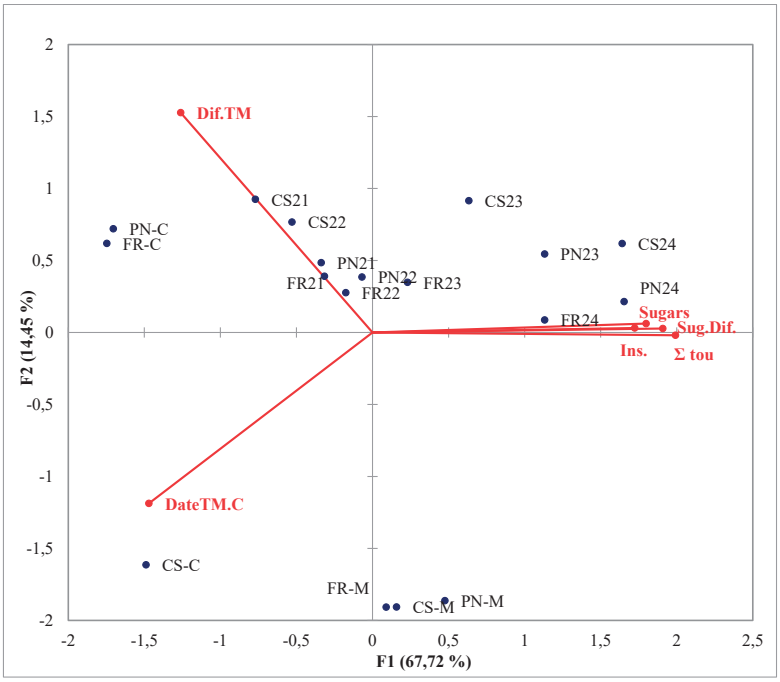


Figure 1. The PCA biplot for the relationships among variables and treatments (2021-2024)
Fr – Feteasca Regala; PN – Pinot Noir; CS – Cabernet Sauvignon; FR-M - Feteasca Regala Mean; PN-M = Pinot Noir Mean; CS-M = Cabernet Sauvignon Mean; FR-C - Feteasca Regala Control; PN-C = Pinot Noir Control; CS-C = Cabernet Sauvignon Control; SugDif = sugars difference to control; DataTM – Technological Maturity Date; Ins – insolation; Σ t°u – temperature amount

Among the three varieties, 'Cabernet Sauvignon' displayed the most pronounced response to environmental factors, particularly in 2023 and 2024, when sugar accumulation and associated variables reached their highest levels. The control sample of 'Pinot Noir' (PN-C), similar to the 'Feteasc Regal' control (FR-C), suggests reduced sugar accumulation and delayed technological maturity under control conditions. The 2021 (PN21) and 2022 (PN22) samples indicate moderate improvements in sugar accumulation and other growth-related parameters compared to the control. The 2023 (PN23) and 2024 (PN24) samples reflect higher sugar accumulation and improved exposure to environmental factors. The position of the Pinot Noir' (PN-M) over the 2021–2024 period suggests an average performance across all years and conditions. Overall, 'Pinot Noir' showed progressive improvements over time, with 2023 and 2024 demonstrating increased sugar accumulation and a more pronounced influence of environmental factors. The 'Feteasc Regal' control sample (FR-C), similar to PN-C, indicates lower sugar accumulation and a

limited response to environmental variables. The 2021 (FR21) and 2022 (FR22) samples showed slight improvements in sugar content and environmental influences compared to the control group. However, the 2023 (FR23) and 2024 (FR24) samples remained positioned behind 'Cabernet Sauvignon' and 'Pinot Noir' along the F1 axis. Notably, FR24 is closer to the positive side of F1, suggesting increased sugar accumulation and more favourable environmental conditions in this year. The position of FR-M (mean for 2021-2024) indicates a weaker response across all growing seasons compared to the other two varieties. 'Feteasca Regală' exhibited the weakest response to environmental factors. Nevertheless, the more favourable position of FR24 indicates some improvement in sugar accumulation in 2024 compared to previous years. These results emphasize the strong influence of environmental conditions - particularly temperature and sunlight exposure - on sugar accumulation and grape maturity, with 'Cabernet Sauvignon' being the most responsive and 'Feteasca Regală' the least.

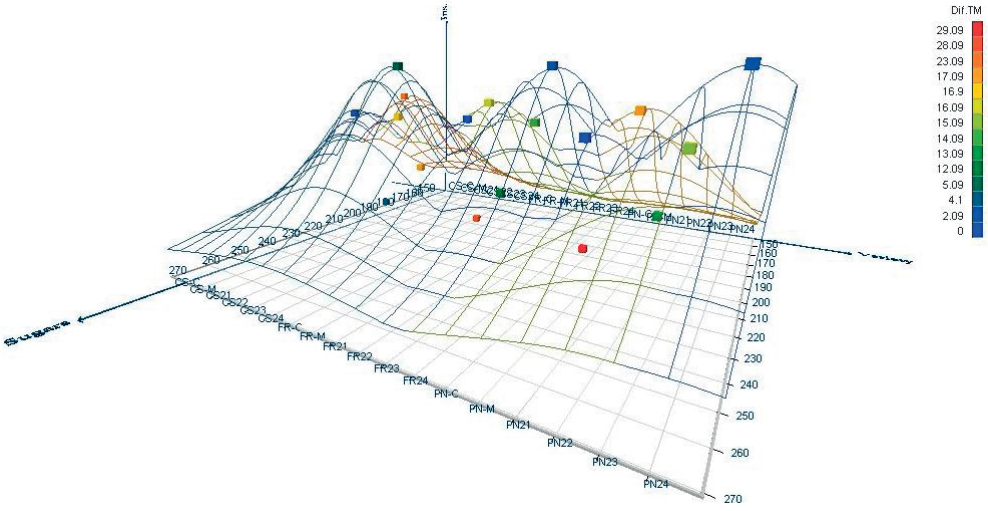


Figure 2. Variation in sugar content, insolation hours, and grape varieties in the Miniș-Măderat Vineyard (2021-2024)
Sugar content (Sugars) – X-axis; Insolation (Ins.) – measured in hours – Y-axis; Grape varieties across different years (Variety) – Z-axis.
The color gradient represents Delta TMC (Temperature Mean Change), with values ranging from 0 (red, indicating no temperature change) to -29 (dark blue, indicating a significant decrease)

The 3D surface plot illustrates the relationship among three variables: sugar content (X-axis), insolation measured in hours (Y-axis), and grape varieties across different years (Z-axis) (Figure 2). Additionally, the colour gradient represents Delta TMC (Temperature Mean Change), with values ranging from 0 (red, indicating no temperature change) to -29 (dark blue, indicating a significant decrease). The analysis of sugar content across grape varieties ('Cabernet Sauvignon' - CS, 'Feteasca Regală' - FR, and 'Pinot Noir' - PN) from 2021 to 2024 reveals a wide range of sugar accumulation. In general, later years (particularly 2023 and 2024) exhibit higher sugar content, as reflected by the rightward shift of data points along the X-axis. Notably, the CS24 ('Cabernet Sauvignon', 2024) and PN24 ('Pinot Noir', 2024) samples demonstrate the highest sugar accumulation, suggesting favourable environmental conditions for sugar synthesis. Increased insolation positively correlates with sugar content across most varieties. Samples from 2024 (CS24, PN24, FR24) display the highest insolation hours, reinforcing the role of increased sunlight in enhancing sugar accumulation. 'Cabernet Sauvignon' shows the most pronounced increase in sugar content and insolation between 2021 and 2024, suggesting a heightened sensitivity to environmental changes. 'Pinot Noir' demonstrates a progressive increase in sugar accumulation,

with a marked improvement in 2023 and 2024. In contrast, 'Fetească Regală' consistently shows the lowest sugar accumulation, although a slight increase is observed in 2024. Overall, the plot reveals a positive correlation between sugar content and insolation hours across all grape varieties, with 2023 and 2024 being the most favourable years for sugar accumulation. 'Cabernet Sauvignon' exhibits the strongest response to environmental variations, while 'Fetească Regală' remains the least responsive. Despite minor temperature fluctuations indicated by insolation emerges as the primary factor influencing sugar content throughout the study period.

CONCLUSIONS

The climatic resources of the Miniș-Măderat wine-growing region have undergone significant changes over the four years of research compared to both the reference year 2001 and the multiyear averages. Solar radiation during all research years was considerably higher than in the reference year, with 2024 standing out, as solar radiation exceeded the reference by more than 600 hours. In terms of precipitation, the research years were extremely variable, ranging from the exceptionally rainy year of 2021 to a normal year in 2024, according to Hellmann's criterion. This irregularity was also evident in the amount of rainfall during the growing season, where all years recorded values lower than the reference. The synthetic bioclimatic indicators calculated based on climatic resources show that, despite recent changes, the Miniș-Măderat wine-growing region continues to offer favourable and highly favourable conditions for viticulture. The Oenoclimatic Aptitude Index (IAOe) indicates that, in recent years, the Miniș-Măderat region provides very favourable conditions for cultivating red grape varieties, despite the clear evidence of global warming as shown by the active and useful thermal balance. The Viticultural Bioclimatic Index (Ibcv), which integrates the effects of three climatic factors (temperature, sunlight, and radiation), reveals that while climatic disturbances are evident, these three factors remain in balance within the Miniș-Măderat region. As a result, the area continues to provide some of the most favourable conditions for grapevine cultivation in Romania. As a consequence of the evident climatic changes compared to the reference year 2001, both the grape ripening dynamics and the qualitative parameters at technological maturity have changed significantly. For all varieties, there was a clear increase in must sugar content, sometimes exceeding 40 grams per litre, resulting in an increase in the alcoholic potential of the must by up to 2.0-2.5% vol alc. Due to the new climatic reality, significant differences were also observed regarding the time of reaching technological maturity. In all four research years, especially in 2024, grape varieties reached technological maturity much earlier, at the beginning of September, recording an advancement of 26 to 29 days compared to the reference year 2001.

REFERENCES

Arias-Pérez, I., Sáenz-Navajas, M. P., De-La-Fuente-Blanco, A., Ferreira, V., & Escudero, A. (2021). Insights on the role of acetaldehyde and other aldehydes in the odour and tactile nasal perception of red wine. *Food Chemistry*, *361*, 130081.

Bucur G.M., Dejeu L., (2022), Research on adaptation measures of viticulture to climate change: overview, *Scientific Papers. Series B, Horticulture. Vol. LXVI, No. 2*, Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

Bucur G. M., Dejeu L. (2024), Phenological and some eno-carpological traits of thirteen new Romanian grapevine varieties for white wine (V*itis vinifera* L.) In the context of climate change, *Scientific Papers. Series B, Horticulture. Vol. LXVIII,* No. 1, Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

Cichi, D. D., Capruciu, R., Stoica, F., Cichi, M., Costea, D. C., & Mărăcineanu, L. C. (2024). Ampelographic and bioproductive characteristics of Cabernet Sauvignon clones in Oltenia winegrowing region, South-West Romania. *Scientific Papers. Series B. Horticulture*, *68*(2), 262-269.

Costa, J. M., Egipto, R., Aguiar, F. C., Marques, P., Nogales, A., & Madeira, M. (2023). The role of soil temperature in mediterranean vineyards in a climate change context. *Frontiers in Plant Science*, *14*, 1145137.

Costea, D. C., Căpruciu, R., Cichi, D. D., & Băducă Cîmpeanu, C. (2023). Research concerning the reaction of vines to variable climatic conditions. *Scientific Papers. Series B. Horticulture*, *67*(2).224-229

De Orduna, R. M. (2010). Climate change associated effects on grape and wine quality and production. *Food research international*, *43*(7), 1844-1855.

Dobrei, A., Nistor, E., Daniela, S., & Dobrei, A. G. (2023). Local microclimates and climate changes influence on cultivation techniques, grapevine production and quality. *International Multidisciplinary Scientific GeoConference: SGEM*, *23*(4.1), 307-316.

Dobrei, A. G., Nistor, E., Dobromir, D., Petcov, D. Z., & Dobrei, A. I. (2021). Evaluation of grape yield and quality of some red wines varieties from Minis-Maderat vineyard. *JOURNAL of Horticulture, Forestry and Biotechnology, Volume 25(2)*, 74-79.

Dominguez, D. L., Cirrincione, M. A., Deis, L., & Martínez, L. E. (2024). Impacts of climate change-induced temperature rise on phenology, physiology, and yield in three red grape cultivars: Malbec, Bonarda, and Syrah. *Plants*, *13*(22), 3219.

Duchêne, E., Huard, F., Dumas, V., Schneider, C., & Merdinoglu, D. (2010). The challenge of adapting grapevine varieties to climate change. *Climate research*, *41*(3), 193-204.

Filimon, R. M., Rotaru, L., Filimon R.V. (2023). Effects of exogenous growth regulators on agrobiological, technological and physiological characteristics of an interspecific grapevine cultivar, *Biological Agriculture & Horticulture, Volume 39*, Issue 2, pp. 91-114, Print ISSN: 0144-8765 Online ISSN: 2165-0616 (IF = 864), WOS:000860609200001.

Fraga, H., Freitas, T. R., Moriondo, M., Molitor, D., & Santos, J. A. (2024). Determining the Climatic Drivers for Wine Production in the Côa Region (Portugal) Using a Machine Learning Approach. *Land*, *13*(6), 749.

Hannah, L., Roehrdanz, P. R., Ikegami, M., Shepard, A. V., Shaw, M. R., Tabor, G., ... & Hijmans, R. J. (2013). Climate change, wine, and conservation. *Proceedings of the national academy of sciences*, *110*(17), 6907-6912.

Jones, G. V., White, M. A., Cooper, O. R., & Storchmann, K. (2005). Climate change and global wine quality. *Climatic change*, *73*(3), 319-343.

Jones, F. A., Bogdanoff, C., & Wolkovich, E. M. (2024). The role of genotypic and climatic variation at the range edge: A case study in winegrapes. *American Journal of Botany*, *111*(1), e16270.

Mustea M., Rotaru Liliana., Irimia L. M., Răileanu M. (2011). Comportarea soiurilor Fetească neagră, Cabernet Sauvignon şi Merlot în centrul viticol Bohotin, din podgoria Iaşi . *Lucrări ştiinţifice USAMV Iaşi, seria Horticultură, vol. 54* I.S.S.N 1454-7376

Palliotti, A., Tombesi, S., Silvestroni, O., Lanari, V., Gatti, M., & Poni, S. (2014). Changes in vineyard establishment and canopy management urged by earlier climate-related grape ripening: A review. *Scientia Horticulturae*, *178*, 43-54.

Sadras, V. O., Moran, M. A., & Bonada, M. (2013). Effects of elevated temperature in grapevine. IBerry sensory traits. *Australian Journal of Grape and Wine Research*, *19*(1), 95-106.

Stroe, M., Budescu, C. (2013). Comparative study regarding the influence of biostimulators on the qualitative and quantitative potential of Cabernet Sauvignon. *Scientific Papers, Series B, Horticulture, Vol. LVII*,, ISSN 2285-5653, pp. 115-120. WOS:000450195500022.

Stroe, M. V. (2021). Genetic diversity the viticultural germplasm fund of Romania – news accessions. *Scientific Papers. Series B. Horticulture, Vol. LXV,* no.1, ISSN 2285-5653, pp. 342-351, WOS:000712336700049.

Van Leeuwen, C., & Darriet, P. (2016). The impact of climate change on viticulture and wine quality. *Journal of Wine Economics*, *11*(1), 150-167.

Van Leeuwen, C., & Destrac-Irvine, A. (2017). Modified grape composition under climate change conditions requires adaptations in the vineyard. *Oeno One*, *51*(2-3), 147-154.

Webb, L., Tozer, C., Bettio, L., Darbyshire, R., Robinson, B., Fleming, A., ... & Prakash, M. (2023). Climate services for agriculture: Tools for informing decisions relating to climate change and climate variability in the Wine industry. *Australian Journal of Grape and Wine Research*, *2023*(1), 5025359.

Williamson, T. N., Fitzpatrick, F. A., Kreiling, R. M., Blount, J. D., & Karwan, D. L. (2024). Sediment budget of a Maumee River headwater tributary: how streambank erosion, streambed-sediment storage, and streambed-sediment source inform our understanding of legacy phosphorus. *Journal of Soils and Sediments*, *24*(3), 1447-1463.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

OPTIMIZING PRODUCTIVITY AND FRUIT QUALITY OF 'MUSCAT HAMBURG' CULTIVAR THROUGH PRUNING INTENSITY

Sergiu-Ayar ENE[1, 2], Ionica DINA[1], Anamaria TĂNASE[1], Traian-Ștefan COSMA[1], Aurora RANCA[1], Răzvan-Ionuț TEODORESCU[2]

[1]Research Station for Viticulture and Oenology Murfatlar, 2 Calea București, Murfatlar, Constanța County, Romania
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author email: razvan.teodorescu@usamv.ro

Abstract

Pruning is a key practice in viticulture, influencing grapevine productivity, fruit quality, and plant health. As the climate evolves, adapting pruning strategies to optimize grape yield and quality becomes increasingly vital. This study was conducted in the grapevine fields of the Research Station for Viticulture and Oenology Murfatlar, Romania, on 'Muscat Hamburg', a table grape cultivar. Three pruning variants were evaluated over a 3-year span (2022-2024): V1 (24 buds), V2 (32 buds), and a control (38 buds). The impact of these variants was assessed on parameters such as total shoots, fertile shoots, fertility percentage, cluster weight, weight of 100 berries, sugars, total acidity and pH. Climate data were analyzed and statistical analysis was performed to compare the variants. Results show that V1 outperformed V2 and the control in fertility percentage (72.9%), cluster weight (195 g), and weight of 100 berries (256 g). Additionally, the glucoacidimetric ratio was highest in V1 (70.1) compared to V2 and the control. And pH values (3.50) were similar across all variants. These findings indicate that pruning with 26 buds optimizes grapevine productivity and fruit quality.

Key words*: pruning, climate change, grapevine productivity, cluster weight, optimisation.*

INTRODUCTION

The manipulation of crop yield is a well-established practice. In commercial vineyards, techniques such as vine pruning, which reduces the number of cluster-bearing buds, and crop thinning, which lowers the number of grape clusters, are commonly employed to control yield. Since yield directly influences the quantity of wine produced during a harvest, and considering the ease with which it can be adjusted, along with the longstanding belief that higher yields negatively impact wine quality, it is surprising that there has been limited research on how crop yield affects the aromas and flavors of wine (Chapmant et al., 2004).

Building upon the established practice of yield manipulation, in Romania, the sustainable development of the viticulture sector, particularly for table grape varieties like 'Muscat Hamburg', depends on the precise management of agronomic practices (Cuharschi et al., 2019). These include vine pruning, green operations, irrigation, and pest control, all aimed at enhancing grape quality and cluster weight (Gatti et al., 2016). Although the overall grape yield may suffer, optimizing the bud load through pruning can lead to significant improvements in the weight of the clusters, which is crucial for producing high-quality table grapes (Davel, 2015).

Variations in the number of growing fruits not only directly affect yield but can also lead to negative impacts on the size and quality of the harvested organs (Kliewer & Dokoozlian, 2005). Bud fertility (the number of bunches per shoot) and the number of flowers per bunch are closely related, with the primary branching of inflorescences playing a significant role in determining the total number of flowers within each inflorescence (Dunn & Martin, 2007).

Some studies show that the primary factors influencing grapevine yield are the number of bunches per vine and the number of berries per bunch, which contribute approximately 60%

and 30% to seasonal yield variation, respectively. In contrast, berry weight accounts for only around 10% of the seasonal yield variation (Clingeleffer et al., 2003).

Various techniques have been employed for yield manipulation in viticulture, including shoot trimming, post-veraison distal leaf removal, late winter pruning, and double pruning, all aimed at mitigating the effects of climate change by delaying maturation and preserving acidity under fluctuating temperatures (De Toda et al., 2013). However, their effectiveness varies depending on environmental conditions and grape varieties, requiring further research to determine their long-term impact on grape quality and vineyard management (Zheng et al., 2017).

Given the variability in their effectiveness depending on environmental conditions and grape varieties, with appropriate training methods and pruning systems, the accumulation of aroma precursors can be promoted, enhancing the wine's sensory profile. Additionally, these systems can influence bunch weight, which directly impacts grape quality (Clingeleffer, 2010).

In response to climate evolution, various pruning systems can help reduce stress on the plant and improve grape quality. These systems are essential for optimizing vine health and fruit composition, supporting the plant in adapting to shifting environmental conditions (Martin & Dunn, 2000).

This study aimed to investigate the impact of varying bud load per cane on key physiological and biochemical parameters, including vine fertility, cluster weight, berry weight, and glucoacidimetric profiles. The study also examined how these pruning strategies, intended to mitigate the effects of climate change, influence grapevine metabolism, phytochemical composition, and overall grape quality.

MATERIALS AND METHODS

The study was conducted from 2022 to 2024 at the Murfatlar Research Station for Viticulture and Oenology, situated in the central part of the Dobrogean Plateau (44°10'36''N; 28°25'22''E) a semi-arid climate, typical of the Dobrogean Plain, due to low rainfall and high evapotranspiration (Chelcea et al., 2016). The research focused on the 'Muscat Hamburg' cultivar (Figure 1), an old variety that exhibits a high adaptability potential to temperate continental climates (Vujović et al., 2017).



Figure 1. 'Muscat Hamburg'

Winter pruning was performed at different lengths over three consecutive years, from 2022 to 2024. Three variants were tested: a control group with 38 buds, V1 with 24 buds, and V2 with 32 buds, each having different pruning lengths.

Inflorescences were identified using the multiplied horizontal sectioning method of the buds, which involved placing the sections on glass slides fused with distilled water for observation under the microscope. The microscope used for these observations was the Kern Optics OBN 135. The number of total (TS) and fertile shoots (FS) was counted, and the number of inflorescences (INF) per variant was assessed. The fertility percentage (F %) was calculated using the formula: F (%) = (100*TS/FS).

Fertility indices were used based on the percentage of fertile shoots, with the fertility coefficient being either absolute or relative. The absolute fertility coefficient (AFC) was calculated using the formula AFC = INF/FS, while the relative fertility coefficient (RFC) was calculated as RFC = INF/TS. Productivity is influenced by cluster weight (CW), which is specific to each variety which is specific to each variety and was determined using a RADWAG PS600 analytical balance with a precision error of ± 0.01 g. The absolute production index (API) was calculated using

the formula API = AFC * CW, and the relative production index (RPI) was calculated as RPI = RFC * CW. In addition, the total berry weight per vine in kilograms (TVW) was calculated using the formula INF x IPR (Pop, 2010). Post-harvest, research was conducted to characterize the quality of the grapes by determining the weight of 100 berries, sugar content (g/L), total acidity (g/L H_2SO_4), pH, and by calculating the glucoacidimetric index (GAI) which is calculated by the sugars/ total acidity ratio (Stanescu, 1960). The evaluation of grape maturity also considered commercial quality standards, including minimum GAI thresholds defined by OIV Resolution VITI 1/2008. (Bucur & Dejeu, 2018).
The climatic data from the vineyard for 2022, 2023, and 2024 were assessed using the iMetos 3.3 weather station by Pessl Instruments, located at the center of the plantation. Additionally, climatic indices were evaluated during the assessment (Irimia et al., 2017; Jolly et al., 2005). The real heliothermal index (IHr) was calculated using the formula IHr = I × Σtu (°C) × 10^{-6} (Branas, 1974). The hydrothermal coefficient (HC) was calculated using the formula HC = Pg / (Σta (°C) × 10), where *Pg* represents the total precipitation during the growing season (Zaldea et al., 2021). The vine bioclimatic index (Ibcv) was then calculated using the formula Ibcv = [(I × Σta (°C))/(Pg × Ndg)]/10, where *Ndg* is the number of days in the growing season with average temperatures above 10 °C (Constantinescu et al., 1964). Finally, the oenoclimate aptitude index (IAOe) was determined using the formula IAOe = I + Σta (°C) − (Pg − 250) (Teodorescu et al., 1987). Statistical analysis was conducted using Microsoft Excel 2021 and IBM SPSS Statistics 26 to process the data collected in this study. Descriptive statistics, including mean and standard deviation, were calculated for both continuous and categorical variables. Additionally, ANOVA was performed to determine significant differences among the pruning variants (Stahle & Wold, 1989).

RESULTS AND DISCUSSIONS

Across the three experimental years, fertility percentages exhibited substantial variation, as shown in Table 1, highlighting the influence of climatic conditions and pruning intensity.

Table 1. Bud fertility and cluster weight in 'Muscat Hamburg' under different pruning variants (2022-2024)

Variant	TS	FS	F%	INF	CW (g)
MH_C_2022	20	10	50.00	13	180
MH_V1_2022	16	10	62.50	15	187
MH_V2_2022	17	8	47.06	14	180
MH_C_2023	21	13	61.90	14	174
MH_V1_2023	16	13	82.48	16	210
MH_V2_2023	18	10	55.56	16	176
MH_C_2024	19	11	57.89	18	170
MH_V1_2024	19	14	73.68	14	189
MH_V2_2024	18	9	50.00	14	176

In 2022, fertility ranged from 47.06% (MH_V2) to 62.50% (MH_V1), with the control (MH_C) showing an intermediate value of 50.00%. A similar trend was observed in cluster weight, where the highest values were recorded for MH_V1 (187 g), while MH_C and MH_V2 maintained equal weights (180 g).
In 2023, improved fertility was observed across all variants, particularly in MH_V1 (82.48%), followed by MH_C (61.90%) and MH_V2 (55.56%), suggesting that the more intensive pruning strategy (V1) promoted enhanced bud differentiation and fruit set. This was accompanied by a significant increase in cluster weight, reaching 210 g in MH_V1, while MH_C (174 g) and MH_V2 (176 g) remained relatively stable.

Table 2. Tests of between-subjects effects on fertility across different pruning variants (2022-2024)

Dependent Variable:			Measurement		
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	782.707[a]	2	391.35	7.547	0.023
Intercept	32528.527	1	32528.52	627.303	0.000
Treatment	782.707	2	391.35	7.547	0.02
Error	311.127	6	51.855		
Total	33622.362	9			
Corrected Total	1093.835	8			

a. R Squared = 0.716 (Adjusted R Squared = 0.621)

In 2024, bud fertility declined in all variants, likely due to excessive rainfall and altered thermal conditions. The highest fertility was again observed in MH_V1 (73.68%), followed by MH_C (57.89%) and MH_V2 (50.00%), mirroring trends from previous years. Cluster weights showed a similar pattern, with MH_V1 (189 g) maintaining superiority, while MH_C

(170 g) and MH_V2 (176 g) displayed minor fluctuations.

Statistical analysis of the fertility data revealed a significant effect of pruning treatment on reproductive potential ($F = 7.547$, $p = 0.023$), as shown in Table 2.

The results indicated that the MH_V1 variant consistently outperformed the other treatments, exhibiting the highest fertility percentages across all years. Specifically, MH_V1 demonstrated a fertility of 82.48% in 2023 and 73.68% in 2024, which was notably higher than both the control (MH_C) and the MH_V2 variants. This suggests that the more intensive pruning applied in MH_V1 is more conducive to optimal bud differentiation and fruit set. This trend is clearly depicted in Figure 2, which shows the fertility percentages for each variant over the three years.

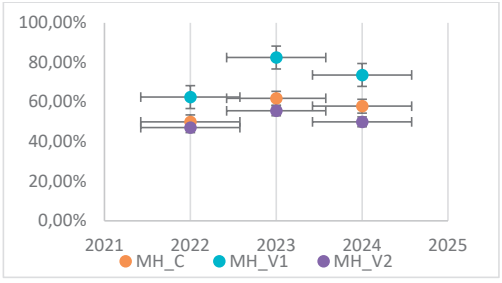


Figure 2. Fertility percentages (%) across different pruning variants (2022-2024)

The analysis further indicates that the treatment factor (pruning variant) explained a substantial portion of the variance in fertility, with the corrected model accounting for 71.6% of the total variability ($R^2 = 0.716$, Adjusted $R^2 = 0.621$). This suggested a strong influence of pruning intensity on bud fertility, with statistically significant differences observed between variants.

Cluster weight (CW) was also assessed to further evaluate the impact of pruning treatments. Table 3 presents the cluster weight data for each variant over the three years.

The ANOVA revealed a significant difference between the pruning variants ($F = 5.89$, $p = 0.038$), where the Between Groups variation explained 758.22 of the sum of squares, and the Within Groups variation accounted for 386. The F crit value was 5.14, confirming the statistical relevance of the observed differences.

Table 3. Cluster weight (g) for different pruning variants (2022-2024)

CW (g)	2022	2023	2024	Avg.	St. dev. ±
MH_C (g)	180	174	170	174.67	5.03
MH_V1 (g)	187	210	189	195.33	12.74
MH_V2 (g)	180	176	176	177.33	2.31

MH_V1 variant consistently resulted in the highest cluster weights, with an average of 195.33 g across the three years. In comparison, the MH_C variant had a lower average cluster weight of 174.67 g, and MH_V2 had an average of 177.33 g. The MH_V1 variant also exhibited a relatively higher standard deviation (± 12.74 g), indicating more variability in cluster weight compared to MH_C (± 5.03 g) and MH_V2 (± 2.31 g), which had more consistent results. These differences highlight the superior performance of MH_V1 in terms of cluster weight, but also the greater variability within that treatment.

The statistical significance of these differences was assessed using ANOVA, with the results presented in Table 4.

Table 4. ANOVA analysis of cluster weight variation across different pruning treatments (2022-2024)

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	758.22	2	379.11	5,89	0.04	5.14
Within Groups	386	6	64.33			
Total	1,144.22	8				

Further examination was conducted on several productivity-related indices, including the absolute fertility coefficient (AFC), relative fertility coefficient (RFC), absolute productivity index (API), and relative productivity index (RPI). As shown in Table 5, the MH_V1 variant exhibited the highest AFC in 2023, while MH_V2 demonstrated the highest RFC in 2022 and 2023.

However, regarding actual productivity, MH_V1 came close to MH_V2, which consistently demonstrated higher productivity. The average total cluster weight for MH_V2 was 4.25 kg, while MH_V1 averaged 3.66 kg. Despite MH_V2's higher overall weight, MH_V1 showed competitive performance, likely due to its more efficient conversion of fertile shoots into productive clusters in 2023.

Table 5. Productivity indices and total cluster weight for different pruning variants (2022-2024)

Variant	AFC	RFC	API	RPI	TVW
MH_C_2022	0.7	1.3	117.0	234.0	3.04
MH_V1_2022	0.9	1.5	175.3	280.5	4.21
MH_V2_2022	0.8	1.8	148.2	315.0	4.41
MH_C_2023	0.7	1.1	116.0	187.4	2.62
MH_V1_2023	1.0	1.2	210.0	258.5	4.14
MH_V2_2023	0.9	1.6	156.4	281.6	4.51
MH_C_2024	0.9	1.6	161.1	278.2	5.01
MH_V1_2024	0.7	1.0	139.3	189.0	2.65
MH_V2_2024	0.8	1.6	136.9	273.8	3.83

The climatic conditions over the three years studied (2022-2024) exhibited notable interannual variability. In 2022, the year was characterized by moderately dry conditions, with a total annual rainfall of 383.7 mm, out of which 206.7 mm occurred during the growing season. The hydrothermal coefficient (HC) was 0.6, and the oenoclimate aptitude index (IAOe) reached 5250.7, indicating a balanced but somewhat dry year. The average temperatures in July and August were 25.7 °C and 26.1 °C, respectively, while the average temperature during the first two-thirds of June was 22.2 °C. The number of days with temperatures exceeding 30 °C was 67, and the bioactive period lasted for 207 days, suggesting a typical thermal regime favorable for grape ripening.

In 2023, the climate turned drier and slightly warmer. Annual precipitation dropped to 318.1 mm, with only 170.9 mm during the growing season. Consequently, the HC decreased to 0.5, reflecting limited water availability, while the IAOe slightly decreased to 5197.2. Despite this, the annual average temperature increased to 14.8 °C, with July and August averaging 26.8 °C and 26.9 °C, respectively. September also showed a notable rise, averaging 22.2 °C. However, the number of hot days (>30 °C) dropped to 62, and the bioactive period slightly shortened to 205 days. Sunshine duration was also lower than in 2022, totaling 1311.8 hours, which may have contributed to a more compressed growing season. Despite these limitations, the useful thermal sum (Σtu) was relatively high at 2398.3 °C, and the vine bioclimatic index (Ibcv) reached 13.9, indicating favorable ripening potential.

By 2024, climatic conditions shifted toward higher humidity, with the annual rainfall increasing to 406.2 mm, and growing season precipitation reaching 345.6 mm, the highest of the three years. As a result, the HC rose to 1.1, and the year was classified as humid.

The Ibcv dropped to 7.3, suggesting lower bioclimatic suitability, likely due to excessive moisture.

However, thermal conditions remained very favorable, with a useful thermal sum of 3266 °C, the highest in the studied interval, and an IAOe of 5277.4, slightly surpassing the previous years. July temperatures peaked at 28.5 °C, and the average during the first two-thirds of June reached 25.8 °C, indicating an early onset of high thermal accumulation.

The number of days above 30 °C increased to 75, and the bioactive period extended to 211 days, reflecting an extended ripening phase. Nonetheless, sunshine duration remained the lowest, at 1301.1 hours, which might have moderated sugar accumulation despite the favorable thermal regime (Table 6).

Table 6. Climatic conditions and viticultural indices for 2022-2024

Year	2022	2023	2024
Global (Σt°g)	5020.6	5416.6	5136.9
Active, (Σt°a)	4549.4	4795.3	4792.9
Useful (Σt°u)	2208.8	2398.3	3266
Avg. T. July (°C)	25.7	26.8	28.5
Avg. T. August (°C)	26.1	26.9	26.1
Avg. T. September (°C)	19.6	22.2	19.8
Absolute min. air T. (°C/date)	-9.6	-9.8	-10.3
Absolute min. soil temperature (°C)	-12.1	-10.1	-12.2
Annual avg. T (°C)	13.7	14.8	14.5
Annual rainfalls (mm)	383.7	318.1	406.2
Growing season rainfalls (mm) (Pg)	206.7	170.9	345.6
Growing season sunshine (hours)	1400.8	1311.8	1301.1
Avg. max. T. August (°C)	32	31.9	31.6
Avg. T during 2/3 of June.	22.2	21.7	25.8
No days > 30°C (Ndg)	67	62	75
Bioactive period (days)	207	205	211
Ihr	4.5	4.4	9.7
HC	0.6	0.5	1.1
Ibcv	12.3	13.9	7.3
IAOe	5250.7	5197.2	5277.4
IH	2520.8	2434.3	3340.65

Variability in grape composition (sugar content and acidity) were closely linked to variations of the climatic conditions in each year (Table 6 and Table 7).

Table 7. Grape composition and quality assessment (2022-2024)

Variant	100 berries (g)	Sugars g/L	Total g/H_2SO_4	pH
MH_C_2022	176.00	181.30	2.80	3.56
MH_V1_2022	215.00	198.68	2.35	3.59
MH_V2_2022	194.00	182.50	2.77	3.60
MH_C_2023	290.00	181.67	3.65	3.55
MH_V1_2023	305.00	203.36	3.15	3.60
MH_V2_2023	300.00	179.76	3.54	3.55
MH_C_2024	211.00	179.94	3.86	3.35
MH_V1_2024	257.00	197.50	3.23	3.30
MH_V2_2024	224.00	184.43	3.66	3.40

MH_V1 variant consistently demonstrated the highest sugar content, with values of 198.68 g/L in 2022, 203.36 g/L in 2023, and 197.50 g/L in 2024. In comparison, the MH_C variant exhibited lower sugar levels, ranging from 181.30 g/L in 2022 to 179.94 g/L in 2024. The MH_V2 variant showed intermediate sugar content, with 182.50 g/L in 2022, 179.76 g/L in 2023, and 184.43 g/L in 2024.

Regarding total acidity, MH_V1 had the lowest acidity in 2022 (2.35 g/L) but experienced a rise in acidity in subsequent years, reaching 3.23 g/L in 2024. The MH_C variant, initially at 2.80 g/L in 2022, saw a gradual increase in acidity over the years, peaking at 3.86 g/L in 2024. The MH_V2 variant started with an acidity of 2.77 g/L in 2022, which increased to 3.54 g/L in 2023 and 3.66 g/L in 2024.

In terms of berry weight, the MH_V1 variant consistently had the heaviest berries, particularly in 2023 (305 g), while the MH_C variant had the lightest berries, especially in 2022 (176 g). The MH_V2 variant exhibited intermediate berry weights, with values of 194 g in 2022, 300 g in 2023, and 224 g in 2024.

The pH levels of the variants also varied across the years. The pH of MH_V1 remained relatively stable, at 3.59 in 2022, 3.60 in 2023, and 3.30 in 2024. Meanwhile, the MH_C variant showed a decline in pH from 3.56 in 2022 to 3.35 in 2024, and the pH of the MH_V2 variant shifted from 3.60 in 2022 to 3.40 in 2024, reflecting a slight increase in acidity over time.

Furthermore, the Glucoacidimetric Index (GAI) was calculated for each variant over the three years of study. As shown in Table 8, the GAI values indicated noticeable differences across variants and years.

Table 8. Descriptive statistics of the glucoacidimetric index (GAI) for different variants (2022-2024)

V.	Range	Min	Max	Mean	St. Dev	Var.
MH_C	18.2	46.6	64.8	53.7333	±9.71665	94.413
MH_V1	23.4	61.1	84.5	70.0667	±12.62154	159.303
MH_V2	15.5	50.4	65.9	55.7	±8.83572	78.07

Descriptive statistics for the GAI across all years showed that MH_V1 had the highest mean GAI of 70.07, followed by MH_V2 with a mean of 55.70, and MH_C with the lowest mean of 53.73. In terms of variability, MH_V1 exhibited the highest standard deviation (±12.62), suggesting greater variation in its GAI values over the years. In contrast, MH_V2 showed the least variability with a standard deviation of ±8.84, while MH_C had a moderate deviation (±9.72). Regarding variance, MH_V1 also had the highest variance (159.30), indicating that the GAI values for MH_V1 were more spread out over the years compared to the other variants. MH_V2 exhibited the lowest variance (78.07), suggesting a more consistent GAI across the three years. MH_C had a variance of 94.41, indicating moderate variability in its GAI over the study period.

In 2022, MH_V1 showed the highest GAI (84.5), followed by MH_V2 (65.9) and MH_C (64.8). Over the course of the study, the GAI values decreased for all variants. Specifically, in 2023, MH_V1 again led with a GAI of 64.6, while MH_V2 and MH_C showed 50.8 and 49.8, respectively. By 2024, MH_V1 maintained the highest GAI (61.1), while MH_V2 and MH_C continued to exhibit lower values (50.4 and 46.6, respectively).

The results suggest that MH_V1 consistently had the best sugar-to-acid ratio across the years, as observed in Figure 3, indicating its potential for producing grapes with a more favorable balance of sweetness and acidity. Furthermore, when comparing these values to commercial maturity standards for table grapes, as outlined by the OIV Resolution VITI 1/2008, it is evident that MH_V1 meets or exceeds the minimum sugar/acid ratio threshold of 20:1 in all years. Its mean GAI of 70.07 and minimum yearly value of 61.1 clearly surpass this requirement. MH_V2 and MH_C also show values above the 20:1 threshold, with minimum GAI values of 50.4 and 46.6

respectively; however, their lower mean GAI (55.70 and 53.73) and higher year-to-year variability suggest that in less favorable conditions, these variants may risk falling closer to the commercial acceptability margin. Therefore, MH_V1 not only demonstrates superior average quality but also greater reliability in maintaining commercial maturity standards across different years.

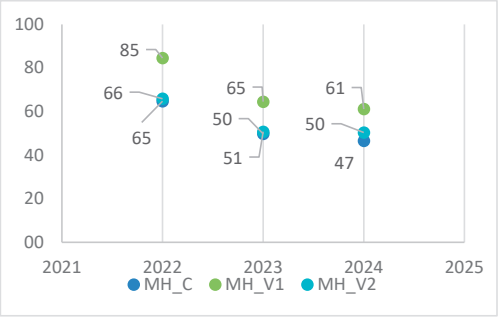


Figure 3. Glucoacidimetric index variation across variants (2022-2024)

However, the greater variance in MH_V1 highlights the influence of climatic variations, making its GAI values more sensitive to environmental changes. In contrast, MH_V2 exhibited a more stable GAI across years, suggesting a more consistent performance regardless of yearly climate fluctuations.

CONCLUSIONS

Across the three experimental years, fertility percentages varied significantly due to climatic conditions and pruning intensity. MH_V1 consistently outperformed MH_V2, with higher fertility rates, indicating that the more intensive pruning strategy promoted better bud differentiation and fruit set.

Although MH_V2 recorded the highest total cluster weight, MH_V1 demonstrated more efficient reproductive performance by converting a higher proportion of fertile shoots into productive clusters, highlighting its superior agronomic efficiency.

Statistical analysis confirmed the significant impact of pruning intensity on reproductive potential. MH_V1 showed consistently better results than MH_V2, reinforcing the conclusion that more intensive pruning had a favorable effect on fertility and productivity.

Climatic variability across the years influenced grape composition. Despite fluctuations in weather conditions, MH_V1 maintained higher sugar levels and a more favorable sugar-to-acid ratio compared to MH_V2, aligning with commercial quality standards.

While MH_V1 exhibited greater sensitivity to environmental changes, MH_V2 showed more stable but lower performance. Overall, MH_V1 proved to be the more productive and qualitatively superior variant, particularly under favorable climatic conditions.

ACKNOWLEDGMENTS

This research was carried out with the support of the Academy of Agricultural and Forestry Sciences "Gheorghe Ionescu-Șișești" through Project no. 629/01.02.2023: "Development of new products, practices, processes and technologies integrated into horticultural production".

REFERENCES

Bucur, G. M., & Dejeu, L. (2018). Research on phenotyping and eno-carpological traits of twenty-three new Romanian table grape varieties (Vitis vinifera L.). In *Conference Proceeding "Agriculture for Life, Life for Agriculture* (Vol. 1, No. 1, pp. 268-275).

Branas, J. (1974). *Viticulture* (Dehan Imprimerie: Montpellier, France).

Chapman, D. M., Matthews, M. A., & Guinard, J. X. (2004). Sensory attributes of Cabernet Sauvignon wines made from vines with different crop yields. *American Journal of Enology and Viticulture*, *55*(4), 325-334.

Chelcea, S., Ionita, M., & Adler, M. J. (2016). Identification of dry periods in the dobrogea region. In *Civil and Environmental Engineering: Concepts, Methodologies, Tools, and Applications* (pp. 324-339). IGI Global.

Clingeleffer, P. R. (2010). Plant management research: status and what it can offer to address challenges and limitations. *Australian Journal of Grape and Wine Research*, *16*, 25-32.

Clingeleffer, P.R., Martin, S.R., Dunn, G.M., & Krstic, M.P. (2001). Crop Development, Crop Estimation and Crop Control to Secure Quality and Production of Major Wine Grape Varieties: A National Approach. *Grape and Wine Research and Development Corporation*: Adelaide, Australia.

Constantinescu, Gh., Donaud, A., & Dragomir, E. (1964). Détermination de la valeur de l'indice bioclimatique de la vigne pour les principaux vignobles de la RP Roumanie. *Revue Roumaine de Biologie, série de Botanique*, *9*, 35-40.

Cuharschi, M., Cebanu, V., Degteari, V., & Condur, M. (2019). Muscat de hamburg varietate de epocă. Istoria, agrobiologia, agrotehnica, protecția contra bolilor și dăunătorilor. *Pomicultura, Viticultura şi Vinificaţia*, *79*(1-2), 31-41.

Davel, A. (2015). Optimising productivity in vineyards and potential effects on grape and wine composition for a specific production goal *(Doctoral dissertation, Stellenbosch: Stellenbosch University*).

De Toda, F. M., Sancha, J. C., & Balda, P. (2013). Reducing the sugar and pH of the grape (*Vitis vinifera* L. cvs.'Grenache'and 'Tempranillo') through a single shoot trimming. *South African Journal of Enology and Viticulture*, *34*(2), 246-251.

Dunn, G. M., & Martin, S. R. (2007). A functional association in *Vitis vinifera* L. cv. Cabernet Sauvignon between the extent of primary branching and the number of flowers formed per inflorescence. *Australian Journal of Grape and Wine Research*, *13*(2), 95-100.

Gatti, M., Pirez, F. J., Chiari, G., Tombesi, S., Palliotti, A., Merli, M. C., & Poni, S. (2016). Phenology, canopy aging and seasonal carbon balance as related to delayed winter pruning of *Vitis vinifera* L. cv. Sangiovese grapevines. *Frontiers in plant science*, *7*, 659.

Irimia, L. M., Patriche, C. V., & Roșca, B. (2017). Changes in oenoclimate aptitude index characterizing climate suitability of Romanian wine growing regions. *Applied Ecology & Environmental Research*, *15*(4).

Jolly, W. M., Nemani, R., & Running, S. W. (2005). A generalized, bioclimatic index to predict foliar phenology in response to climate. *Global Change Biology*, *11*(4), 619-632.

Kliewer, W. M., & Dokoozlian, N. K. (2005). Leaf area/crop weight ratios of grapevines: Influence on fruit composition and wine quality. *American Journal of Enology and Viticulture*, *56*(2), 170-181.

Martin, S. R., & Dunn, G. M. (2000). Effect of pruning time and hydrogen cyanamide on budburst and subsequent phenology of *Vitis vinifera L.* variety Cabernet Sauvignon in central Victoria. *Australian Journal of Grape and Wine Research*, *6*(1), 31-39.

Stahle, L., & Wold, S. (1989). Analysis of variance (ANOVA). *Chemometrics and intelligent laboratory systems*, *6*(4), 259-272.

Stanescu, C. (1960). The gluco-acidimetric index of grapes as a criterion for the technological evaluation of grape varieties.

Teodorescu, Ş., Popa, A., & Sandu, G. (1987). *Oenoclimatul României*. Editura Stiintifica si Enciclopedica, Bucuresti.

Vujović, D., Maletić, R., Popović-Đorđević, J., Pejin, B., & Ristić, R. (2017). Viticultural and chemical characteristics of 'Muscat Hamburg' preselected clones grown for table grapes. *Journal of the Science of Food and Agriculture*, *97*(2), 587-594.

Zaldea, G., Nechita, A., Damian, D., Ghiur, A. D., & Cotea, V. V. (2021). Climate changes in recent decades, the evolution of the drought phenomenon and the impact on vineyards in North-eastern Romania. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, *49*(4), 12448-12448.

Zheng, W., del Galdo, V., García, J., Balda, P., & de Toda, F. M. (2017). Use of minimal pruning to delay fruit maturity and improve berry composition under climate change. *American Journal of Enology and Viticulture*, *68*(1), 136-140.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

VALIDATION OF *GRAPEVINE PINOT GRIS VIRUS* ELIMINATION IN GRAPEVINE BY *IN VITRO* CHEMOTHERAPY

Ionela-Cătălina GUȚĂ, Elena-Cocuța BUCIUMEANU

National Research and Development Institute for Biotechnology in Horticulture Ştefăneşti - Argeş, 37 București-Pitești Road, Ștefănești, Argeș County, Romania

Corresponding author email: gutaionelacatalina@yahoo.com

Abstract

Distributed worldwide, Pinot Gris Grapevine Virus (GPGV) is considered a major pathogen of grapevine. Virus-free propagation material is the first step in the control of viral diseases, often requiring the application of methods to regenerate healthy plants. In vitro chemotherapy is the sanitation method used in this work, its validation being necessary to improve the efficiency of obtaining healthy biological material. To obtain GPGV-free grapevines, the treatment was applied to recover valuable clones from INCDBH Ștefănești, Fetească albă 97 Șt. and Cabernet Sauvignon 131 Șt. The apices and axillary buds were collected during the active growth period in two stages from plants grown in the protected space and in the field. A subculture of these explants on medium with ribavirin and oseltamivir, followed by three multiplication and one rooting on medium without antivirals, led to the regeneration of new plants with elimination rates of 50-100%, effective elimination being achievable with a precision of 21%, independent of genotype, explant type and sampling period.

Key words*: Vitis, GPGV, sanitation, precision, ELISA.*

INTRODUCTION

Grapevine Pinot gris virus (GPGV), a member of the genus Trichovirus (family *Betaflexiviridae*), was first identified in Pinot gris in the Trentino vineyards of Italy by next-generation sequencing (NGS) techniques (Giampetruzzi et al., 2012), although symptoms of the new disease called Grapevine Leaf Mottling and Deformation (GLMD) (Martelli, 2014) have been reported since 2003.

The reports on GPGV have revealed the widespread of this virus in many European and non-European wine-producing countries, such as Korea, China, United States and Canada.

Molecular characterization and phylogeny of different isolates have indicated the existence of symptomatic and latent GPGV variants (Saldarelli et al., 2015; Bertazzon et al., 2017). In addition, a correlation between GPGV concentration and GLMD expression has also been proposed (Bertazzon et al, 2016). Furthermore, GPGV has been detected in several non-*Vitis* hosts (*Silene latifolia*, *Chenopodium* sp., *Asclepias syriaca*, *Rosa* sp., *Rubus* sp., *Fraxinus* sp.) (Gualandri et al., 2017; Demián et al., 2018). However, mechanical transmission to herbaceous plants has not been observed (Malagni et al., 2016).

Being transmitted by grafting (Saldarelli et al., 2015), GPGV is mainly spread through propagation material. Without regular testing of mother plants of fruiting varieties and the absence of symptoms on rootstocks, the virus spreads and cannot be safely controlled. As GPGV is widespread in Slovakia (Glasa et al., 2014); Moravia (Eichmeier et al., 2016) and Hungary, it is believed that there is a possibility that GPGV could spread from Eastern Europe to Italy and from Europe to other areas of the world (Bertazzon et al., 2016). On the other hand, GPGV is assumed to originate from Asia, with China being the most probable source of the virus (Hily et al., 2020).

GPGV has been detected in the body of the eriophyid mite *Colomerus vitis* (Pagenstecher) and it is transmitted to healthy grapevines by this infested mite (Malagnini et al., 2016).

Preliminary data on the presence of GPGV in Romania were obtained by analyzing grapevine material collected after 2010 year from germplasm collections in several European countries (Bertazzon et al., 2016). During 2019-2020 period, 199 grapevine samples were

analyzed by ELISA (Enzyme-linked immunosorbent assay) for the presence of GPGV, *Grapevine fanleaf virus* (GFLV), *Grapevine leafroll-associated virus 1+3* (GLRaV-1+3) and *Grapevine fleck virus* (GFkV). Of these, GPGV was detected in 107 samples (53.76%), in single or mixed infections with GFkV or GLRaV-1+3 (Guță & Buciumeanu, 2021).

Currently, in Europe, GPGV is considered a major pathogen of grapevines (Cieniewicz et al., 2020). GPGV control can be achieved by using healthy propagation material, using virus elimination methods (Gualandri et al., 2015; Guță & Buciumeanu, 2022), vector control (Gualandri et al., 2015) and removing alternative hosts (https://www.awri.com.au/wp-content/uploads/2015/09/GPGV-fact-sheet.pdf).

The paper aims to evaluate the influence of genotype, origin and type of explant on the application of *in vitro* chemotherapy for the elimination of *Grapevine Pinot Gris virus* in grapevine.

MATERIALS AND METHODS

The biological material was represented by grapevine plants (*Vitis vinifera* L.) belonging to the Fetească albă 97 Șt. and Cabernet Sauvignon 131 Șt. genotypes, from two locations: plants grown in vegetation pots in a protected space (germplasm collection G0) and plants selected in experimental fields.

The plants were virological tested ELISA (Clark & Adams, 1977) with commercial reagents Bioreba (Switzerland). They were ELISA - positive for GPGV and negative (control) for GPGV. All plants were free of GFLV, GLRaV-1+3 and GFkV.

The experiment consisted of using two types of explants (apices and nodal fragments) from a grapevine infected with GPGV and a control plant, one stored under controlled conditions in the G0 storage greenhouse and the other in the field. Six apexes and six nodal fragments were collected from each plant. The reason for starting from one plant was that all explants taken from it should have similar viral concentrations. Since plants stored in a protected space start the vegetation earlier as compared to those in the field, the initiation of the cultures was carried out in two stages: April and May (explants taken from the protected space), May and June (explants taken from the field).

For the initiation of aseptic cultures, growth tips were collected and after an insistent wash under jet of water for 1 h, they were sterilized with 6% sodium hypochlorite for 7 minutes. These plant fragments constituted the source of biological material for the excision of intensely regenerative apices and nodal fragments.

In vitro chemotherapy as a viral elimination method consisted of explants cultivating, apices and nodal fragments on grapevine specific culture medium (MS basic medium - Murashige and Skoog, 1962), containing 1 mg/L benzylaminopurine (BAP) + 0.5 mg/L indolylacetic acid (AIA) and 20 mg/L glucose, solidified with agar-agar (7g/L), supplemented with the mixture of viricides, ribavirin 10-30 mg/L and oseltamivir 30-60 mg/L, for one subculture (30-40 days), followed by the subcultivation on media (multiplication, rooting) without viricides, until the regeneration of new grapevine plants, 3 subcultures (SI, SII, SIII) (according to Patent no. 123133/2010 – Procedure for obtaining virus-free grapevine plants).

Ribavirin (1-β-D-ribofuranosyl-1, 2,4 triazone-3-carboxamide) pure substance (produced by Sigma-Aldrich, USA) (R) and oseltamivir ([(3E,4R,5S)-4-acetamido-5-amino-3-(1-ethylpropoxy)-1-cyclohexane-1-carboxylic acid)] (O) as capsules containing oseltamivir phosphate (Tamiflu, Hofmann-La Roche, Germany) have been used.

Culture media were sterilized by autoclaving for 20 minutes at 120°C and 1 atm.

After inoculation, under aseptic conditions, in a laminar airflow hood, the tissue cultures were stored in the growth chamber where the conditions for triggering organogenesis processes were ensured (24°C, 16/8 photo-period, day/night).

The multiplication rate (X) was expressed as the number of resulting explants reported to the number of initiated explants.

The evolution of the inocula during the three post-treatment subcultures was expressed by quantifying the multiplication rates [number of multiplication formations (adventitious bud glomeruli and shoot primordia)], which were

statistically interpreted by regression analysis for the 95% confidence interval.
The phytotoxic effect of viricides was assessed by cultivation of explants on medium without antivirals, after the culture on medium containing ribavirin and oseltamivir.
The efficiency of the GPGV elimination method by *in vitro* chemotherapy was expressed as no. ELISA negative plants/no. tested plants x100.
The calculation of precision expressed as the coefficient of variation (standard deviation/mean of measurements) as a validation parameter reflects the repeatability of the results when applying the method.

RESULTS AND DISCUSSIONS

After 30 days on aseptic medium containing the mixture of viricides ribavirin and oseltamivir, the cultures belonging to the two genotypes studied behaved relatively similarly, considering the accumulation of disruptive factors: the phytotoxic effect of the chemotherapeutics, the genetic regenerative potential of the genotypes studied and last but not least the presence of viral infection.
In the case of Fetească albă 97 Șt.:
- Of the six apices grown on the medium with antivirals, only two developed organo-regenerative processes that led to the continuation of the culture, so that, after the first subculture, the multiplication rate was 1:1 (three explants resulted from each of the two viable explants);
- Under the influence of the chemotherapeutics, the nodal fragments behaved similarly: two explants out of the five initiated retained their viability and generated three explants each, that were subcultured on regular medium, the multiplication rate being 1:1.2;
- The phytotoxic effect of antivirals was assessed by the cultivating the explants on medium without antivirals, without omitting the presence of viral infection that can influence the regenerative potential of the genotype. The apices showed a viability rate of 1:0.5. The multiplication rate of the apex culture on basic medium was 1:1.66;
- The nodal fragments were treated similarly: the viability rate was 1:0.6; the multiplication rate was 1:2.2; 4 explants each from two initiated on control medium were subcultured on medium with chemotherapeutics.
In the case of Cabernet Sauvignon 131 Șt.:
- The treatment with ribavirin and oseltamivir for of GPGV elimination in the Cabernet Sauvignon 131 Șt. had a similar effect to the previous genotype (Table 1).

Table 1. *In vitro* chemotherapy for GPGV elimination to Cabernet Sauvignon 131 Șt. genotype. Values are averages of three replicates ± SD

Treatment	Explant type	Initiation No. of explants initiated	Subculture 1 Multiplication rate (X)*
R+O	Apex	5.0 ± 0.573	2.0± 0.897
	nodal fragment	5.0 ± 0.798	2.0 ± 0.768
Control	Apex	4.0 ± 0.836	2.5 ± 0.258
	nodal fragment	4.0 ± 0.798	3.0 ± 0.675

*multiplication rate = number of resulting explants reported to the number of initiated explants

For the construction of the regression curves of multiplication rate, the polynomial equation of degree 2 was chosen at which the coefficient of determination r^2 has the value 1 in all the analyzed cases, which shows a linear functional dependence between the analyzed variables, in our case, each explant in a subculture corresponds to a multiplication rate (Figures 1, 2, 3, 4).

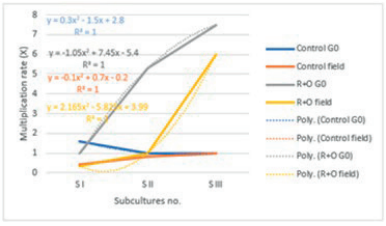


Figure 1. Multiplication rate of apices belonging to Fetească albă 97 Șt. genotype during 3 consecutive subcultures (SI, SII, SIII)

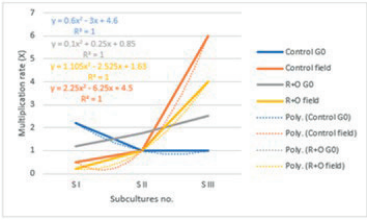


Figure 2. Multiplication rate of nodal fragments belonging to Fetească albă genotype 97 Șt. during 3 consecutive subcultures (SI, SII, SIII)

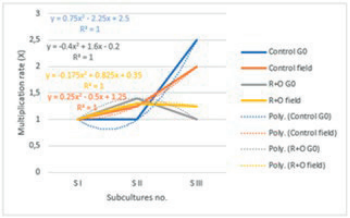


Figure 3. Multiplication rate of apices belonging to Cabernet Sauvignon 131 Șt. genotype during 3 consecutive subcultures (SI, SII, SIII)

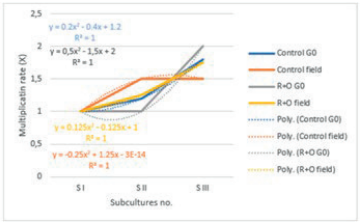


Figure 4. Multiplication rate of nodal fragments belonging to Cabernet Sauvignon 131 Șt. genotype during 3 consecutive subcultures (SI, SII, SIII)

Visually, the phytotoxic effect was manifested by chlorosis of explants cultivated on media with viricides after 30 days of treatment (Figure 5). Released from the effect of viricides, explants subcultured on regular multiplication medium during three consecutive subcultures proliferated adventitious buds that differentiated into shoot primordia that rose, having normal appearance. These were excised and transferred to rooting medium.



Figure 5. Chlorosis of explants (apices and nodal fragments) of Cabernet Sauvignon 131Șt. (right) compared to the untreated control (left) after 30 days of culture on medium with ribavirin and oseltamivir

The regeneration rates reflect the regenerative potential of the genotype in the presence of viral infection and the effect of chemotherapeutics on the evolution of regenerative processes during consecutive subcultures, without omitting the fact that each explant initiated in culture is a on one hand, and on the other hand for obtaining virus-free plants distinct individual with specific behaviour (Table 2).

Table 2. Regeneration by *in vitro* chemotherapy of Feteasca albă 97Șt. and Cabernet Sauvignon 131Șt. genotypes infected with GPGV

Genotype	Treat-ment	Explant type*	Period and place of explant sampling				Average	Average regeneration rate**
			April, protected space	May, protected space	May, field	June, field		
Feteasca albă 97Șt.	Control	apex	3	2	2	3	2.50	0.41
		nodal fragment	6	4	0	1	2.75	0.45
	R+O	apex	5	4	4	3	4.00	0.66
		nodal fragment	3	5	0	1	2.25	0.38
Cabernet Sauvignon 131Șt.	Control	apex	10	11	8	9	9.50	1.58
		nodal fragment	8	6	5	4	5.75	0.95
	R+O	apex	6	5	4	3	4.50	0.75
		nodal fragment	5	3	4	2	3.50	0.58

*cultures were initiated with 6 inocula/type of explant
**regeneration rate = number of *in vitro* regenerated (rooted *in vitro*)/number of initiated inocula

Tissue culture is a very effective approach for obtaining a large number of plants in a short time However, some research has shown that viral elimination in grapevine was not possible by shoot tip culture (Wang et al., 2016; Sim et al., 2019). Therefore, tissue culture has been combined with thermotherapy, chemotherapy, electrotherapy, etc. (Panattoni et al., 2013).
The grapevine genotypes behave differently when applying the micropropagation method.

Studies undertaken to evaluate the regenerative potential of some grapevine clones from germplasm collection belonging to the National Research & Development Institute for Biotechonoly in Horticulture Ștefănești - Argeș led to their grouping into three classes, following the quantification of the multiplication rate, *in vitro* rhizogenesis, and the acclimatization of the *in vitro* regenerated plants. Thus, the Fetească albă 97 Șt. genotype was declared to have a low regenerative potential, at the opposite pole being the Cabernet Sauvignon 131Șt. genotype (Vișoiu et al., 2008). On the other hand, it is self-evident that the presence of a viral infection in the explant donor plants influences the behaviour of these genotypes in the *in vitro* culture. In Cabernet Sauvignon infected with the viral complex *Grapevine rupestris stem pitting-associated virus* (GRSPaV), *Grapevine leafroll-associated virus 2* (GLRaV-2) and GFkV, no plants could be regenerated by micropropagation (Hu et al., 2022).

The influence of viral infection on the behaviour of a grapevine genotype in the *in vitro* culture was studied in Fetească neagră genotype. *In vitro* studies conducted with explants collected from healthy grapevine and those infected with GFLV, GLRaV1+3 or GFkV highlighted the influence of the presence of the virus on the quantitative and qualitative characteristics of the culture. Significant differences in the multiplication rate were recorded between healthy and virus-infected material. On the contrary, shoot elongation and rooting capacity were not significantly influenced by virus infection. The type of virus did not influence the behaviour of the genotype during the cultures, highlighting the fact that the Fetească neagră genotype has a tolerant response to various viral infections under *in vitro* conditions (Guță et al., 2009b). The use of chemotherapeutics in aseptic culture media to block viral multiplication and to regenerate new virus-free plants caused problems regarding phytotoxicity on explants from the beginning. Different viricides, different concentrations of them and different treatment times have been studied so that the method generates viral elimination: ribavirin (Weiland et al., 2004; Hu et al., 2018); tiazofurin, 6-thioguanine, oseltamivir (Panattoni et al., 2011), dihydroxypropyladenine (Panattoni et al., 2007a); mycophenolic acid (Panattoni et al., 2007b), salicylic acid (Khassein et al., 2024).

Increasing the treatment time (1-3 subcultures) induced a significant decrease in the multiplication rate for each concentration of the viricides ribavirin and oseltamivir in GFkV - infected Canner genotype. The phytotoxic effect diminished with the transfer of the plants to the rooting medium without antivirals (Guță et al., 2009a). In the process of GLRaV-1 and *Grapevine virus A* (GVA) elimination in Servant variety, using 80 µmol/L ribavirin in the culture medium for 30-90 days, the phytotoxic effect on the multiplication formations (axillary buds, shoot primordia) was also highlighted, which diminished at the transfer to regular medium (Guță & Buciumeanu, 2011). Studies on the GFLV elimination in Fetească albă genotype, GLRaV-1+3 in Ranâi Magaraci, and GFkV in Canner mentioned the occurrence of explants vitrification and necrosis phenomena as an effect of the presence of viricides in the culture medium (Guță & Buciumeanu, 2012). Throughout time, *in vitro* chemotherapy with ribavirin and oseltamivir used single each one or in combination has proven to be effective in viral elimination, the phytotoxic effect being neutralized by subsequent cultivation on regular medium without antivirals, and by the regenerative potential specific to the grapevine genotype (Guță et al., 2014; 2017a; 2017b).

Successful application of the *in vitro* chemotherapy method must consider the cultivation of a sufficient number of explants that lead to the regeneration of new plants, some of which are virus-free. After acclimatization, as a result of the process of adapting the vitroplants to ex vitro conditions, the number of regenerated plants decreased, but all experimental variants were generally represented. These were individually tested by ELISA to establish the virological status, namely the presence of GPGV (Table 3).

Table 3. Virological status of grapevine plants regenerated by *in vitro* chemotherapy

Geno-type	Treat-ment	Explant type *	No. of regenerated plants							
			Period and place of explant sampling							
			April, protected space		May, protected space		May, field		June, field	
			tested**	ELISA negative***	tested**	ELISA negative***	tested**	ELISA negative***	tested**	ELISA negative***
Fetească albă 97Șt.	Control	apex	3	1	1	0	2	0	1	0
		nodal frag.	3	0	2	1	-	-	1	0
	R+O	apex	1	1	4	4	3	2	1	1
		nodal frag.	2	1	3	2	0	-	1	1
Cabernet Sauvig-non 131Șt.	Control	apex	8	0	3	0	6	0	4	0
		nodal frag.	7	0	6	0	2	0	2	0
	R+O	apex	6	5	2	1	3	3	1	1
		nodal frag.	3	3	1	0	2	2	1	1

*cultures were initiated with 6 inocula/type of explant
**plants regenerated from *in vitro* rooted plants after going through the acclimatization process
***regenerated plants were ELISA tested for GPGV

The efficiency of the GPGV elimination method by *in vitro* chemotherapy was calculated as no. ELISA negative plants/no. of tested plants x 100 (Table 4).

Table 4. Efficiency of the method of GPGV elimination, simple infection, by *in vitro* chemotherapy (expressed as no. of ELISA negative plants/no. of plants tested x100)

Genotype	Treat-ment	Explant type	GPGV elimination rate (%)			
			Period and place of explant sampling			
			April, protected space	May, protected space	May, field	June, field
Fetească albă 97Șt.	Control	apex	33	0	0	0
		nodal frag.	0	50	-	0
	R+O	apex	100	100	66	100
		nodal frag.	50	66	-	100
Cabernet Sauvignon 131Șt.	Control	apex	0	0	0	0
		nodal frag.	0	0	0	0
	R+O	apex	83,3	50	100	100
		nodal frag.	100	0	100	100

The calculation of precision, as the variability of the results obtained when repeating the experiments of GPGV-free plants regeneration by *in vitro* chemotherapy, dependent on the genotype and the type of explant, independent of the period and place of sampling of the explants subjected to chemotherapy treatment, showed that the apexes give the best results in both genotypes studied. Cultivation of apices from donor plants grown in protected culture or in the field, regardless of the sampling period, led to the regeneration of GPGV-free plants with a variation of ±0.19 in Fetească albă 97Șt., respectively ± 0.28 in Cabernet Sauvignon 131Șt. as compared to the nodal fragments (Table 5).

Eliminating the influence of genotype led to a precision of 0.22 at an average of 87.41 and SD of 19.52 when cultivating apices on viricidal medium.

There are cases when a small amount of biological material requires the use of both apices and nodal fragments in the application of *in vitro* chemotherapy.

Table 5. Validation the parameters of the GPGV elimination method by *in vitro* chemotherapy in two grapevine genotypes infected with GPGV

Genotype	Explant type	Average*	Standard deviation** (SD)	Precision*** (Variation coefficient VC)
Fetească albă 97Șt.	apex	91.5	17.0	0.19
	nodal fragment	54.0	41.6	0.77
Cabernet Sauvignon 131Șt.	apex	83.3	23.6	0.28
	nodal fragment	75.0	50.0	0.66

*average of viral elimination rate (number of GPGV - ELISA negative plants/number of plants tested x100)
**SD = standard deviation of the average of the 4 values corresponding to the four initiation periods
***VC = SD/average

The precision calculated when standardizing all parameters that could influence the successful application of the viral elimination method (genotype, explant type, donor plant, sampling period) was 0.21 for an average of 75.95 and a standard deviation of 16.10. This indicates a minimal influence of the type of explant on the repeatability of the method.

CONCLUSIONS

In vitro chemotherapy is an effective method for regeneration of ELISA negative grapevine plants for GPGV.

The method can be applied by culturing apexes and nodal fragments on medium supplemented with antivirals taken from donor plants grown in protected areas or in the field during the grapevine vegetation period.

In the case of the genotypes Fetească albă 97 Șt. and Cabernet Sauvignon 131 Șt., GPGV was eliminated at rates ranging from 50 to 100% with a precision of ± 0.21.

ACKNOWLEDGEMENTS

The study is part of the Nucleus Program 23.41/2023-2026, Project 01 01 "Procedure of *Grapevine Pinot gris virus* elimination under conditions of economic efficiency, in order to increase the biological value of viticultural propagation material" which is supported jointly by Ministry of Research, Innovation and Digitalization.

REFERENCES

Bertazzon, N., Filippin, L., Forte, V., & Angelini, E. (2016). Grapevine Pinot gris virus seems to have recently been introduced to vineyards in Veneto, Italy. Archives of Virology, *161*(3), 711-714.

Bertazzon, N., Forte, V., Filippin, L., Causin, R., Maixner, M., & Angelini, E. (2017). Association between genetic variability and titre of Grapevine Pinot gris virus with disease symptoms. *Plant Pathology*, 66, 949-959.

Cieniewicz, E.J., Qiu, W., Saldarelli, P., & Fuchs, M. (2020). Believing is seeing: lessons from emerging viruses in grapevine. *Journal of Plant Pathology*, 102, 619-632.

Clark, M.F., & Adams, A.N. (1977). Characteristics of the microplate method of the enzyme-linked immunosorbent assay for the detection of plant viruses. Journal of General Virology, 34, 475-483.

Demián, E., Czotter, N., &Várallyay, É. (2018). Detection of Grapevine pinot gris virus in different non-*Vitis* hosts in Hungary. Proceedings of the 19th ICVG, Santiago, Chile, April 9-12, 2018, 24-25.

Eichmeier, A., Peňázová, E., Pavelková, R., Mynarzová, Z., & Saldarelli, P. (2016). Detection of Grapevine Pinot gris virus in certified grapevine stocks in Moravia, Czech Republic. *Journal of Plant Pathology*, 98(1), 155-157.

Gualandri, V., Asquini, E., Bianchedi, P., Covelli, L., Brilli, M., Malossini, U., Bragagna, P., Saldarelli, P., & Si-Ammour, A. (2017). Identification of herbaceous hosts of the Grapevine Pinot gris virus (GPGV). *European Journal of Plant Pathology*, 147, 21-25.

Gualandri, V., Bianchedi, P., Morelli, M., Giampetruzzi, A., Valenzano, P., Bottalico, G., Campanale, A., & Saldarelli, P. (2015). Production of Grapevine pinot gris virus-free germplasm: techniques and tools. *Proceedings of the 18th ICVG*, 7-11 September 2015, Ankara, Turkey, 246-247.

Giampetruzzi, A., Roumi, V., Roberto, R., Malossini, U., Yoshikawa, N., La Notte, P., Terlizzi, F., Credi, R., & Saldarelli, P. (2012). A new grapevine virus discovered by deep sequencing of virus- and viroid-derived small RNAs in cv. Pinot gris. *Virus Research*, 163, 262-268.

Glasa, M., Predajňa, L., Komínek, P., Nagyová, A., Candresse, T., & Olmos, A., (2014). Molecular characterization of divergent grapevine Pinot gris virus isolates and their detection in Slovak and Czech grapevines. *Archives of Virology*, *159*(8), 2103-2107.

Grapevine Pinot Gris virus. Fact Sheet, Viticulture. Retrieved January 21, 2025, from https://www.awri.com.au/wp-content/uploads/2015/09/GPGV-fact-sheet.pdf.

Guta, I.C., & Buciumeanu, E.C. (2011). Grapevine chemotherapy for elimination of multiple virus infection. *Romanian Biotechnological Letters*, *16*(5), 6535-6539.

Guță, I.C., & Buciumeanu, E.C. (2012). Results of chemotherapy and electrotherapy on virus elimination in grapevine. *Proceedings of the 17th ICVG*, Davis, California, USA, October 7-14, 2012, 264-265.

Guță, I.C., & Buciumeanu, E.C. (2021). Grapevine Pinot gris virus infecting grapevine in Romania – Short Communication. *Horticultural Science* (Prague), *48*(1), 47-50.

Guță, I.C., & Buciumeanu, E.C. (2022). Procedeu de eliminare a virusului Grapevine Pinot gris virus la viță-de-vie prin cultură de meristem. In: Tabăra V. (coord.), *Oferta cercetării științifice pentru transfer tehnologic în agricultură, industria alimentară și silvicultură, XXV*, 173-175. Bucharest, RO: Ceres Publishing House.

Guță, I.C., Buciumeanu, E.C., Gheorghe, R.N., & Teodorescu, Al. (2009a). Examination of phytotoxic effect of viricides on grapevine in controlled medium. *Scientific Papers U.S.A.M.V.B., Serie B, Horticulture*, LIII, 559-563.

Guță, I.C., Buciumeanu, E.C., Tătaru, L.D., & Topală, C.M. (2017a). Regeneration of grapevine virus-free plants by *in vitro* chemotherapy. *Acta Horticulturae*, 1188, 319-322.

Guță, I.-C., Buciumeanu, E.-C., Topală, C.M. & Tătaru, L.D. (2017b). The use of *in vitro* chemotherapy in grapevine virus elimination. *Acta Horticulturae*, 1155, 425-430.

Guță, I.-C., Buciumeanu, E.-C., & Vișoiu, E. (2009b). Comparative study of *in vitro* behavior of grapevine (*V. vinifera* L., Fetească neagră cv.) under the influence of various infections. *Extended abstacts 16th ICVG*, Dijon, France, 31 Aug.- 4 Sept., 2009, 247.

Guță, I.C., Buciumeanu, E-C., & Vișoiu, E. (2014). Elimination of Grapevine fleck virus by *in vitro* chemotherapy. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, *42*(1), 115-118.

Hily, J.-M., Poulicard, N., Candresse, T., Vigne, E., Beuve, M., Renault, L., Velt, A., Spilmont, A.S., & Lemaire, O. (2020). Datamining, genetic diversity analyses, and phylogeographic reconstructions redefine the worldwide evolutionary history of Grapevine Pinot gris virus and Grapevine berry inner necrosis virus. *Phytobiomes Journal*, *4*(2), 165–177.

Hu, G., Dong, Y., Zhang, Z., Fan, X., Ren, F., Li, Z., & Zhang, S. (2018). Elimination of Grapevine rupestris stem pitting-associated virus from *Vitis vinifera* 'Kyoho' by an antiviral agent combined with shoot tip culture. *Scientia Horticulturae*, 229, 99-106.

Hu, G., Dong, Y. Zhang, Z., Fan, X., & Ren, F. (2022). Effect of *in vitro* culture of long shoot tip on variant structure and titer of grapevine viruses. *Plants*, 11, 1907.

Khassein, A., Suleimanova, G., Malakhova, N., & Kizildeniz, T. (2024). Grapes recovery from grapevine fan leaf virus by chemotherapy using salicylic acid. *Research on Crops*, *25*(4), 605-610.

Malagnini, V., de Lillo, E., Saldarelli, P., Beber, R., Duso, C., Raiola, A., Zanotelli, L., Valenzano, D., Giampetruzzi, A., Morelli, M., Ratti, C., Causin, R., & Gualandri, V. (2016). Transmission of grapevine Pinot gris virus by *Colomerus vitis* (Acari: Eriophyidae) to grapevine. *Archives of Virology*, 161, 2595-2599.

Martelli, G.P. (2014). Directory of Virus and Virus-like Diseases of the Grapevine and their Agents. *Journal of Plant Pathology*, 96(1S), 105-120.

Murashige, T., & Skoog, F. (1962). A Revised Medium for Rapid Growth and Bio Assays with Tobacco Tissue Cultures. *Physiologia Plantarum*, *15*(3), 473–497.

Panattoni, A, D'Anna, F, Cristani, C, Triolo, E. (2007a). Grapevine vitivirus A eradication in *Vitis vinifera* explants by antiviral drugs and thermotherapy. *Journal of Virological Methods*, *146*(1-2), 129-135.

Panattoni, A., D'Anna, F., & Triolo, E. (2007b). Antiviral activity of tiazofurin and mycophenolic acid against Grapevine Leafroll-associated Virus 3 in *Vitis vinifera* explants. *Antiviral Research*, *73*(3), 206-211.

Panattoni, A., Luvisi, A., & Triolo, E. (2011). Selective chemotherapy on Grapevine leafroll-associated virus-1 and -3. *Phytoparasitica*, 39, 503–508.

Panattoni, A., Luvisi, A., & Triolo, E. (2013). Review. Elimination of viruses in plants: twenty years of progress. *Spanish Journal of Agricultural Research*, *11*(1), 173-188.

Saldarelli, P., Giampetruzzi, A., Morelli, M., Malossini, U., Pirolo, C., Bianchedi, P., & Gualandri, V. (2015). Genetic variability of Grapevine Pinot gris virus and its association with grapevine leaf mottling and deformation. *Phytopathology*, *105*(4), 555–563.

Sim, S.T., Khuu, N., Shoulders, J.R., Pudlo,W., Hoang, N.H., & Golino, D.A. (2019). Elimination of rose viruses using microshoot tip tissue culture. *Acta Horticulturae*, 1232, 241–246.

Wang, M.R., Li, B.Q., Feng, C.H., & Wang, Q.C. (2016). Culture of shoot tips from adventitious shoots can eradicate apple stem pitting virus but fails in apple stem grooving virus. *Plant Cell, Tissue and Organ Culture*, 125, 283-291.

Weiland, C.M., Cantos, M., Troncoso, A., & Perez-Camacho, F. (2004). Regeneration of virus-free plants by *in vitro* chemotherapy of GFLV (Grapevine fanleaf virus) infected explants of *Vitis vinifera* L. Cv 'Zalema'. *Acta Horticulturae*, 652, 463-466.

Vișoiu, E., Zmărăndoiu, I.L., Buciumeanu, E.C., & Guță, I.C. (2008). Use of *in vitro* culture as a method for valorification of grapevine genetic resources. *Book of abstracts, XXXVIII*, ESNA Annual Meeting Krakow, Poland, 27-31 August, 2008, 307.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

INVESTIGATION OF THE PHENOLOGICAL PHASES OF THE PINOT NOIR CLONE 777 UNDER THE INFLUENCE OF CLIMATE CHANGE IN THE SOUTHERN VINE-GROWING REGION IN BULGARIA

Marko MIHAYLOV[1], Boyan STALEV[2]

[1]University of Forestry, 10 Kliment Ohridski, 1797, Sofia, Bulgaria
[2]Agricultural University of Plovdiv, 12 Mendeleev Blvd, Plovdiv, Bulgaria

Corresponding author email: marko.mihaylovltu@gmail.com

Abstract

The impact of climate change on the phenological phases of the Pinot Noir variety clone 777 was studied during the period 2022-2023. The plantation was established in 2008 in the land of the village of Nikolaevo, Sliven district, in the southern wine-growing region of Bulgaria. The vines are formed on a one stem cordon with a pruning severity of 6 spurs, at planting distances of 2.4 x 0.9 m and non-irrigated conditions. The climate is transitionally continental with low mountain influence and diverse relief. In 2022, budburst begins in the first ten days of March, lasting about 35 days, flowering varies from 17 to 22 days, veraison occurs from 18 to 23. In 2023 budburst begins in the first ten days of April with a duration of 5 to 9 days, flowering began on June 04, with the standardized variants flowering 9 days longer, veraison is from 20 to 23 days. The grape harvest was carried out in the third ten days of August for 2022 and the first ten days of September for 2023. The difference in phenophases is due to the uneven distribution of rainfalls and unpredictable temperature dynamics.

Key words*: viticulture, climate, phenology, Pinot Noir clone 777.*

INTRODUCTION

Phenological phases include different stages of the annual life cycle of the vine, which occur under the influence of climatic factors. They are important both for proper cultivation and for the management of all agrotechnical activities in vineyard plantations (Popov, 2017).

The phenological stages of the vine are highly dependent on the interaction between climatic, soil and agrotechnical factors and varietal characteristics. Their successful management, including adaptation to climate change, is essential for maintaining stable yields and high grape quality. The main factors influencing the individual phases can be divided into climatic factors (temperature, rainfalls, humidity and solar radiation) soil factors (soil type, soil nutrient composition and pH) agrotechnical factors (pruning, yield regulation, irrigation, fertilization) and varietal characteristics including adaptability and genetic characteristics (Keller, 2010).

Climate change has a serious impact on agriculture, including viticulture. Changes in temperatures, rainfalls, the frequency of extreme weather events, and the length of seasons directly affect grape growth, yield, and quality (Fraga et al., 2012).

Climate change poses a serious threat to viticulture, but at the same time provides opportunities for innovation. Through the development of new technologies (smart farming, artificial intelligence, biotechnology and genetic research) some of the challenges can be overcome. Despite the difficulties, adaptation to new climatic conditions and the creation of resistant grape varieties will be key factors for maintaining grape and wine production in the future (Prada et al., 2024; Cojocaru, 2020; Georgieva & Kazandjiev, 2013).

An important problem for viticulture that needs to be solved in the near future is related to access to water, especially during droughts, which will be increasingly common. The problem of access to water is becoming increasingly serious. Due to global warming, water resources are limited, which requires the creation of varieties that are better adapted to drought (Atak, 2024).

Climate change in recent years has had a significant impact on grapevine growth and development. The main effects are related to

increasing temperatures, changes in the amount and distribution of rainfalls, the occurrence of extreme weather events, and changes in grape quality (Wasko et al., 2021; Biasi et al., 2019; Leah et al., 2018; Stott, 2016; Van Leeuwen & Darriet, 2016).

High temperatures usually accelerate the ripening process of grapes, increase the sugar content and alcohol in the wines produced, but often lead to a decrease in acidity, which is where the balance is lost (Droulia & Charalampopoulos, 2021; Venios et al., 2020).

It is possible that new wine-growing regions will be established in the coming decades that were previously unsuitable for growing grapes (Candiago et al., 2022).

According to data from the Ministry of Agriculture of the Republic of Bulgaria the areas planted with the Pinot noir wine variety amounted to 95.19 ha. These plantations are distributed among 24 farms, with 25.50 ha of them being young and not yet bearing fruit (Ministry of Agriculture, 2020).

The southern wine-growing region is one of the most important for grape and wine production in Bulgaria and covers the largest area occupied by vineyards. It is characterized by a warm climate, suitable for growing red wine varieties (Ministry of Agriculture, 2020).

The main objective of this research is related to the study of the phenological stages through which the Pinot noir variety undergoes changes in climate in the southern wine-growing region.

MATERIALS AND METHODS

The experiment was conducted during the period 2022-2023. The object of the study is the red wine variety Pinot noir, clone 777 planted in 2008 in the land of the village of Nikolaevo, Bulgaria at planting distances of 2.4/0.9 m. The vines were formed on a one stem cordon. The vines are pruned with 6 spurs with 2 buds with a total severity of 12 buds. The experiment was conducted under non-irrigated conditions. For each of the variants, data from 45 vines were collected and analyzed. A total of 3 variants were included in the experimental scheme.

V_0 – control with thinning only on the stems.

V_1 – leaving 15 inflorescence at a shoot length of 20 cm. Thinning was performed along the stem and cordon.

V_2 – leaving 15 clusters in the berry growth phase. Thinning was performed along the stem and cordon.

Phenological observations were recorded using BBCH scale and methodology described in the Ampelographic exercise manual (Meier, 1997; Roychev, 2014).

The content of the main nutrients – nitrogen, phosphorus and potassium were recorded using the following methods: BDS/ISO 10390:2011; ISO/TS 14256-1:2003; GOST 26209:1991 (BDS/ISO, 2011; ISO/TS, 2003; Riem, 1991).

Climatic data were obtained from a Meteobot meteorological station located in the vineyard.

RESULTS AND DISCUSSIONS

The average annual air temperature in the studied area is 12.4°C. The average monthly temperatures during the winter period are positive (1.2°C). This guarantees normal overwintering of winter buds and their successful development during the vegetation period. The area is characterized by mild winters and relatively hot summers with an average temperature of 23.2°C (Table 1).

Table 1. Meteorological data from the land of the village of Nikolaevo, Bulgaria in 2022-2023

Air temperature, °C			Air humidity, %			Average annual rainfalls, mm
Average annual	Average monthly for the winter period	Average monthly for the summer period	Average annual	Average monthly for the winter period	Average monthly for the summer period	
12.4	1.2	23.2	66	70	55	596

Autumn is longer than spring. The average annual air humidity is 66%. During the winter months it stays above 70%, and during the summer months it drops to 55%. On average, about 596 mm of rainfalls per year, with a maximum in summer of 165 mm and a secondary maximum in spring, and a minimum in winter of 134 mm.

The climatic conditions in the land of the village of Nikolaevo offer a continental climate with clearly defined seasons. The summer period is

dry, which can be a prerequisite for stress for the vines if there is insufficient irrigation.

Agrochemical analysis provides information on the chemical composition of the soil at a depth of 30 to 60 cm. The slightly acidic reaction of the soil is suitable for the absorption of nutrients such as nitrogen, phosphorus and potassium, which have a direct impact on the development of the vine. The low content of carbonates indicates that the soil is suitable for the cultivation of vines, as it is known that high levels of carbonates can limit the absorption of some microelements such as iron (Table 2).

Table 2. Agrochemical analysis of soil

Sampling depth, cm	pH	Active carbonates g/kg	N mg/1000 g	P2O5 mg/100 g	K2O mg/100 g
30-60	6.62	1.5	20.0	6.95	12.38

The mineral nitrogen content in the soil was recorded at a value of 20.0 mg N/1000 g of soil at a depth of 30 to 60 cm. The mineral nitrogen content is too low for growing vines, which is why fertilization with 174 kg per hectare of nitrogen was carried out during the study period. Regarding the content of mobile phosphates in the soil, it was found that it is poorly stocked 6.95 mg P_2O_5/100 g of soil. After the soil analysis, it was found that the content of available potassium in the soil is at the lower limit of stock 12.38 mg K_2O/100 g of soil, but it is sufficient for normal vegetation growth.

The soil conditions in the land of the village of Nikolaevo are favorable for growing vines with a direction of production of red table and sparkling wines of the Pinot noir variety. The vine is subject to continuous water erosion. This leads to the washing out of the mobile forms of N, P_2O_5 and K_2O in the root-dwelling soil horizon. The lower pH determined during the analysis does not represent a limitation for the development of grapevine culture under the soil conditions in the land of the village of Nikolaevo.

Soil analysis shows that it is suitable for growing grapes with its slightly acidic reaction. The content of phosphorus and potassium is moderate, but enrichment with nitrogen and phosphorus may be necessary for optimal growth.

The results of the phenological observations are reflected in Table 3.

Table 3. Timing of the phenological phases of development in the Pinot noir clone 777

Variant	Year	Beginning of bud swelling	First leaf unfolded and spread away from shoot	Inflorescence clearly visible	Beginning of flowering 10% of flowerhoods fallen	Berries groat-sized	Beginning of ripening	Berries ripe for harvest	Beginning of leaf-fall
V_0	2022	10 Mar.	22 Apr.	11 May	29 May	27 Jun.	18 Jul.	28 Aug.	10 Oct.
	2023	05 Apr.	20 Apr.	05 May	04 Jun.	19 Jun.	31 Jul.	07 Sept.	10 Oct.
V_1	2022	10 Mar.	22 Apr.	11 May	29 May	27 Jun.	25 Jul.	28 Aug.	10 Oct.
	2023	06 Apr.	19Apr.	08 May	04 Jun.	28 Jun.	07 Aug.	07 Sept.	10 Oct.
V_2	2022	10 Mar.	22 Apr.	11 May	29 May	24 Jun.	24 Jul.	28 Aug.	10 Oct.
	2023	06 Apr.	19 Apr.	08 May	04 Jun.	25 Jun.	06 Aug.	07 Sept.	10 Oct.

The different pruning severity on the vines in the experimental scheme has a specific influence on the timing of the individual phenophases in the individual variants. The phases occur relatively intensively.

In 2022, budburst begins on March 10 for all variants. In 2023, budburst is slightly delayed – It begins on April 5-6, depending on the variant. The duration of budburst is about 35 days for 2022, and for 2023 it varies from 5 to 9 days, depending on the variant. The difference of about 3-4 weeks between the two years may be due to differences in spring temperatures. The later budburst in 2023 is an indicator of a cooler or longer winter.

The first leaf in 2022 appears around April 22 for all variants. In 2023, the leaves appear earlier, on April 19-20. Despite the later budburst in 2023, the first leaves appear almost simultaneously with 2022, which shows that the vines have compensated for the delay thanks to faster growth in April.

The flowering of the Pinot noir clone 777 conditioned by the applied green operations, has led to a change in its dynamics. In 2022, at V_0 it is 17 days, at V_1 and V_2 it is 22 days. In 2023, flowering began on June 04 in all variants, with the variants with severity flowering 9 days longer. The rapid course of flowering is due to the favourable climatic conditions in 2022.

However, this is not the case in the second year of the experiment. The subsequent phases of development show that the vine crop has the ability to compensate for the time needed for the growth processes to occur. The pea size berry growth phase is formed in the second and third ten days of June, with the difference between the variants not significant and it is formed for several days.

Veraison occurs for about 18 days in the control variant for 2022, while for V_1 it is 21 and 23 in V_2. In the reporting year 2023, the results reported based on the phenological forecast follow a similar trend again, with the most dynamic process being completed in V_0 20 days followed by V_1 21 days and the variant V_2 flowered for 23 days. The grape harvest was carried out in the third ten days of August for 2022 and the first ten days of September for 2023, it was carried out simultaneously in all variants.

The budburst begins depending on the climatic features from the end of April to the beginning of May. This process occurs with a permanent retention of temperatures in the range from 6 to 9°C for 15 days (Figures 1 and 2).

The first dates of recording with the strongest growth are the shoots of variant V_2 followed by V_1 and V_0 during the two years of the experiment.

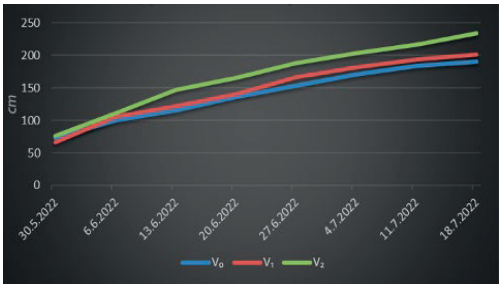


Figure 1. Growth dynamics of the shoots (cm) in 2022

The control variant V_0, which is not applied thinning along the cordon, forms weaker growth. When considering the following measurements of linear growth, a trend of a smooth increase in length is established in all variants. As the vegetation progresses and temperatures increase, growth increases, and by the third ten days of June in the reported 2022, most of the growth of all variants is formed.

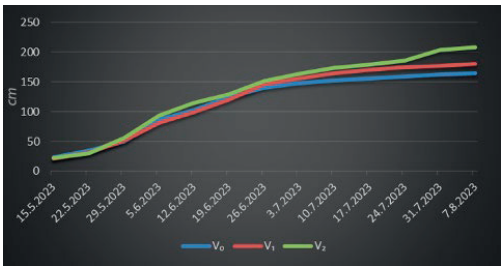


Figure 2. Growth dynamics of the shoots (cm) in 2023

In 2023, the formation of greater vegetative growth is demonstrated in all variants in the experiment, while the trend formed in the first year in terms of variants is maintained. The graphs also clearly show the influence of severity of the bunches in the fourth phase of the vegetative growth of the vine. The variant with severity of 15 bunches in the phase of berry growth significantly outperforms the vines with 15 inflorescence at the beginning of the vegetation. While in the control, growth dynamics remain smaller, regardless of the increase in average daily air temperatures. This is also due to the fact that in this variant, no suckers were planted along the cordons.

CONCLUSIONS

The Pinot noir clone, grown in the land of the village of Nikolaevo, Bulgaria, shows good adaptability, as it compensates for delays in the early phases and reaches technological maturity with minimal delay.

The variants with standardization V_1 and V_2 have a better balance between growth and yield, which makes them more effective for producing quality grapes.

The vines that are not standardized V_0 go through the different phenological phases faster, but are characterized by weaker shoot growth.

The relatively early ripening of the grapes suggests suitable conditions for the production of quality wines in the area of the village of Nikolaevo, Bulgaria.

The main differences in the timing of the phenological phases between 2022 and 2023 are mainly due to the climatic factors of the village of Nikolaevo, Bulgaria.

The data obtained show that through appropriate agrotechnical measures such as pruning and severity it is possible to optimize the quality and

quantity of the yield, regardless of climatic challenges.

ACKNOWLEDGEMENTS

I express my gratitude to the financial opportunities provided by the National Program "Young Scientists and Postdoctoral Fellows - 2. Thanks to the Agricultural University – Plovdiv, Bulgaria and the University of Forestry - Sofia, Bulgaria, as well as to the winegrowers from South-Western Bulgaria for the opportunity to work together.

REFERENCES

Atak, A. (2024). Climate change and adaptive strategies on viticulture (*Vitis* spp.). *Open Agriculture*, *9*(1), 2022-2058.

BDS/ISO (2011). *Soil quality - Determination of pH.* 10390: 2011.

Biasi, R., Brunori, E., Ferrara, C., & Salvati, L. (2019). Assessing impacts of climate change on phenology and quality traits of *Vitis vinifera* L.: the contribution of local knowledge. *Plants, 8*(5), 121–130.

Candiago, S., Winkler, J., Giombini, V., Giupponi, C., & Vigl, E. (2022). An ecosystem service approach to the study of vineyard landscapes in the context of climate change: A review. *Sustainability Science,* 18, 997–1013.

Cojocaru, O. (2020). Climate change in the republic of Moldova - causes and their induced consequences. *Scientific Papers. Series A. Agronomy*, *LXIII* (2), 18–23.

Droulia, F., & Charalampopoulos, I. (2021). Future climate change impacts on European viticulture: a review on recent scientific advances. *Atmosphere*, 12, 495–525.

Fraga, H., Malheiro, A., Moutinho-Pereira, J., & Santos, J. (2012). An overview of climate change impacts on European viticulture. *Food and Energy Security*, 1, 94–110.

Georgieva, V., & Kazandjiev, V. (2013). Climate change and agrometeorological conditions for growing winter crops in Bulgaria. *Scientific Papers. Series A. Agonomy*, *LVI*(1), 459–466.

ISO/TS, (2003). 14256-1:2003 *Soil quality - Determination of nitrate, nitrite and ammonium in field-moist soils by extraction with potassium chloride solution.*

Keller, M. (2010). *The science of grapevines: anatomy and physiology*. Burlington, USA: Academic Press.

Leah, T., Cerbari, V., Baliuk, S., Zakharova, M., & Nosonenko, O. (2018). Physical properties features of alluvial irrigated soils of Dniester and Dnieper river basins. *Scientific Papers. Series A. Agronomy, LXI*(1), 90–95.

Meier, U. (1997). *Growth stages of mono- and dicotyledonous plant*s. BBCH Monograph. Berlin, DE: Blackwell.

Ministry of Agriculture (2020). Agrostatistics Vineyard structure survey – Bulgaria.

Popov, K. (2017). *Viticulture.* Sofia, BG: University of Forestry Publishing house.

Prada, J., Dinis, T., Soriato, E., Vandelle, E., Soletkin, O., Uysal, S., Dihazi, A., Santos, C., & Santos, A. (2024). Climate change impact on Mediterranean viticultural regions and site-specific climate risk-reduction strategies. *Mitigation and Adaptation Strategies for Global Change*, 29, 1–43.

Riem, E. (1991). *GOST 26209-91. Determination of mobile compounds of phosphorus and potassium method (DL-method)*. Moscow, RU: Official edition.

Roychev, V. (2014). *Ampelography exercise guide*. Plovdiv, BG: Agrarian University Publishing House.

Stott, P. (2016). How climate change affects extreme weather events. Research can increasingly determine the contribution of climate change to extreme events such as droughts. *Science, 352*, 1517–1518.

Van Leeuwen, C. & Darriet, P. (2016). The impact of climate change on viticulture and wine quality. *Journal of Wine Economics*, 11, 150–167.

Venios, X., Korkas, E., Nisiotou, A., & Banilas, G. (2020). Grapevine responses to heat stress and global warming. *Plants, 9*, 1754–1765.

Wasko, C., Nathan, R., Stein, L., & O'Shea, D. (2021). Evidence of shorter more extreme rainfalls and increased flood variability under climate change. *Journal of Hydrology*, 603, 126–168.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

STUDY ON THE OENOLOGICAL POTENTIAL OF SOME NATIVE YEASTS STRAINS ISOLATED FROM WINE CENTER STEFANESTI

Petronela Anca ONACHE, Andreea Elena MANOLESCU, Dorin Ioan SUMEDREA, Andrei TĂNASE , Stan BONTEA

National Research & Development Institute for Biotechnology in Horticulture Ştefăneşti - Argeş, 37 Bucureşti-Piteşti Road, Ştefăneşti, Argeş County, Romania

Corresponding author email: dsumedrea@yahoo.com

Abstract

A study was conducted in 2023-2024 on the local yeast microflora in the Ștefanesti wine center. Investigations led to the isolation of yeasts belonging to the families Saccharomycetaceae, Saccharomycodaceae, Sporidiobolaceae, and Metschnikowiaceae, including genera such as Saccharomyces and Metschnikowia, among others, morphologically characterized at the strain level. These were used to reproduce natural fermentation conditions in a controlled manner and to provide the possibility of studying the oenological parameters of wine. Yeast strains isolated from the native microbiome are the basis for the implementation of new biotechnologies in winemaking. The fermentation potential of native strains by inoculation in monoculture was studied. The results obtained confirm that native yeast strains can be a possible basic source for wine biotechnology. Ten types of native yeasts useful for wine production were selected and isolated, among which the following yeast strains stood out with useful characteristics for fermentation: 12St_G and 15St_F.

Key words*: wine, fermentative potential, native yeast strains, yeasts.*

INTRODUCTION

Wine production is an ancient tradition that was achieved through the spontaneous fermentation of grape juice (Rantsiou, 2017), which occurs due to the presence of indigenous yeasts of different genera and species (Nechita, 2022; Fleet, 2003; Romano, 2003). The number of species of yeasts during fermentation depends on several factors (Rantsiou, 2017; Pretorius, 1999), which lead to differences in the quality of the wine by geographical areas, but also from year to year (Pretorius, 2000). To avoid this situation many winemakers used pure commercial cultures Saccharomyces cerevisiae inoculated in must (Stojanovic, 2023; Pretorius, 2000). Commercial cultures selected for the fermentation of wines of different varieties, destroy the uniqueness of the bouquet, typical oenological characteristics of the wine and its originality from the micro-area in which it is produced (Capece, 2019; Cordero-Bueso, 2013). However, it has been suggested that native strains of *S. cerevisiae* are better suited to the climatic conditions of the micro-area in the wine production region (Lopez, 2002) and therefore can more easily dominate the natural biota (Stojanovic, 2023; Nechita, 2022; La Jeune, 2006). In recent years, there has been a tendency to increase research on winemaking as closely related as possible to the terroir of the winemaking area. Current trends in scientific research as well as in the modern food and wine industry suggest continuous improvement of product quality (Stojanovic, 2023). This trend leads winemakers to adopt another technology of alcoholic fermentation, not using selected yeasts and using native yeasts, selected from the microflora naturally present in the area of origin of the wine (Lopez, 2007), existing in the vineyard, on the grapes, soil if the vineyard is old and on rachis or vegetable material of the vine. This approach might seem a step back from the scientific and technological stage of oenological science, but given its growing spread and economic relevance, it deserves scientific attention (Guzzon, 2018). The important wine regions have already made progress in adapting to the new requirements by introducing technological processes that will improve the complexity and quality of wine (Bisson, 2002).

The indigenous non-*Saccharomyces* yeast strains are now increasingly being brought to

the attention of winemakers (Mancic, 2022; Jolly, 2014), due to the good fermentative characteristics and production of aromatic compounds, specific to the geographical area of origin that give wine a recognized note (Mancic, 2022; Laroque, 2021; Rollero, 2021; Garcia, 2016). In addition, it is believed that specific strains of domestic yeast can be associated with specific regions and give wines a more typical, regional character. With unique and authentic wines gaining ground, the pursuit of new, untapped yeasts suitable for the wine industry is becoming inevitable. Many non-*Saccharomyces* yeasts have been characterized and applied in the wine industry (Mancic, 2022; Laroque, 2021; Rollero, 2021; Garcia, 2016).

Among them, the genus *Hanseniaspora*, morphologically characterized as consisting of bee yeast with bipolar buds, has a special potential in wine production (Mancic, 2022; Testa, 2021; Cadez, 2002). This species of yeast has good oenological characteristics, with a positive effect on the color, taste, aroma and stability of the wine (Mancic, 2022; Testa, 2021).

Recent studies have shown that grape varieties and the climate of a geographical area influence native yeast strains (Mancic, 2022; Martin, 2018; Brilli, 2014). Recently, the correlation between native microbial strains and organoleptic characteristics of wine has seen an increasing increase in attention from winemakers (Dumonde-Neves, 2017; Bokulich, 2016; Knight, 2015). However, there is controversy about the origin of the native strains of *S. cerevisiae*. Several studies have shown that the direct isolation of *S. cerevisiae* strains from undamaged grapes and vineyards is very difficult (Agarbati, 2019; Agarbati, 2022; Mercado, 2007).

In this context, the present study investigates the diversity of yeasts from the *Saccharomyces* and non-*Saccharomyces* genera from the Stefanesti wine-growing area, by isolating and selecting new strains from soil, plant material (grape stalks and berries) and grape must. The study aimed at the oenological characterization of spontaneous fermentation performed by 10 yeast isolates, belonging to both the *Saccharomyces* genus and other genera, selected from the Stefanesti wine-growing area.

MATERIALS AND METHODS

For the oenological characterization of the isolated and selected native yeast strains, they were previously subjected to microscopic and molecular characterization, according to the method described by Manolescu et al. (2024). Aseptic soil samples were taken from the three distinct area from viticultural centres of INCDBH Stefanești. Samples of plant materials (grape stalks and berry) and grape musts were collected. All juice grape samples were sterilized before they were used for fermentation with native yeast strains. The grape varieties from which musts resulted were: `Riesling Italian` - RI, `Fetească Regală` - FR, `Muscat Ottonel` - MO, `Sauvignon Blanc` - SB, `Tămâioasă Romanească` - TR, `Merlot` - M, `Fetească Neagră` - FN, `Blauer Zweigelt`- BZ, `Burgund Mare' - BM, and `Cabernet Sauvignon' - CS (Table 1).

Table 1. Code strains of native yeasts and specific features

Yeast code	Source of Yeast insulation	Strains identification	Specific features
1Șt_G	**FR** (washing water berry - Goleasca farm)	*Saccharomycetaceae*	Ovoidal, matte, slightly mucoid, cream
3Șt_F	**FR** (washing water berry - Fitotron farm)	*Sporidiobolaceae*	Round, glossy, orange-pink, double cell wall
5Șt_G	FR (soil washing water - Goleasca farm)	*Saccharomycetaceae*	Round, convex, slightly mucoid matte, cream
6Șt_G	**TR** (fresh juice - Călinești farm)	*Saccharomycetaceae*	Round, convex, glossy, cream-white
7Șt_G	**FR** (string washing water –Goleasca farm)	*Saccharomycetaceae*	Spherical-round, convex, glossy, cream-white
9Șt_G	**MO** (Fresh juice - Goleasca farm)	*Saccharomycodaceae*	Ellipsoidal, convex, glossy, cream with yellow-orange pigments
11Șt_G	**FR** (fresh juice - Goleasca farm)	*Saccharomycodaceae*	Oval-round, convex, glossy, cream-white
12Șt_G	**BZ** (Fresh juice - Goleasca farm)	*Saccharomycetaceae*	Elliptical-apicular, convex, slightly mucoid matte, cream
14Șt_G	**MO** (Fresh juice - Călinești farm)	*Saccharomycetaceae*	Spherical, slightly convex, slightly mucoid matte, cream-gray
15Șt_F	**FN** washing water berry - Fitotron farm)	*Metschnikowiaceae*	Ovoidal-elongated, convex, cream-white, light pink pigmentation

The grape juice has been sterilized by autoclaving. Selected native strains were tested in fermentation processes with inoculation in monoculture (10^6 CFU/ml), *Saccharomyces* and non-*Saccharomyces* strains were used. The fermentation processes were dynamically monitored and were carried out at a temperature of 20 ± 1°C. The main physico-chemical parameters were determined in the obtained wines: alcoholic strength (% vol); total acidity (g/L tartaric acid); volatile acidity (g/l acetic acid), unfermented sugars (g/L), and pH according to the methods OIV, 2025.

RESULTS AND DISCUSSIONS

The musts were characterized before being inoculated with native yeasts (Table 2).

Table 2. The characteristics of the musts used for experiments

Musts used	Sugars, g/L	Total acidity, g/l H_2SO_4	pH
RI	232	1.7	3.64
FR	221	1.75	3.92
MO	209	1.95	3.73
TR	192	3.2	3.62
SB	220	2.65	3.64
BZ	198	4.1	3.62
CS	221	2.85	3.52
BM	205	4.8	3.6

After the isolation and selection of the native yeasts from the Stefanesti geographical area, experiments were made with them in the must varieties from Table 2. In the experiment, for each batch of must, a witness was used for which we used as a fermentation agent a commercial compound usually used in the production of white wine and red wine. Data obtained from monitoring fermentation processes carried out on strains of native yeast are graphically shown in Figures 1 and 2.

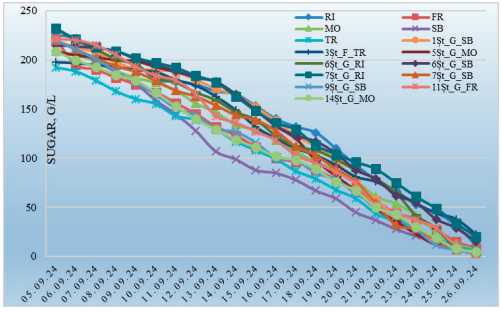


Figure 1. Evolution of yeast fermentation, native and commercial, to white varieties of must used

Figure 1 shows the evolution of fermentation for each native yeast and commercial yeast in white musts.

Figure 2 includes the fermentation of native and commercial yeasts in red musts.

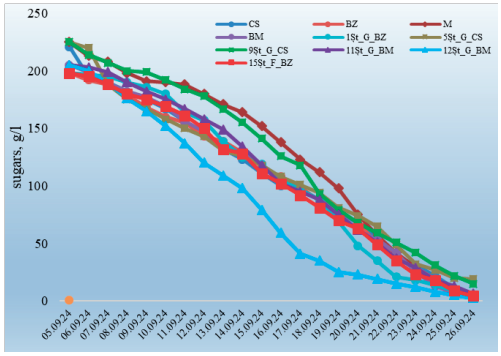


Figure 2. Evolution of yeast fermentation native and commercial to red varieties of must used

The fermentation processes in monoculture with native yeast strains were completed in 20 days, the average fermentation speed values being between 25 and 30 g/L sugars in 48 hours. Yeast strain *12St_G*_BM fermentation completed in 10-15 days, with a fermentation rate of 32.5 g sugars/48 hours.

The newly selected yeast strains began alcoholic fermentation 12-24 hours after introduction into the must. At this stage, the musts began to ferment tumultuously, and the temperature of the wort slowly increased by about 1°C. The relatively small duration of the period of preference is an advantage of the alcoholic fermentation process, it is always preferred by yeast strains that exhibit this feature. In the evolution of the fermentation curve, the sugar degradation is accentuated in the tanks considered control, in which commercial yeast was used for fermentation.

The phase of tumultuous fermentation began after 48 hours and continued for 10-15 days, when the temperature of the wort gradually increased, due to the increase in metabolic activity while increasing the number of yeasts. At this stage, the temperature was checked once a day, intervening to keep it between 15 and 16°C for white wines and 25-26°C for red wines. In the case of native yeasts, an average sugar degradation was observed, which eventually resulted in wines with good oenological and sensory characteristics.

From the analysis of the compositional characteristics of the wines obtained presented in Table 3, it is noted that, regardless of the selected yeast strains, the alcohol concentrations were between 14.01% vol and 11.13% vol, obtaining mostly dry wines, which proves the alcoholic character of the selected strains of *Saccharomyces* and non-*Saccharomyces* isolated yeast from the Stefanesti wine-growing area. The total acidity values ranged from 4.44 g/L to 7.21 g/L tartaric acid, being considered normal for white wines and between 5.2 and 8.01 for red wines. Differences were found in the case of volatile acidity, it was between 0.28 g/l acetic acid in `Feteasca Regală` inoculated with native yeast *11St_G* and the highest of 0.88 g/l acetic acid in `Burgund Mare` inoculated with *11St_G* and `Blauer Zweigelt` inoculated with yeast *9St_G*.

Table 3. The physico-chemical properties of wines obtained by inoculation of native strains

Yeast code	Wine Type	AS, %vol	TA, g/L AT	VA, g/L AE	S, g/l	Ph	SC
1St_G	SB	12.91	5.35	0.44	2.9	3.75	+++
	BZ	12.10	6.8	0.45	2.9	3.32	+++
3St_F	TR	13.18	4.94	0.45	22.5	3.6	+++
	BZ	11.4	6.96	0.81	5.5	3.26	++
5St_G	MO	13.77	6.24	0.32	3	3.57	++
6St_G	RI	13.68	5.18	0.32	1.5	3.26	+++
	SB	11.85	5.35	0.56	11.5	3.65	+++
7St_G	RI	11.94	7.21	0.46	7.5	3.25	+++
	SB	12.22	6.4	0.54	3.5	3.69	+++
	CS	12.69	5.99	0.65	4.5	3.24	+++
9St_G	SB	13.94	6.21	0.81	3	3.83	+++
	CS	13.23	7.8	0.80	1.5	2.73	+++
	BZ	11.27	8.01	0.88	1.3	3.23	++
	CS	14.01	6.99	0.65	5.5	2.73	++
11St_G	FR	13.02	4.54	0.28	2	3.81	+++
	BM	11.13	7.8	0.88	6.5	3.29	+++
12St_G	BZ	11.28	6.5	0.38	2.5	3.27	+++
14St_G	MO	12.0	4.4	0.32	5	3.57	+++
15St_F	BM	11.8	5.2	0.42	4.5	3.29	+++

AC - alcoholic concentration; TA - total acidity, g/L tartaric acid; VA - volatile acidity g/L acetic acid; S - unfermented sugars, g/L; CS - sensory characteristics; ++ - good; +++ - very good

From a sensory point of view, the following native yeasts used for the production of wines were highlighted: *1St_G*, *5St_G*, *6St_G*, *7St_G*, *12St_G*, *13St_G*, *14St_G* and *15St_F*. They were rated as clear, bright, straw yellow with medium intensity. Flavourings were classified as normal in a simple mixture, with the overall flavour intensity being assessed as mean. Olfactory, a slight smell of lemon, pear and quince, but also pineapple in white wines. In the case of red wines besides the intense color of bright red, the flavors were of blackcurrant at CS and with a smoky flavor at BM, blackberry flavors at BZ. The intensity of the basic aroma was balanced in the case of acidity and softness in the case of sweetness, without notes of bitterness and with a normal body for the type of wine. The mild astringency given by a low tannic intensity makes these wines to be appreciated as balanced, correct, with medium aromatic persistence.

CONCLUSIONS

After testing the oenological value of the newly selected yeast strains we determined that they can be appreciated as a useful biological material for the practice in micro- wine making of white and red wines. The fermentative processes, in the experimental variants realized, have highlighted the relevance of the native strains selected as possible sources for the early culture in micro-wine making. The compositional analysis of the wines obtained proved their alcoholic character, obtaining dry wines with a moderate volatile acidity.

The relatively small duration of the pre-fermentation period is an advantage of the alcoholic fermentation process, it is always preferred by yeast strains that exhibit this feature (*12St_G*, *15St_F*).

From the 10 native yeast strains tested: *12St_G* and *15St_F* are valuable in the fermentation process given their reduced foaming capacity.

ACKNOWLEDGEMENTS

This research work was carried out with the support of Ministry of Research, Innovation and Digitization through the Core Program, grant 37N/2023, project PN 23.41.01.04.

REFERENCES

Agarbati, A., Canonico, L., Ciani, M., Comitini, F. (2019) The impact of fungicide treatments on yeast biota of Verdicchio and Montepulciano grape varieties. *PLoS ONE*, 14, e0217385.

Agarbati, A., Canonico, L., Comitini, F., Ciani, M. (2022). Ecological Distribution and Oenological Characterization of Native *Saccharomyces cerevisiae* in an Organic Winery. *Fermentation*, 8, 224. https://doi.org/10.3390/fermentation8050224

Bisson, L.F., Waterhouse, A.L., Ebeler, S.E., Walker, M.A., Lapsley, J.T. (2002). The Present and Future of the International Wine Industry. *Nature*, 418, 696–699.

Bokulich, N.A., Collins, T.S., Masarweh, C., Allen, G., Heymann, H., Ebeler, S.E., Mills, D.A. (2016). Associations among wine grape microbiome, metabolome, and fermentation behavior suggest microbial contribution to regional wine characteristics. *mBio*, 7, e00631-16

Brilli, L., Buscioni, G., Moriondo, M., Bindi, M., Vincenzini, M. (2014) Influence of interannual meteorological variability on yeast content and composition in sangiovese grapes. *Am. J. Enol. Vitic*., 65, 375–380.

Cadez, N., Raspor, P., De Cock, A.W.A.M., Boekhout, T., Smith, M.T. (2002). Molecular identification and genetic diversity within species of the genera *Hanseniaspora* and *Kloeckera*. *FEMS Yeast Res*., 1, 279–289.

Capece, A., Pietrafesa, R., Siesto, G., Romaniello, R., Condelli, N., Romano, P. (2019). Selected Indigenous *Saccharomyces cerevisiae* Strains as Profitable Strategy to Preserve Typical Traits of Primitivo Wine. *Fermentation*, 5, 87.

Cordero-Bueso, G., Esteve-Zarzoso, B., Cabellos, J.M., Gil-Díaz, M., Arroyo, T. (2013). Biotechnological potential of *non-Saccharomyces* yeasts isolated during spontaneous fermentations of Malvar (*Vitis vinifera* cv. L.). *Eur. Food Res. Technol*., 236, 193–207.

Drumonde-Neves, J., Franco-Duarte, R., Lima, T., Schuller, D., Pais, C. (2017). Association between grape yeast communities and the vineyard ecosystems. *PLoS ONE*, 12, e0169883

Fleet G.H.(2003). Yeast interactions and wine flavour. *Int. J. of Food Microbiol*., 86: 11–22.

García, M., Esteve-Zarzoso, B., Arroyo, T. (2016). Non-*Saccharomyces* yeasts: Biotechnological role for wine production. In *Grape and Wine Biotechnology*; Morata, A., Loira, I., Eds.; Intech Open: London, UK.

Guzzon, R., Labagnara, T., Toffanin, A. (2018). Oenological characterisation of indigenous strains of S. cerevisiae isolated in a biodynamic winery in the Cortona DOC area. *Microbiology*, *68, 963–967*. Published November 13, 2018, from https://doi.org/10.1007/S13213-018-1405-4 http://doi:10.3390/fermentation5040087

https://www.oiv.int/what-we-do/standards

Knight, S., Klaere, S., Fedrizzi, B., Goddard, M.R. (2015). Regional microbial signatures positively correlate with differential wine phenotypes: Evidence for a microbial aspect to terroir. *Sci. Rep*., 5, 14233.

La Jeune, C., Enry, C., Demuyter, C., Lollier, M. (2006). Evolution of the population of *Saccharomyces cerevisiae* from grape to wine in a spontaneous fermentation. *Food Microbiol*. 2, 709–716.

Larroque, M.N., Carrau, F., Fariña, L., Boido, E., Dellacassa, E., Medina, K. (2021) Effect of Saccharomyces and non-*Saccharomyces* native yeasts on beer aroma compounds. *Int. J. Food Microbiol*., 337, 108953.

Lopes, C.A., Rodríguez, M.E., Sangorrín, M., Querol, A., Caballero, A.C. (2007). Patagonian wines: the selection of an indigenous yeast starter. *J. Ind. Microbiol. Biotechnol*., 34: 539–546.

Lopes, C.A., van , M., Querol, A., Caballero, A.C. (2002). *Saccharomyces cerevisiae* wine yeast populations in a cold region in Argentinean Patagonia. A study at different fermentation scales. *J. Appl. Microbiol*., 93: 608–615.

Mancic, S., Stamenkovic Stojanovic, M., Cvetkovic, D., Malicanin, M., Danilovic, B., Karabegovic, I. (2022). Oenological Characterization of Native *Hanseniaspora uvarum* Strains. *Fermentation*, 8, 92. https://doi.org/10.3390/fermentation8030092

Manolescu, A., Onache, P.A., Popescu, C.F., Sumedrea, D.I., Dumitru, A.M. (2024). Identidication of new yeast isolates from the Stefanesti – Arges vineyard through PCR ITS -RFLP techniques. *Scientific Papers. Series B, Horticulture, Vol. LXVIII*, Issue 2, 325–330.

Martin, V., Valera, J.M., Medina, K., Boido, E.; Carrau, F. (2018). Oenological impact of the *Hanseniaspora* / *Kloeckera* yeast genus on wines - A review. *Fermentation*, 4, 76.

Mercado, L., Dalcero, A., Masuelli, R., Combina, M. (2007). Diversity of Saccharomyces strains on grapes and winery surfaces: Analysis of their contribution to fermentative flora of Malbec wine from Mendoza (Argentina) during two consecutive years. *Food Microbiol.*, 24, 403–412.

Nechita, A., Filimon, R., Nechita, C.B., Pașa, R., Filimon, R., Zaldea, G. (2022). The study of the fermental potential of some yeast strains isolated from the Iasi vineyard. *Lucrari stiintifice, Seria Horticultura, USV Iasi*, 65 (2).

Pretorius, I.S. (2000). Tailoring wine yeast for the new millennium: novel approaches to the ancient art of winemaking. *Yeast*, 16: 675–729.

Pretorius, I.S., van der Westhuizen, T.J., Augustyn, O.P.H. (1999). Yeast biodiversity in vineyards and wineries and its importance to the South African wine industry-a review. *South African J. Enol. Vitic*., 20: 61–75.

Rantsioua, K., Marengoa, F., Englezosa, V., Torchiob, F., Giacosa S., Rollea, L, Gerbia, V., Cocolin, L. (2017). *Saccharomyces cerevisiae* Biodiversity in Monferrato, North West Italy, and Selection of Indigenous Starter Cultures for Barbera Wine Production. *Ital. J. Food Sci., 29*(3), 518-536. https://doi.org/10.14674/IJFS-767

Rollero, S., Bloem, A., Brand, J., Ortiz-Julien, A., Camarasa, C., Divol, B. (2021). Nitrogen metabolism in three non-conventional wine yeast species: A tool to modulate wine aroma profiles. *Food Microbiol*., 94, 103650.

Romano P., Capece A., Serafino V., Romaniello R. and Poeta C. (2008). Biodiversity of wild strains of *Saccharomyces cerevisiae* as tool to complement and optimize wine quality. *World J. Microbiol. Biotechnol*., 24: 1797–1802.

Stamenkovic Stojanovic, M., Mancic, S., Cvetkovic, D., Malicanin, M., Danilovic, B., Karabegovic, I. (2023). Impact of Commercial Inactive Yeast Derivatives on Antiradical Properties, Volatile and Sensorial Profiles

of Grašac Wines. *Fermentation*., 9, 494-509. https://doi.org/10.3390/fermentation9050494

Testa, B., Coppola, F., Lombardi, S.J., Iorizzo, M., Letizia, F., Di Renzo, M., Succi, M., Tremonte, P. (2021) Influence of Hanseniaspora uvarum as27 on chemical and sensorial characteristics of aglianico wine. *Processes*, 9, 326.

Testa, B., Coppola, F., Lombardi, S.J., Iorizzo, M., Letizia, F., Di Renzo, M., Succi, M., Tremonte, P. (2021). Influence of *Hanseniaspora uvarum* as on chemical and sensorial characteristics of aglianico wine. *Processes*, 9, 326.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

EVALUATION OF THE TECHNOLOGICAL POTENTIAL OF WINE GRAPE VARIETIES IN THE CONTEXT OF CLIMATE CHANGE IN THE DEALU MARE VINEYARD

Liliana PÎRCĂLABU, Steliana Paula BARBU, Marian ION, Georgeta TUDOR, Alexandra BUCUR

Research and Development Institute for Viticulture and Enology Valea Călugărească, 2 Valea Mantei Street, Valea Călugărească Commune, Prahova County, Romania

Corresponding author email: stelianap.barbu@gmail.com

Abstract

Climate change involves the adaptation of crop systems to the effects of climate variability, which influence vines, given the dependence of weather changes during the growing season, as well as increasing the duration and intensity of meteorological phenomena. The researches were carried out in the Valea Călugărească viticultural center within three viticultural plantations with the varieties Sarba, Negru aromat and Feteasca neagra. In the last the 25 years, the climate has changed in the meaning that heating resources increased and the rainfall resources decreased. These changes in the viticultural climate have determined an earlier development of the vegetative phenophases, especially the early phenophases and grape ripening (by approximately 7-14 days), which has particularly influenced the quality of grape production. Regarding the yield of grape production, it was found that the average rate of decrease was 0.86 kg/vine, the loss of acidity by 0.99 g/l and a higher accumulation of sugar 30-60 g/l.

Key words*: climatic change, grapevine, phenology, grape production.*

INTRODUCTION

In recent decades, increasing attention has been given to climate change and its associated risks. Given the importance of grapes for wine production and the current circumstances of climate change, it is essential to avoid modification of composition and freshness of wine concerning consumer preferences.

The impact of climate change on grape production is difficult to predict due to the large number of variables involved in grape cultivation.

Viticulture could benefit from increased atmospheric CO_2 levels, but at the same time, excessive heat, drought, and solar radiation can have negative effects on grapevines.

Climate change not only affects yield and production quality but can also change the progression of the vine's vegetative phenophases.

Higher temperatures caused by climate change (ex. + 2°C) encourage the ripening period to occur during a warmer time of the year (ex. + 2°C), resulting in an overall increase of + 4°C during grape ripening (Alston JM et al., 2011; Delay E et al., 2015). Currently, with the increase in the average air temperature and solar radiation, grapes are harvested about two weeks earlier than the period from 1970-1999.

Climate studies conducted in the Romanian vineyard region have highlighted a trend of changing viticultural climate over the past two decades, manifested by an increase in temperature regime and an unfavorable distribution of precipitation during the growing season of the vine (Busuioc et al., 2004).

The intensity and duration of soil drought phenomena vary depending on the complex interaction of agrometeorological factors, particularly temperature, which is undoubtedly a strong driving force for the development of grapevines (Viviane Becart, 2022; Jones and Alves, 2012), extremely high maximum temperatures in the air and soil, associated with low relative air humidity (atmospheric drought), a deficient precipitation regime influences the vegetative behavior of grapevines and consequently, the quality of grape musts and the composition of wines.

Although the grapevine is considered a plant that is resilient to water stress, these phenomena can significantly affect the vegetative development of the plants, their productive potential, and especially the quality of the grape

harvest, with direct implications for the quality and typicity of the wines produced in the viticultural areas DOC (Tate, 2001; Jones G.V. et al., 2005; Drappier et al., 2017).

MATERIALS AND METHODS

The study aimed to evaluate the impact of climate change on the technological potential of the Negru aromat (NA) and Fetească neagră (FN) grape varieties, which are part of the basic assortment for the production of quality red wines typical of the Dealu Mare vineyard, Sarba variety for aromatic white wines and table grape varieties Victoria and Xenia. Climate data used in this analysis are for the Research Institute for Viticulture and Enology Valea Călugărească station for 2000 to 2024. The studied viticultural climate parameters were: air temperature, precipitation, solar radiation, and relative humidity.

The characteristics of the precipitation regime were analyzed based on the criteria of Hellmann, which highlights the characteristics of periods with drought years and those with rainy years through mathematical calculations.

Assessments were made on the grape harvest from a quantitative and qualitative point of view at the technological maturity, at the same stage for all the experimental variants.

The following analyses were performed: sugar content - determined by refractometry (OIV, 2021a); total acidity - determined by titration with NaOH (OIV, 2021b), gluco-acidimetric index = sugar ratio/total acidity (expressed in g/l tartric acid).

Grape quality parameters were defined through correlation analysis.

To establish the correlations between phenolic maturity and the technological characteristics of grapes, the Spearman correlation coefficient was calculated, based on the relative position of a value compared to the others.

RESULTS AND DISCUSSIONS

The meteorological data recorded in the period 2000-2024 show a thermal regime characterized by annual average temperatures oscillating between 11.4°C and 14.0°C. Compared to the multi-year average, years 2022, 2023 and 2024 they recorded an increase in the annual average temperature of 0.3°C, respectively 1.2°C. Concerning the average temperature registered throughout the vegetation period, the variations oscillated between + 0.2°C (2023) and +1.7°C, (2024), average annual temperatures with 0.3°C, respectively 1.2°C (Figure 1).

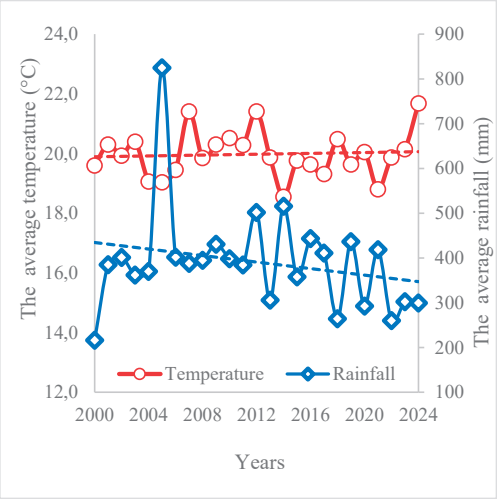


Figure 1. The average temperature (°C) and rainfall (mm) during the vegetative period, from 2000-2024

Regarding the frequency of occurrence of absolute maximum temperatures (>30°C), in the year 2022 in the month of June increased by 3.8 days, respectively with 19.8 days (2024), (26 days compared to the multi-year average of 6.2 days). During July and August, the frequency of appearance of absolute maximum temperatures exceeding 30°C, has been increased by 19.6% in the year 2022, respectively 26.6% (2024) (25.4 days cumulative average over the period 1970-1999, 45 days in the year 2022 and 52 days in the year 2024).

The Valea Călugărească viticultural center is characterized by a precipitation variation spectrum within the IS_1 class, resulting in conditions of moderate drought. The variation of precipitation amounts during the growing season is characterized by a maximum in the year 2005 (824.7 mm) and a minimum in the year 2000 (216 mm). The analysis of the data reveals that the categories of years with the greatest weight are those in the normal rating category composed of 10 years, with a weight of 40% and precipitation between 583.3 and 712.7 mm/year, the category with the slightly dry rating, composed of 2 years, with a weight of 8%

and precipitation between 532.5 and 554.8 mm/year and dry with a weight of 12% and precipitation between 467.7 mm and 505.2 mm, and the category with the very dry rating, composed of 2 years, with a weight of 8% and precipitation between 364.1 mm and 424.3 mm
Percentage-wise, for the analyzed time interval, 32% of the years are characterized by a rating from slightly dry to extremely dry, 40% of the years are characterized by normality, and 28% of the years are characterized by a rating from slightly wet to extremely wet.
The duration of sunshine during the growing season recorded significant increases of +285 hours (2023) and +316 hours (2024), against a background of reduced relative air humidity by 7% (2024) (Figure 2).

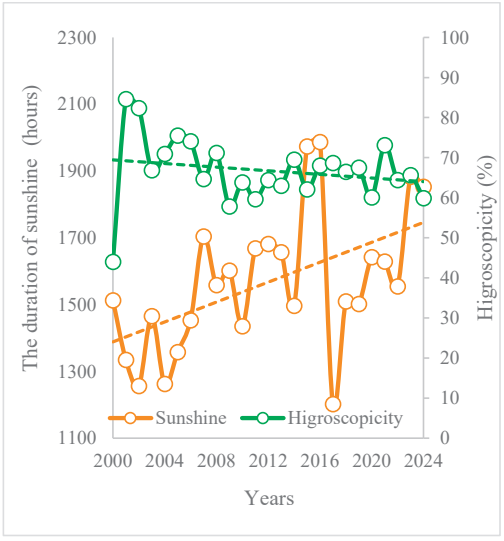


Figure 2. The average duration of sunshine (hours) and the higroscopicity (%) during the vegetative period from 2000-2024

Analysis over a 25-year period (2000-2024) of varietal phenology Feteasca neagra, Negru aromat and Sarba, the analysis highlights that budburst started towards the beginning of April, April 10th (2024) - April 18th-20th (2000), flowering at the beginning of June, June 9th (2024) - June 20th (2000), early ripening in the first half of August, August 7th (2024) and the second half of August, August 21st (2000), and grape ripening was reached at the beginning of September, September 2nd (2024), and September 20th (2000), respectively.

Furthermore, a uniform progression of vegetative phenophases was noted across all varieties.
These changes in the viticultural climate have led to an earlier progression of vegetative phenophases, particularly early ripening and grape ripening (by approximately 7-14 days), which has notably influenced the quality of grape production.
In the case of the Feteasca neagra variety, grape production showed variations ranging from 3.524 kg/vine (in 2000) to 1.926 kg/vine (in 2024), an average reduction of 0.063 kg/vine/year, while the sugar content registered an increase of 3.28 g/l/year sugar, with variations between 174.8 g/l (2000) and 256.7 g/l (2024). Regarding acidity, the average value decreased by 0.076 g/l/year (Figure 3).

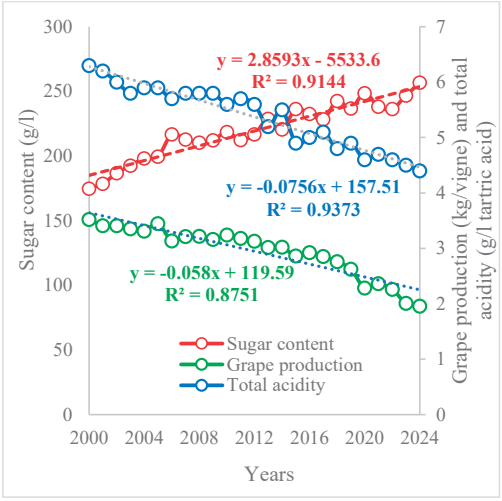


Figure 3. The grape production (kg/vine), sugar content (g/l) and total acidity (g/l tartric acid) of the Feteasca neagra variety

The high sugar content (256.7 g/l) and total acidity (4.4 g/l tartric acid) corresponding to the year 2024, resulted in the gluco-acidimetric index reaching a value of 58.4.
Significant positive correlations were identified between sunshine duration and sugar content (+0.57), as well as negative correlations with grape production (-0.50) and total acidity (-0.53) (Table 1).
Based on the significance of correlation coefficients, coefficients with a value greater than 0.42 are considered significant.

Table 1. Correlation coefficients between climatic parameters, grape production, and its quality in the Feteasca neagra variety

Climatic parameters	Grape production	Sugar content	Total acidity
Temperature (°C)	-0.12	0.13	-0.07
Rainfall (mm)	0.36	-0.22	0.31
The duration of sunshine (hours)	**-0.50**	**0.57**	**-0.53**
Higroscopicity (%)	0.12	-0.21	0.17

Table 2. Correlation coefficients between climatic parameters, grape production, and its quality in the Negru aromat variety

Climatic parameters	Grape production	Sugar content	Total acidity
Temperature (°C)	-0.19	0.11	-0.10
Rainfall (mm)	0.37	-0.22	0.14
The duration of sunshine (hours)	**-0.55**	**0.57**	**-0.54**
Higroscopicity (%)	0.23	-0.21	0.15

In the case of the Negru aromat variety, grape production showed oscillations between 2.022 kg/vine hub (2024) and 3.682 kg/vine hub (2002). Over the course of 25 years, the average grape production decreased by 0.066 kg/vine hub/year, while the sugar content increased by 2.99 g/l/year. A decreasing trend was also observed in total acidity, with approximately 0.080 g/l/year (Figure 4).

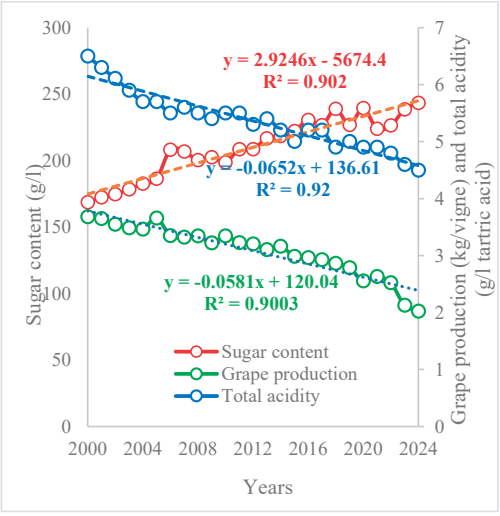


Figure 4. The grape production (kg/vine hub), sugar content (g/l) and total acidity (g/l tartric acid) of the Negru aromat variety

The high sugar content (243.4 g/l) and total acidity of 4.5 g/l tartric acid resulted in the gluco-acidimetric index reaching values well above those optimal for producing quality wines, with a value of 54.09 (2024).

The obtained results demonstrate the existence of positive correlations between sunshine duration and sugar content (+0.57), as well as negative correlations with grape production (-0.55) and total acidity (-0.54) (Table 2).

The same decreasing trend was observed in the grape production of the Sarba variety, with variations between 3.986 kg/vine hub (2000) and 2.512 kg/vine hub (2024), as well as in the total acidity of the must, from 6.7 g/l tartric acid (2000) to 4.4 g/l tartric acid (2024).

The sugar content showed an average increase of 3.200 g/l/year, registering contents from 162.8 (2000) to 242.8 g/l (2024) (Figure 5).

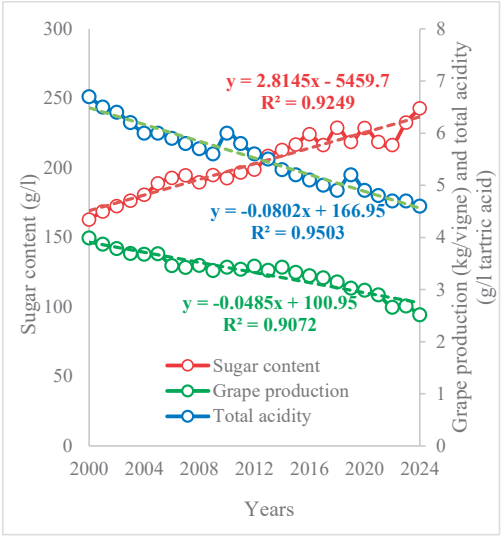


Figure 5. The grape production (kg/vine hub), sugar content (g/l) and total acidity (g/l tartric acid) of the Sarba variety

The gluco-acidimetric index values obtained for the Sarba variety are well above those optimal for producing high-quality aromatic wines, with a value of 52.8 (2024).

Significant positive correlations were also identified between sunshine duration and sugar content (+0.58), as well as negative correlations with grape production and total acidity (-0.51), (Table 3).

Table 3. Correlation coefficients between climatic parameters, grape production, and its quality in the Sarba variety

Climatic parameters	Grape production	Sugar content	Total acidity
Temperature (°C)	-0.18	0.09	-0.02
Rainfall (mm)	0.28	-0.17	0.19
The duration of sunshine (hours)	**-0.51**	**0.58**	**-0.51**
Higroscopicity (%)	0.19	-0.18	0.15

The excessive thermal regime and severe decrease in soil humidity, evident since the early ripening period of the 2024 grape harvest, had a favourable effect on sugar accumulation in table grapes, while grape production was negatively influenced due to the berry shrivelling phenomenon caused by a greater water loss through transpiration compared to that supplied through soil water absorption. Thus, the grape production of the Victoria variety decreased from 4.100 kg/vine hub (2000) to 2.266 kg/vine hub (2024), (Table 4), and in the case of the Xenia variety, the variations were from 6.000 kg/vine hub (2000) to 4.216 kg/vine hub (2024) (Tabel 5).

Table 4. Grape production and its quality in the Victoria variety

Period	Grape production (kg/vigne)	Sugar content (g/l)
2000-2009	3.553	160.6
2010-2019	3.015	169.6
2020	2.684	173.2
2021	2.576	175.4
2022	2.532	178.8
2023	2.438	182.6
2024	2.266	189.6

Table 5. Grape production and its quality in the Xenia variety

Period	Grape production (kg/vigne)	Sugar content (g/l)
2000-2009	5.568	161.4
2010-2019	4.902	171.7
2020	4.516	176.8
2021	4.502	178.4
2022	4.482	182.6
2023	4.424	184.2
2024	4.216	192.8

The sugar content recorded in table grape varieties had an average value of 160.6 g/l (2000-2009) and 189.6 g/l (2024) for the Victoria variety, and from 161.4 g/l (2000-2009) to 192.8 g/l (2024) for the Xenia variety.

The reduced volume of rainfall in the summer months caused the soil water reserve in 2024 to drop below 50% of the plant's available moisture range, starting in August. These values persisted throughout September, manifesting the phenomenon of grape shrivelling.

Based on the analysis of time series, long-term trends of climate change were identified, predicting a decrease in grape production for the analyzed varieties, an increase in sugar content (a trend that can be explained by higher temperatures and more hours of sunshine), as well as a decrease in acidity (Table 6).

Table 6. Long-term trends of climate change impact on grape production and its quality in the analyzed varieties

Variable	Tendency
Grape production FN	-0.058/an
Grape production NA	-0.058/an
Grape production Sarba	-0.048/an
Sugar content FN	+2.86/an
Sugar content NA	+2.92/an
Sugar content Sarba	+2.81/an
Total acidity FN	-0.075/an
Total acidity NA	-0.065/an
Total acidity Sarba	-0.080/an

All trends are extremely statistically significant ($p<0.05$), meaning that the changes observed over time are not random.

The decrease in production and acidity, as well as the increase in sugar, are confirmed as real tendencies.

Based on prediction models (Random Forest & XGBoost), we determined which climatic parameters most significantly influence grape production and its quality.

Production is primarily influenced by precipitation (30%) and temperature (24-26%), confirming the impact of water and heat on the grape harvest (Table 7).

Table 7. The prediction model (Random Forest) for grape production of the analyzed varieties

Climatic parameters	Feteasca neagra	Negru aromat	Sarba
Temperature (°C)	0.26	0.24	0.22
Rainfall (mm)	0.32	0.28	0.30
The duration of sunshine (hours)	0.20	0.22	0.21
Higroscopicity (%)	0.22	0.26	0.27

Sugar content is largely determined by sunshine duration (40-45%) and hygroscopicity (30%), confirming that more sun → more sugar (Tabel 8).

Table 8. The prediction model (Random Forest) for the sugar content of the analyzed varieties

Climatic parameters	Feteasca neagra	Negru aromat	Sarba
Temperature (°C)	0.15	0.18	0.16
Rainfall (mm)	0.10	0.12	0.08
The duration of sunshine (hours)	0.45	0.42	0.40
Higroscopicity (%)	0.30	0.28	0.36

Acidity is most influenced by hygroscopicity (75-81%), suggesting that air humidity plays a significant role (Tabel 9).

Table 9. The prediction model (Random Forest) for the total must acidity of the analyzed varieties

Climatic parameters	Feteasca neagra	Negru aromat	Sarba
Temperature (°C)	0.07	0.06	0.08
Rainfall (mm)	0.05	0.04	0.06
The duration of sunshine (hours)	0.10	0.09	0.11
Higroscopicity (%)	0.78	0.81	0.75

CONCLUSIONS

The data suggests that the climate is changing in the direction of higher sugar content, but with lower production and reduced acidity. This could affect the quality of wines produced from these varieties.

Precipitation and temperature are the most significant climatic parameters affecting grape production, with sugar concentration being influenced by duration of sunshine and total must acidity by hygroscopicity.

Long-term trends indicate a decrease in production with values ranging from 0.048 kg/vine hub/year (Sarba) to 0.058 kg/vine hub/year for Feteasca neagra and Negru aromat, as well as a decrease in total acidity by 0.065 g/l/year (Negru aromat), 0.075 g/l/year (Feteasca neagra) and -0.080 g/l/year for the Sarba variety. The sugar content of the must will register increasing trends with +2.81 g/l/year (Sarba), 2.86 g/l/year (Feteasca neagra) and 2.92 g/l/year for Negru aromat. Statistically, the long-term trends are very significant ($p<0.05$).

REFERENCES

Alston JM, Fuller KB, Lapsley JT, Soleas G. (2011). Too much of a good thing? Causes and consequences of increases in sugar content of California wine grapes. *Journal of Wine Economics*, 6:135-159. DOI: 10.1017/S1931436100001565

Busuioc Aristiţa, Dumitrescu Alexandru, Soare Elena, Alina Orzan (2004). Analysis of the aridity index variability in Romania. *Romanian Journal of Meteorology*, *6*(1-2): 19-27.

Delay, E., Piou C., Quenol H. (2015): The Mountain Environment, a Driver for Adaptation to Climate Change. *Land Use Policy*, 48, 51–62.

Drappier Julie, Cecile Thibon, Amelie Rabot (2017). Relationship between wine composition and temperature: Impact on Bordeaux wine typicity in the context of global warming. *Rev Food Sci Nutr*, 59: 14-30, 2017, DOI: http://dx.doi.org/10.1080/1040 8398. 2017.1355776

Jones G.V., Michael A. White, Owen R. Cooper, Karl Storchmann, 2005: Climate Change and Global Wine Quality, *Climatic Change*, *73*(3): 319-343

Jones, G., and Alves, F. (2012). Impact of climate change on wine production: A global overview and regional assessment in the Douro Valley of Portugal. *International Journal of Global Warming*, *4*(3–4), 383–406.

OIV (2021a). Evaluation by refractometry of the sugar concentration in grape, musts, concentrated grape musts and rectified concentrated grape must (Recueil OIV ed. 1990 revised by 377/2009). Section 2- *Physical analysis in Compendium of International Methods of Analysis*, OIV-MA-AS2-02.

OIV (2021b). Total acidity (revised by 551/2015). Section 3 - *Chemical analysis in Compendium of International Methods of Analysis*, OIV-MA-AS313-01.

Tate A. B. (2001). Global warming's impact on wine. *Journal of Wine Research*, *12*(2): 95-109.

Vivien Becart, Romain Lacraix, Carole Puech, Inaki Garcia de Cortazar-Atauri. (2022). Assessment of changes in Grenache grapevine maturity in a Mediterranea context over the last half-centuri, *OENO One*, vol. 56, no. 1.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

RESEARCH ON THE AGROBIOLOGICAL AND TECHNOLOGICAL VALUE OF SOME OLD ROMANIAN VINE VARIETIES IN THE CONTEXT OF CLIMATE CHANGES IN THE ODOBEŞTI VINEYARD, VRANCEA COUNTY

Marioara PUŞCALĂU, Ionica BOSOI, Alina Camelia DÎRLOMAN

Research and Development Station for Viticulture and Oenology Odobeşti,
61 Ştefan cel Mare Street, 625300, Odobeşti, Romania

Corresponding author email: oana_boss2002@yahoo.com

Abstract

The issue of returning to the ancestral varieties from the old sortiment specific to Romanian vineyards in the context of global warming and the conservation of biodiversity in wine-growing areas, represents one of the main directions of scientific research. The old Romanian grape varieties are currently preserved in ex situ germplasm collections (ampelographic collections) at the research units in the viticulture field. This paper presents preliminary results regarding the agrobiological and technological characteristics of four old autochthonous grape varieties (Vitis vinifera L.) for white wines ('Galbenă de Odobeşti', 'Cruciuliță', 'Pîrciu' and 'Gordin'), in the climatic conditions of the year 2024 in Odobeşti vineyard, Vrancea county. In the drought conditions of year 2024, these varieties demonstrated a high fertility potential (63.2-89.6% fertile shoots) and valuable technological characteristics, with a production that varied between 2.26 kg/vine for 'Pârciu' variety and 4.43 kg/vine in 'Cruciulita' variety, under the conditions of a good accumulation of sugars in the berries (>190 g/L) and a balanced total acidity (>5.1 g/L as tartaric acid).

Key words*: ancestral varieties, stress factors, agrobiological and technological value.*

INTRODUCTION

The practice of intensive viticulture in recent decades has greatly affected the biodiversity of the vine, causing the loss of local varieties and intravarietal variability, a phenomenon known as genetic erosion (OIV-VITI Resolution 677-2022). Worldwide, it is estimated that there are between 6,000 and 7,000 vinifera varieties, of which only a small part are authorized for planting (5-10%), so that currently there is a trend towards standardization of wine production. In the past, each wine-growing region had its own assortment of grape varieties (traditional) that differentiated the wine-growing areas in terms of varietal and the wines obtained. These old varieties are emblematic of each wine-growing area and are an integral part of the vine and cultural history of the respective areas (Dhananjay et al., 2021). The neglect or gradual abandonment of these historical grape varieties, and the emphasis on so-called "international" grape varieties, was mainly due to the susceptibility to disease and low yields exhibited by the native varieties.

In fact, in recent years there has been a global effort to rehabilitate forgotten grape varieties, threatened with extinction, due to the dominance of the great international grape varieties that represent only a small part of the biodiversity of the vine (Mannini, 2004; Volynkin et al., 2019; Fatalivev et al., 2023).

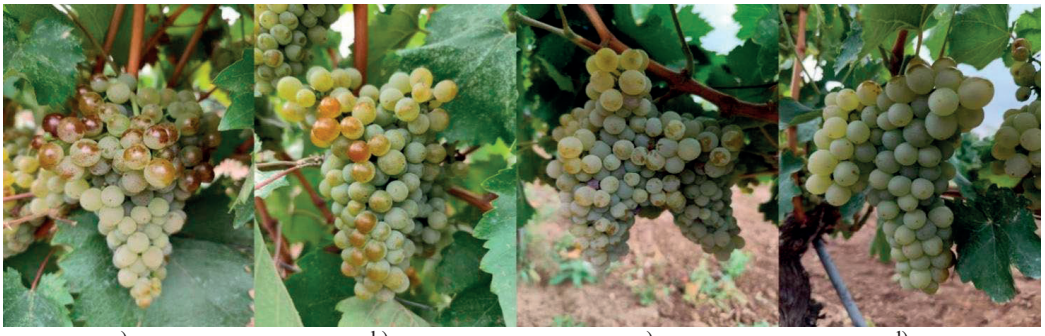
In Spain, for example, after three decades of research, the Ancestral Varieties project is responsible for the rediscovery of almost 50 varieties, six of which could have real oenological potential. The main objective of this initiative is not only to bring back old varieties generations after they were last used, but it is also an exercise in viticultural archaeology to recover our heritage. The advantages of growing these varieties include their ability to better withstand heat, drought and disease (Karoglan Kontić et al., 2009). They also tend to reach maturity either later or earlier and avoid sugar levels rising out of sync with overall phenolic ripening and excessively high alcohol levels (Kallithraka et al., 2005; Makuev, 2022; Faralli et al., 2024).

Global warming, as an extreme climatic phenomenon, is also felt in the cultivation of

vines, which leads to the reconsideration of the assortments of cultivated varieties (Jones, 2005; Fraga et al., 2012; Santos et al., 2020).
This is why even for the varieties from the old assortments of Romanian vineyards, that gave the note of originality specific to each vineyard, disappeared from the local wine assortment or less cultivated today (′Cruciuliță′, ′Pîrciu′, ′Gordin′, ′Braghină′, ′Negru vârtos′, ′Plăvaie′, ′Mustoasă′, ′Berbecel′, ′Razachie′ etc.), laborious work is required that requires both identification and conservation, but also reevaluation in current ecoclimatic conditions. Thus, it will be necessary to consider redefining zoning works and introducing ancestral varieties that have proven useful over time into the current assortments in Romanian vineyards (Oșlobeanu et al., 1991).
Research on the behavior of ancestral Romanian varieties under climate changes is one of the current topics for viticultural research in our country (Rotaru et al., 2018).

MATERIALS AND METHODS

The research was carried out within the ampelographic Collection of the Research and Development Station for Viticulture and Oenology (RDSVO) Odobești, with geographical coordinates 45º45′ north latitude, 27º06′ east longitude and an altitude of 150 m. The study was carried out in year 2024.
The biological material was represented by four old Romanian grapevine varieties, which were part of the old assortment of the Odobești vineyard, Vrancea county: ′Galbenă de Odobești′, ′Cruciuliță′, ′Pârciu′ and ′Gordin′ (Figure 1). Each genotype was represented in this study by three repetitions with five vines each. The studied cultivars were grafted onto the Kobber 5 BB rootstock. The pruning system practiced was the Dr. Guyot, with a fruit load between 30-36 buds/vine, distributed on strings of 8-9 buds and fruiting cones with 2-3 buds, and a semi-tall management form.



a) b) c) d)

Figure 1. The ancestral vine varieties studied: a) ′Galbenă de Odobești′; b) ′Cruciuliță′; c) ′Pârciu′; d) ′Gordin′

To evaluate the agrobiological potential under the conditions of the 2024 wine year, observations and determinations were made regarding: the phenological spectrum, growth vigor through measurements of shoots during the period of intense growth; fertility and productivity elements by calculating the percentage of fertile shoots (FS%), fertility coefficients (relative fertility coefficient - Rfc and absolute fertility coefficient - Afc) and productivity indices (relative productivity index - Rpi and absolute productivity index - Api); behavior to the main vine diseases and stress factors (frost, drought) by scoring from 1 to 9 according to the resistance scale developed by the OIV (2009). To determine the technological potential, determinations were made regarding the quantity (kg/vine, t/ha) and quality of grape production (g/L sugars using the refractometric method OIV-MA-AS2-02; 2012), g/L as tartaric acid using the potentiometric method), analysis of the physicochemical composition of 100 berrys, technological indices of grapes. The results were statistically interpreted using the Student's test (TTEST) in the Microsoft Excel program, for analysis of variance, using the average of the four cultivars as a control.
The climatic data for the study period were provided by the AgroExpert weather station and

the multiannual climatic database of the R.D.S.V.O. Odobești (1946-2023).

RESULTS AND DISCUSSIONS

Climatic conditions. The evolution of the thermal regime and precipitation in the viticultural ecosystem of the Odobești vineyard in the 2024 wine year is presented in Table 1. Global warming, a phenomenon that has strongly characterized the last decades, has considerably influenced the evolution of the annual thermal regime and during the vegetation season. From a thermal and hydrological point of view, the 2024 wine year was considered one of the warmest and driest years in the last decade. The thermal regime of the vegetation period was deeply excessive, the values recorded for the thermal balances - Σ °t active; Σ °t useful - (3879.7 respectively 2089.7), being clearly superior to the multiannual values (3231.6 respectively 1554.2). The average air temperature (21.4°C), the average maximum temperatures (27.9°C) and the average minimum temperatures in this period (15.3°C) recorded values much higher than the multiannual data for these elements (18.2°C, 24.6°C, and 12.5°C, respectively). During this period, 71 days with maximum temperatures above 30°C were recorded, of which 24 days with temperatures above 35°C.

In the wine year 2024, the rainfall regime was deeply deficient throughout the vegetation period, except for September, when a surplus of 12.3 mm was recorded. The amount of precipitation recorded in this period (245.8 mm) represents only 63.7% of the multiannual value for this period (385.7 mm), with 6 days with precipitation greater than 10 mm recorded. The relative air humidity recorded a value almost 10 percent lower (54.3%) than the multiannual value (63.7%). The duration of sunshine during this period accumulated 1719.0 hours, higher than the multiannual value (1498.1 hours).

Table 1. The main climatic conditions during the grapevine growing season (SCDVV Odobești, 2024)

Month	Average temperatures (°C)		Average extreme temperatures (°C)		Sum of temperatures useful -Σ°tu (°C)		Rainfall sum (mm)		Sunstroke (hours)		Hygroscopicity of air (%)	
	multi-annual	2024	min.	max.	Multi-annual	2024	multi-annual	2024	multi-annual	2024	multi-annual	2024
April	11.1	15.5	10.0	21.6	69.4	172.7	48.4	33.9	188.8	261.5	62.8	55.9
May	16.8	16.6	10.4	22.8	217.6	205.1	72.5	28.8	238.6	269.0	65.4	56.7
June	20.2	24.2	17.5	31.2	313.7	425.3	85.9	50.4	265.3	315.5	64.0	56.9
July	22.2	26.8	20.1	33.5	377.1	520.0	76.8	51.8	301.1	352.0	63.2	47.8
Aug.	21.7	25.4	19.2	32.5	362.8	477.8	58.9	24.0	284.3	316.0	62.0	48.3
Sept.	17.1	19.6	14.7	25.6	213.6	288.8	45.0	57.3	220.0	205.0	66.5	60.2
Average /sum	18.2	21.4	15.3	27.9	1554.2	2089.7	387.5	246.2	1498.1	1719.0	64.0	54.3

Phenological spectrum

The phenological evolution of the four ancestral indigenous varieties was influenced by the specific climatic conditions of the 2024 wine year (Table 2).

Due to the high temperatures recorded in March and April, disbudding was recorded early in the first decade of April, with 10-15 days earlier compared to 2023, between April 2 for the ′Cruciuliță′ variety and April 6 for the ′Gordin′ variety (Figure 2).

Flowering was recorded in the third decade of May, with 8-12 days earlier than in 2023, between May 24 for the ′Galbenă de Odobești′ variety and May 27 for the ′Pârciu′ variety (Figure 3).

Grape veraison was recorded in the last decade of July - the first decade of August, with 10 - 15 days earlier compared to 2023, between July 30 for the ′Galbenă de Odobești′ variety and August 3 for the ′Gordin′ variety.

Due to dry climatic conditions, the full ripening of the grapes was recorded between August 21 for the ′Pârciu′ variety and 26 August for the ′Galbenă de Odobești′ variety, with 12 to 18 days earlier compared to 2023.

The leaf fall for the four varieties occurred in the last decade of October, thus, under the conditions of the 2024 wine year, the duration of the vegetation period for the studied varieties varied between 198 and 208 days.

Table 2. The phenological spectrum of the ancestral varieties studied (Odobești, 2024)

Ancestral grape variety	Phenological phases					During the vegetation days
	Disbudding	Flowering	Grapes veraison	Technological maturity	Fall leaves	
'Galbenă de Odobești'	3 - 7.IV	22 - 26.V	28.VII - 1.VIII	25 -27.VIII	25 - 29.X	202 - 206
'Cruciuliță'	31.III - 4.IV	23 - 27.V	29.VII - 2.VIII	24 - 26.VIII	23 - 26.X	203 - 206
'Pârciu'	3 - 7.IV	25 - 29.V	30.VII - 2.VIII	20 - 22.VIII	20 - 25.X	198 - 203
'Gordin'	4 - 8.IV	24 - 28.V	1 - 4.VIII	25 -27.VIII	26 - 30.X	203 - 208



Figure 2. 'Galbenă de Odobești' variety - phenological aspects

Fertility and productivity characteristics. The main characteristics of the fertility elements of the old grape varieties studied in the Odobești wine-growing area under the conditions of the 2024 wine year are presented in Table 3.

Under the climatic conditions of 2024, the ancestral grape varieties 'Galbenă de Odobești', 'Cruciuliță', 'Pîrciu' and 'Gordin' recorded values of the fertile shoot coefficient and of the relative and absolute fertility coefficients close to the values described by the specialized literature in the Odobești vineyard. The fertile shoot coefficient (FS) varied between 63.2% for the 'Pârciu' variety and 89.1% for the 'Cruciuliță' variety.

The fertility potential of these varieties was also confirmed by the values obtained for the relative and absolute fertility coefficient (Rfc and Afc). The highest values of fertility coefficients (relative and absolute) were recorded for the 'Cruciuliță' variety (Rfc -1.63 and Afc - 1.83). Relative to values for the average weight of the berry clusters, the productivity indices (relative and absolute) recorded higher values for 'Cruciuliță' variety (296.0 respectively 332.3) and 'Gordin' variety (288.8 respectively 321.1) and lower values for the varieties 'Galbenă de Odobești' (227.5 respectively 262.2) and Pârciu (144.7 respectively 229.5).

Table 3. The fertility and productivity characteristics (Odobești, 2024)

Ancestral grape variety	Fertile Shoots (%)	Fertility coefficients		Weight of bunch (g)	Productivity indices	
		Rfc	Afc		Ipr	Ipa
'Galbenă de Odobești'	87.0	1.28	1.47	177.7	227.5	261.2
'Cruciuliță'	89.1	1.63	1.83	181.6	296.0	332.3
'Pârciu'	63.2	0.87	1.38	166.3	144.7	229.5
'Gordin'	89.6	1.52	1.69	190.0	288.8	321.1



Figure 3. 'Cruciuliță' variety - phenological aspects

The growth vigor. To evaluate the vegetative growth of the varieties under study, measurements were periodically (every 7 days)
taken on the shoots during the vegetative growth period, respectively after the flowering phenophase until their intensive growth phase (period May 24 - July 1) (Table 4).

Table 4. The growth vigor according to OIV descriptors (Odobești, 2024)

Ancestral grape variety	No. of shoots/vine	Average shoot length (cm)	Shoot growth vigor (OIV 351)
′Galbenă de Odobești′	19.3	110.9	5 (medium)
′Cruciuliță′	18.3	101.2	3-5 (weak-medium)
′Pârciu′	16.3	77.0	3- (weak)
′Gordin′	17.4	90.8	3- (weak)

Due to the dry climatic conditions of the 2024 wine year, the vegetative growth of the four varieties was weak, below the values recorded in the specialized literature. At an average number of shoots per vine ranging between 16.3 for ′Pârciu′ variety and 19.3 for ′Galbenă de Odobești′ variety, the average shoot length varied between 77.0 cm for ′Pârciu′ variety and 110.9 cm for ′Galbenă de Odobești′ variety, being classified according to the OIV 351 descriptor as varieties with weak and weak to medium growth.

The biological resistance to the main cryptogamic diseases was established by assessing the grades from 1 to 9 according to the resistance scale developed by O.I.V (International Organisation of Vine and Wine). Under the vegetation conditions of 2024 and the application of six phytosanitary treatments, the four ancestral Romanian varieties showed good (7-9) and very good (9) tolerance to the attack of the main grapevine diseases (Table 5).

Table 5. Behavior to the main grapevine diseases (OIV descriptor list for grape varieties and Vitis species, 2nd edition - 2009), Odobești, 2024

Ancestral grape variety	Downy mildew (*Plasmopara viticola*)		Powdery mildew (*Uncinula necator*)		Gray rot (*Botrytis cinerea*)	
	Leaf OIV 452	Grape OIV 453	Leaf OIV 455	Grape OIV 456	Leaf OIV 458	Grape OIV 459
′Galbenă de Odobești′	7-9	7-9	7-9	7-9	9	9
′Cruciuliță′	9	7-9	7-9	7-9	9	9
′Pârciu′	9	9	9	7-9	9	9
′Gordin′	9	7-9	7-9	9	9	9

Drought resistance (OIV 403). According to the OIV descriptor list for grape varieties and Vitis species, 2nd edition, 2009. Under the conditions of the 2024 growing season, considered based on recorded climatic data, one of the driest years in the Odobești vineyard, the studied ancestral Romanian grapevine varieties did not present specific manifestations of thermal and water stress (withering of the shoot tips, yellowing leaves at the base of the trunk, wilting of grapes etc.), demonstrating high to very high tolerance to the phenomenon of atmospheric and pedological drought (Table 6).

Table 6. Behavior at drought according to OIV descriptors (Odobești, 2024)

Ancestral grape variety	OIV 403	Expression level
′Galbenă de Odobești′	9	Very high
′Cruciuliță′	7-9	High - Very high
′Pârciu′	7-9	High - Very high
′Gordin′	7-9	High - Very high

Quantity and quality of production. The results obtained regarding the technological potential of the four old Romanian varieties in the wine year 2024 revealed that the level of climatic factors existing in the ecosystem, but also the genetic factor, influenced the values of the elements that define the potential and actual productivity, grape production, but especially its quality (Table 7). Under the conditions of the 2024 wine year, the average grape mass recorded values lower than those recorded in the specialized literature for these varieties in the Odobești vineyard, ranging between 166.3 g for ′Pârciu′ variety and 190.0 g for ′Gordin′ variety (Figures 4 and 5).

Determined by the average weight of the grape and the number of grapes per vine, the grape production per vine varied between 2.26 kg for ′Pârciu′ variety and 4.43 kg for ′Cruciuliţă′ variety, resulting in an average production calculated per hectare ranging between 8.560 kg for ′Pârciu′ variety and 16.770 kg for ′Cruciuliţă′ variety, productions lower than those recorded in the specialized literature for these varieties.

The dry climate recorded in the Odobești wine-growing area during grape ripening influenced the accumulation of sugars in the berries, the values recorded being higher than those specified in the specialized literature

(Constantinescu et al., 1959; 1962) for the varieties studied, these being between 190 g/L for 'Gordin' variety and 219 g/L for 'Pârciu' variety, with intermediate values for 'Cruciuliță' variety (202 g/L), and 'Galbenă de Odobești' variety (210 g/L).

Table 7. The quantitative and qualitative characteristics of the grapes (Odobești, 2024)

Ancestral grape variety	No. grapes/ vine	Weight of bunch (g)	Grape production		Sugar content of the must (g/L)	Total acidity (g/L tartaric acid)	Gluco-acidometric index
			kg/vine	kg/ha			
'Galbenă de Odobești'	20.2	177.7	3.59	13.590	210	5.26	39.9
'Cruciuliță'	24.4	181.6	4.43	16.770	202	5.62	35.9
'Pârciu'	13.6	166.3	2.26	85.600	219	4.16	52.6
'Gordin'	22.4	190.0	4.26	16.130	190	5.10	37.3
Average - control	20.1	178.9	3.64	13.762	205.3	5.04	41.4



Figure 4. 'Pârciu' variety - phenological aspects

In contrast, for the total acidity of the must, lower values were recorded compared to those specified in the specialized literature for these varieties, this being between 4.16 g/L tartaric acid for 'Pârciu' variety and 5.62 g/L tartaric acid for 'Cruciuliță' variety, with intermediate values for 'Galbenă de Odobești' variety (5.26 g/L tartaric acid) and 'Gordin' variety (5.10 g/L tartaric acid). The values recorded for the sugar content and total acidity of the must determined higher values of the glucoacidimetric index, whose ranging between 35.9 for 'Cruciuliță' variety and 52.6 for 'Pârciu' variety.



Figure 5. 'Gordin' variety - phenological aspects

The statistical interpretation of the experimental data obtained for the main technological characteristics of the studied varieties compared to the average values - control, was performed by applying the Student test in Microsoft Excel (TTEST).

Regarding grape production/vine, the P probability values obtained through the Student test show that 'Pârciu' (P = 0.002211) and 'Cruciuliță' (P = 0.042744) varieties present statistical differences compared to the control variant - the average (Table 8).

And the P probability values obtained through the Student test for the acidity indicator show that the varieties 'Pârciu' (P = 0.001505) and 'Cruciuliță' (P = 0.010836) present statistical differences compared to the control variant - the average.

Table 8. Student's t-test probability value (P) for establishing statistical difference from control

Ancestral grape variety	Student's t-test probability value (P) P critical <0.05		
	Grape production	Sugar content of the must	Total acidity
′Galbenă de Odobești′	0.887285	0.560830	0.185383
′Cruciuliță′	0.042744	0.709985	0.010836
′Pârciu′	0.002756	0.061367	0.001505
′Gordin′	0.061057	0.066501	0.441804

The ′Pârciu′ variety shows significant negative statistical differences, and the ′Cruciuliță′ variety shows positive statistical differences. The P probability values obtained through the Student test for the sugar content in the juice show that the studied varieties do not present statistical differences compared to the control variant - the average (P>Pcritic).

Analysis of the physicochemical composition of 100 berrys. The average weight of 100 berrys recorded values that varied between 126 g for ′Cruciuliță′ variety and 172 g for ′Galbenă de Odobești′ variety, with intermediate values for ′Pârciu′ (137 g) and ′Gordin′ (154 g) varieties, lower by 25 to 31% compared to the values specified in the specialized literature (Table 9).
Of the average weight of 100 berrys, the weight of the flesh represented between 88.3% for ′Pârciu′ variety and 92.2% for ′Gordin′ variety, and the weight of the skin represented between 3.3% for ′Gordin′ variety and 8.0% for ′Pârciu′ variety. The number of seeds contained in 100 berrys varied between 151 in ′Galbenă de Odobești′ variety and 214 in ′Cruciuliță′ variety, and the average seed weight recorded values between 4.94 g in ′Pârciu′ variety and 6.56 g in ′Gordin′ variety.

Table 9. Analysis of the physicochemical composition of 100 berrys (Odobești, 2024)

Ancestral grape variety	Weight (g)	Volume (cm3)	No. of seeds	Seeds weight (g)	Skin weight (g)	Flesh weight (g)
′Galbenă de Odobești′	172	162	151	5.76	11.45	154.89
′Cruciuliță′	126	115	214	5.27	6.51	114.22
′Pârciu′	137	120	165	4.94	10.73	121.33
′Gordin′	154	145	174	6.56	5.13	142.31

The analysis of the values obtained for the main technological indices shows that, with the exception of ′Galbenă de Odobești′ variety, the grape structure index recorded values below 30, indicating that the grapes are not well formed, with a low yield in normally developed berries (Table 10).

Table 10. Technological indices of grapes at harvest (Odobești, 2024)

Ancestral grape variety	Grape composition index	Berrys index	Berry composition index	Yield index
′Galbenă de Odobești′	31.9	55.1	9.1	4.4
′Cruciuliță′	21.4	80.3	9.7	4.2
′Pârciu′	26.7	66.7	7.7	3.2
′Gordin′	27.8	62.8	12.2	3.5

The values obtain for the berry composition index (55.1-80.3), highlight the fact that the studied varieties have reached the specific parameters of the production direction in which they fall, and the yield index presented values specific to wine grape.

CONCLUSIONS

From a climatic point of view, the 2024 wine year in the Odobești vineyard was an extremely dry one with a profoundly excessive thermal regime and a deficient precipitation regime, accumulated over the last 5-6 years.
In the conditions of the 2024 wine year, the ancestral romanian varieties ′Gordin′, ′Cruciuliță′ and ′Galbenă de Odobești′ showed high fertility (over 85%), and ′Pârciu′ variety showed medium fertility (63%), confirming the data from the specialized literature.
Under the conditions of the 2024 wine year, the average grape mass recorded values lower than those recorded in the specialized literature for these varieties in the Odobești vineyard, ranging between 166.3 g for ′Pârciu′ variety and 190.0 g for ′Gordin′ variety.

The production of grapes per vine varied between 2.26 kg for ′Pârciu′ variety and 4.43 kg for ′Cruciuliță′ variety, lower values compared to those specified in the R.S.R. Ampelography for these varieties**.**

The dry climate recorded in the Odobești wine-growing area during grape ripening determined the accumulation of higher amounts of sugars in the berries (190-219 g/L) in conditions of decreased values for the total acidity of the must (4.16-5.62 g/L tartaric) compared to data in the literature.

The statistical interpretation of the experimental data for the main technological characteristics of the studied varieties compared to the average values - control, shows statistical differences for grape production and total acidity in the must in the case of ′Cruciuliță′ and ′Pîrciu′ varieties.

The average weight of 100 berries recorded values lower by 25 to 31% compared to the values specified in the Ampelography of R.S.R., of which the pulp weight represented between 88.3-92.2%, the skin mass between 3.3-8.0%, and the seed mass between 3.7- 4.5%.

Taking into account the extremely dry climatic conditions in the Odobești wine-growing area, it is necessary to continue research on the evaluation of the agrobiological and technological potential of these ancestral Romanian varieties, in order to obtain conclusive data on their agrobiological and technological value in order to capitalize on them in the context of climate change.

ACKNOWLEDGEMENTS

The work was carried out within the project S.P. ADER 6.3.12/20.07.2023, funded by the Ministry of Agriculture and Rural Development of Romania through the Sectorial Plan for Research and Development in the Agricultural Field – ADER 2026.

REFERENCES

Constantinescu, Gh., Negreanu, E., Lăzărescu, V., Poenaru, I., Alexei, O., Boureanu, C. (1959). *Ampelografia Republicii Populare Romîne*, vol. II. Bucharest, RO: Academiei Republicii Populare Romîne Publishing House.

Constantinescu, Gh., Negreanu, E., Lăzărescu, V., Poenaru, I., Alexei, O., Mihalca, Gh. (1962). *Ampelografia Republicii Populare Romîne*, vol. IV and V. Bucharest, RO: Academiei Republicii Populare Romîne Publishing House.

Dhananjay, Dr., & Gawande, N. (2021). A retrospection of Indian grape varieties. *The Pharma Innovation Journal* 2021; SP-10(11), 408-414.

Fataliyev, H., Lazgiyev, Y., İmamguliyeva, M., Haydarov, E., Fataliyeva, Sh., Huseynova, Sh., Agayeva, S., İsganderova, S., Askarova, A., & Askarova, İ. (2023). Comparative evaluation and studing of some indigenous and introduced red grape varieties. *Food Science & Technology (2073-8684),* ISSN 2073-8684, Vol 17, Issue 2, p.18.

Faralli, M., Mallucci, S., Bignardi, A., Varner, M., & Bertamini, M. (2024). Four decades in the vineyard: the impact of climate change on grapevine phenology and wine quality in northern Italy. *OENO One 58* (3). DOI: https://doi.org/10.20870/oeno-one.2024.58.3.8083.

Fraga, H., Malheiro, A. C., Moutinho-Pereira, J., & Santos, J.A. (2012). An over view of climate change i mpacts on European viticulture, *Food and Energy Security 1*(2), 94-110.

Jones, G.V., Duchene, E., Tomasi, D., Yuste, J., Braslavksa, O., Schultz, H., Martinez, C., Boso, S., Langellier, F., Perruchot, C., Guimberteau, G. (2005). Changes in European Winegrape Phenology and Relationships with Climate. *Proceedings GESCO, Geisenheim, Germany,* 54-61.

Kallithraka, S., Mohdaly, A.A, Makris, P. D., & Kefalas, P. (2005). Determination of major anthocyanin pigments in Hellenic native grape varieties (*Vitis vinifera* sp.): association with antiradical activity. *Journal of Food Composition and Analysis, 18*(5), 375-386.

Karoglan, Kontić, J., Preiner, D., Šimon, S., Zdunić, G., Poljuha, D., & Maletić, E. (2009). Sanitary Status of Croatian Native Grapevine Varieties. *Agriculturae Conspectus Scientificus*, *74*(2), 99-103.

Mannini, F., (2004). Italian indigenous grapevine cultivars: guarantee of genetic biodiversity and economic resources. *ISHS Acta Horticulturae 652,* 87-95.

Makuev, G.A., Isrigova, T.A., Mukailov, M.D., & Salmanov, M.M., Magomedov, M.G., & Gadzhieva, M. (2022). Technological assessment of native grapes varieties for winemaking in the conditions of Southern Dagestan. *EESTE-2021 IOP Conf. Series: Earth and Environmental Science,* 979, 1-7. Publishing doi:10.1088/1755-1315/979/1/012018 1.

Oşlobeanu, M., Macici, M., Georgescu, M., Stoian, V., (1991). *Zonarea soiurilor de viţă de vie în România,* Bucharest, RO: Ceres Publishing House.

Rotaru, L., Colibaba, L.C., & Aelenei, S.I. (2018). The Agrobiological and technological value of ancient Romanian grape varieties (*Vitis vinifera* L.) cultivated in Iaşi vineyard. *Lucrări Ştiinţifice, Seria Horticultură USAMV Iaşi,, vol. 61*(1), 135-138.

Santos, J.A., Fraga, H., Malheiro, A.C., Moutinho-Pereira, J., Dinis, L.T., Correia, C., Moriondo, M., Leolini, L., Dibari, C., Costafreda-Aumedes, S.,

Kartschall, T., Menz, C., Molitor, D., Junk, J., Beyer, M., & Schultz, H. (2020). Review of the Potential Climate Change Impacts and Adaptation Options for European Viticulture. *Appl. Sci., 10*(9), 3092, 1-28.

Volynkin, V., Polulyakh, A., Levchenko, S., Vasylyk, I., & Likhovskoi, V. (2019). Autochthonous grape species, varieties and cultivars of Crimea. *ISHS Acta Horticulturae 1259,* 91-98.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

INFLUENCE OF SOME SOIL HERBICIDES ON THE VEGETATIVE MANIFESTATIONS OF GRAFTED CUTTINGS OF SIYANA VINE VARIETY

Svetlana STOYANOVA, Galina DYAKOVA, Ralitsa MINCHEVA, Evgeniya ZHEKOVA

Agricultural Academy, Institute of Agriculture and Seed Science "Obraztsov chiflik",
1 Prof. Ivan Ivanov Street, 7007, Rousse, Bulgaria

Corresponding author email: e.d.zhekova@abv.bg

Abstract

The article shows the results of the influence of three soil herbicides: pendimethalin (Stomp New 330EK), s-metolachlor (Dual Gold 960EK) and pendimethalin and clomazone (Alcans Sync Tech) on the vegetative manifestations of the grafted vine cuttings. The study was carried out during the period 2018-2021, in the experimental vine rooting nursery of IASS "Obratzov Chiflik", with Siyana dessert variety. Dual Gold 960EK and Stomp New 330EK were found to have high selectivity, provide good control of annual weeds, do not suppress budding and do not reduce the vigor of the main shoot growth. The tested herbicides did not negatively affect the synthesis of photosynthetic pigments. Alcans Sync Tech caused a moderate to moderately strong phytotoxic effect, expressed in a chlorotic halo of the first leaves of the cuttings, which was overcome at a later stage and did not affect their development. Despite slight fluctuations in the values of the ratio of chlorophylls to carotenoids, there was no evidence of premature aging due to stress factors.

Key words*: weeds, herbicides, efficacy, phytotoxicity, vine rootstock.*

INTRODUCTION

The essence of modern concepts for the development of sustainable agriculture consists in finding the appropriate, optimal productive level for each crop, by applying such systems, that guarantee obtaining stable yield for a long period of time and preserving natural resources, including the main means of production – the soil.

For the successful rooting of vine cuttings, soil preparation is important. The expected high and quality yield, from standard grafted vines, can be obtained only if all the requirements in the technologies for their cultivation are observed - control of weeds, diseases and enemies. One of the most dangerous competitors of vine cuttings are weeds, and it is very important to control them in the early phases of the crop's development (immediately after planting the cuttings in the rooting nursery) to avoid competition for water, light and nutrients substances (Correia et al., 2012; Gitti et al., 2012; Nosratti et al., 2020).

Weeds are the vine's main competitor for the absorption of the main biotic factors. Boychev (1980) and Prodanova-Marinova & Staneva (2018) found that the multiplication of weeds in a vine rooting nursery transpired much more intensively than the vine and extract larger amounts of nutrients from the soil. According to Dimitrov & Prodanova-Marinova (2019) and Staneva & Rankova (2017), in addition to the fact that weeds are good accumulators of nutrients, their percentage content of nitrogen, phosphorus, potassium and magnesium in the leaf mass is higher, compared to the crops. The high degree of weed infestation usually leads to less developed shoots and a decrease in the quantity and quality of vine planting material.

There are studies that show that oxyfluorfen, pendimethalin, s-metolachlor, etc. can be used. Flumioxazin shows high efficacy against tilling-type weed associations (typical for the rooting nursery) and selective action in the production of grapevine planting material (Ivanova & Rankova, 2014; Rankova & Tityanov, 2014; 2017; Tsvetanov et al., 2014). A number of authors also report high efficacy and good selectivity of some soil herbicides (fluroxypyr, glyphosate, flumioxazin, isoproturon) used immediately after planting the grafted cuttings (Lange et al., 1970; Chelebiev & Encheva, 2002; Marble et al., 2016). The influence of trifluralin, napropamide, oxyfluorfen, pendimethalin and other active substances on the rooting process

and vegetative manifestations of young vines also was studied (Leonie et al., 2014). The production of vine planting material is a process that is influenced by a number of factors of a biological, ecological and technological nature (Risina, 2007; Schildberger et al., 2007).

Herbicides are an excellent assistant to the farmer in the weed control, but not infrequently they can also be a double-edged sword. If herbicides are not well known and the technology of their use is not observed, the crop can suffer significant losses, which naturally has direct economic consequences for farmers. Regardless of the selectivity of herbicides, they have a stressful effect on crop plants, which directly affects yield (Christopher et al., 2021; Ionescu and Popescu, 2024; Matei et al., 2024).

The objective of the study was to determine the influence of some soil herbicides on the growth and development of grafted vine cuttings of Siyana variety in a vine rooting nursery.

MATERIALS AND METHODS

During the period 2018-2021, in the experimental vine rooting nursery of the Institute, on soil type leached chernozem, the influence of three soil herbicides Stomp New 330EK (330 g l^{-1} pendimethalin), Dual Gold 960EK (960 g l^{-1} s-metolachlor) and Alcans Sync Tech (298 g l^{-1} pendimethalin; 43 g l^{-1} clomazone) on the vegetative manifestations of the grafted cuttings of Siyana vine cultivar was studied. The active soil fertility is characterized with a good potassium (33.17 mg $100g^{-1}$ soil), insufficient nitrogen (16.84 mg $100g^{-1}$ soil), poor phosphorus (6.15 mg $100g^{-1}$ soil) nutritional regime and slightly acidic soil reaction pH (in KCL 5.6-6.7, degree of saturation with bases 89-92%) (Sabev & Stanev, 1963; Dimitrov, 1990). The experiment was carried out with Siyana dessert vine variety, grafted on SO4 rootstock (Berlandieri x Riparia). Before rooting, the stratified grafted cuttings were waxed up to 2/3 of their length, then rooted to a depth of 15-17 cm (up to the non-waxed part). The vines were planted in the spring, when the soil temperature at a depth of 10-15 cm reached 11-12ºC (for the region of Obraztsov chiflik, it was in the third ten days of May). Paraffinized grafted cuttings were rooted according to the technology of open cultivation, adopted by IASS "Obratzov Chiflik", with drip irrigation and micro-jet misting in the first month after planting for rooting (Todorov, 2005). The planting scheme was in two-row beds with a distance between rows of 50 cm, and in rows - 7-8 cm. Variants included 264 pcs grafted vines planted in four replications of 66 each, with an experimental plot area of 2.31 m^2 and compared with a control (untreated) variant with grafted vines of the same variety. The following variants were tested during the experiment: control (untreated); Stomp New 330EK (1.98 kg ha^{-1} a.c.); Dual Gold 960EK (1.44 kg ha^{-1} a.c.) and Alcans Sync Tech (0.85 kg ha^{-1} a.c.). A sprayer was used for the treatment with the herbicides, at a working solution consumption of 400 l ha^{-1}, applied at doses indicated in Table 1, and the control variant was kept clean of weeds by manual weeding. The selectivity by the 9 score scale of EWRS was evaluated on the 7-th and the 14-th day after the herbicide application (at score 0 there were not damages on the crop, and at score 9 the crop was completely destroyed).

The grafted cuttings of all variants were fertilized equally with nitrogen, phosphorus and potassium fertilizers.

Table 1. Herbicides and doses of treatment

Variants	Active substance, g l^{-1}	Doses - commercial product, l ha^{-1}	Dose - a.c., kg ha^{-1}
Control		-	-
Stomp New 330EK	330 g l^{-1} pendimethalin	6.00	1.98
Dual Gold 960EK	960 g l^{-1} s-metolachlor	1.50	1.44
Alcans Sync Tech	298 g l^{-1} pendimethalin; 43 g l^{-1} clomazone	2.50	0.85

To clarify the influence of the tested soil herbicides on the grafted vine cuttings, the following parameters were traced:

✓ Emergence in dynamics - counting the emerging cuttings at the end of the first, second, third, fourth and fifth ten days, after planting them in the rooting nursery. The percentage of emergence was calculated based on the total number of cuttings planted.

✓ Length of the main shoot - the length of the main shoot (cm) was measured at the end of each ten-day period from the thirtieth day after planting to the beginning of wood maturation.

✓ Growth speed of the main shoot in dynamics - the growth speed of the main shoot was calculated in mm/day for each ten-day period. 10 vines per replication were measured.
✓ Content of photosynthetic pigments in the leaves (chlorophyll a, chlorophyll b and carotenoids c) - was determined by the spectrophotometric method (mg 100 g-1) described by Delvin et al. (1981), by extracting pigments with an 85% acetone extract. An average sample was used for a variant of maximally developed leaves (6th - 8th leaf from the shoot tip to the base) on the 30th day after the treatment with the herbicides.

The data on the average daily temperature, during the study period, were taken from the stationary meteorological cell in IASS "Obratzov Chiflik", located near the Experimental Vineyard.

The mathematical processing of the results was carried out by two-factor analysis of variance (ANOVA), with the statistical program SPSS 19.0 (Ganeva, 2016). The significance of the assessment and the strength of the influence of the factors was determined, as a part of the intergroup variation in the total variation, and calculated after the method of Plohinsky (Lakin, 1990).

RESULTS AND DISCUSSIONS

Evaluating the impact of average monthly temperatures during the vegetation of the vines set for rooting, the studied years were relatively favorable for their development (Table 2). The average monthly air temperature according to the multi-year norm (over a 109-year period) was 15.8ºC. The data showed that the studied years had air temperatures above the multi-year norm. July (2018) and June (2021) were exceptions, with average monthly temperatures below the multi-year average temperature, with a deviation of -0.21ºC and -0.22ºC. The annual variation of the temperatures showed that the years of the study could be considered as hot and differed with significantly higher average monthly air temperatures compared to the multi-year period. The period of active vegetation of the vines for rooting (from May to November), during the years of the study, had a clear trend of higher temperature deviations, compared to the multi-year norm (+0.04ºC to +8.0ºC), which did not have an influence on the development of rooting vine cuttings. Venkataraman & Krishnan (1992) found that the phenology of a crop is largely dependent on genetic and environmental factors, including temperature and sunshine.

Table 2. Average monthly air temperature (°C), for the period 2018-2021

Period		Vegetation period							Average V - XI
		V	VI	VII	VIII	IX	X	XI	
Average for MP		16.4	20.2	22.5	23.9	17.9	7.2	2.7	15.8
Deviation, ± °C from MP	2018	5.00	1.68	-0.21	0.04	1.30	6.30	2.80	2.40
	2019	0.60	2.18	0.49	0.54	1.70	6.60	8.20	2.90
	2020	0.10	0.38	1.59	1.44	2.70	7.50	3.10	2.40
	2021	0.40	-0.22	2.09	0.04	-0.20	8.00	4.20	2.00

Legend: MP (multi-year period); The period from 1896 to 2006 was used for a multi-year period, as climate norm, since that period included decades with differences in the meteorological factors.

In the area of the vine rooting nursery, 2 groups of weeds which formed tilling-type associations were determined: late spring - *Setaria viridis* Beauv., *Echinochloa crus-galli* L., *Digitaria sanguinale* L., *Solanum nigrum* L., *Chenopodium album* L., *Polygonum aviculare* L., *Tribulus terrestris* L., *Portulaca oleracea* L., *Xanthium strumarium* L., *Amaranthus retroflexus* L., *Persicaria lapathifolia* L., *Anthemis arvensis* L.; perennial rhizomes - *Convolvulus arvensis* L., *Cirsium arvense* Scop. и *Sonchus arvensis* L. Table 3 shows the average weed density, averaged over the study period, by species, 30 and 60 days after the fertilization with Stomp New 330EK, Dual Gold 960EK and Alcans Sync Tech. The perennial species *Convolvulus arvensis* L., *Cirsium arvense* Scop. and *Sonchus arvensis* L. were very little affected by the soil herbicides.

When double counting was done in the density of the weed associations, the applied soil herbicides had a significant long-lasting effect. The preservation of the total density of the weeds until the 60th day after the treatment, was due to the efficient action of the soil herbicides. The smallest density of weeds and accordingly the most significant herbicidal effect throughout the entire period of study were reported by the variants with Alcans Sync Tech and Stomp New 330EK (Table 3).

The same trend has been proven by other authors, who noted that the use of soil herbicide

products has a positive effect on the density of weed associations in a vine rooting nursery (Prodanova, 2012; 2015). Sarpe et al. (2007) and Andr et al. (2014) also reported a similar effect on *Amaranthus retroflexus* L., *Setaria viridis* Beauv. and *Solanum nigrum* L.

Table 3. Weed density by species, 30 and 60 days after treatment with soil herbicides (pcs. m^{-2}), average for the period 2018-2021

Types of weeds	30 days				60 days			
	Control	Stomp New 330EK	Dual Gold 960EK	Alcans Sync Tech	Control	Stomp New 330EK	Dual Gold 960EK	Alcans Sync Tech
Late spring weeds								
Setaria viridis Beauv.	53	-	-	-	72	-	-	-
Echinochloa crus galli L.	28	-	-	-	40	-	-	-
Digitaria sanguinale L.	14	-	-	-	20	-	-	-
Solanum nigrum L.	4	-	-	-	12	-	-	-
Chenopodium album L.	2	-	-	-	10	-	-	-
Persicaria lapathifolia L.	26	-	-	-	10	-	-	-
Portulaca oleracea L.	14	4	2	-	43	4	2	-
Xanthium strumarium L.	8	6	9	7	12	6	9	7
Amaranthus retroflexus L.	2	-	-	-	20	-	-	-
Anthemis arvensis L.	-	-	-	-	6	-	-	-
Polygonum aviculare L.	2	-	-	-	15	1	-	3
Tribulus terrestris L.	3	-	-	-	6	2	1	-
Total weeds	156	10	11	7	266	10	11	7
Perennial rhizomes								
Convolvulus arvensis L.	15	-	1	1	17	-	1	-
Cirsium arvense Scop.	2	-	-	-	2	-	-	-
Sonchus arvensis L.	-	-	1	1	6	-	4	5
Total weeds	17	-	2	2	25	-	5	5
Total number of weeds	173	10	13	9	291	21	26	21

In addition to the good herbicidal activity against weeds, the tested soil herbicides also showed good selectivity towards emerging grafted cuttings. In the counts made, the number of the emerging grafted cuttings in the treated variants was greater, compared to that in the control variant. Table 4 shows the data about the dynamics of the emerging grafted vine cuttings, average over the period of the experiment, recorded from the 30th to the 70th day after planting and their treatment with herbicides in the vine rooting nursery.

The percentage of emerging of the grafted cuttings, from the 30th to the 70th day after the treatment with Stomp New 330EK, Dual Gold 960EK and Alcans Sync Tech, was higher than that of the control variant. Statistical differences were found, at the level of significance α=0.05, for the variants Stomp New 330EK, Dual Gold 960EK (on the 50th day of treatment) and Alcans Sync Tech (on the 70th day of treatment). Good statistical significance (α=0.01) was found on the 30th (Stomp New 330EC and Dual Gold 960EC) and 70th (Dual Gold 960EC) days of variant treatment. Very good significant differences, at α=0.001, had the variants treated with Alcans Sync Tech on the 30th day and Stomp New 330EK 330EK on the 70th day of the use of the products.

In Table 5, the analysis of variance showed that, on average over the study period, the use of herbicides (factor B) had a significant effect (α=0.01) on the emerging of grafted cuttings and with a power of impact of 34.29%. The unexplained influence due to random factors was 29.87%.

Table 4. Vine germination dynamics (%) of grafted cuttings in vine rooting nursery, on average for the period 2018-2021.

Days / Variants	30-th day	Difference	40-th day	Difference	50-th day	Difference	60-th day	Difference	70-th day	Difference
Control	85.95	-	91.32	-	90.85	-	92.35	-	93.62	-
Stomp New 330 EC	90.12**	4.17	92.15n.s.	2.00	92.45*	1.60	93.90n.s.	1.55	95.37***	1.75
Dual Gold 960 EC	90.35**	4.40	92.77n.s.	1.45	92.45*	1.60	93.45n.s.	1.10	94.9**	1.27
Alcans Sync Tech	91.07***	5.12	93.32n.s.	0.82	91.80n.s.	0.95	93.82n.s.	1.47	94.72*	1.10
LSD										
α=0.05	2.45	-	3.00	-	1.05	-	1.73	-	0.86	-
α=0.01	3.44		4.12		1.48		2.43		1.20	
α=0.001	4.86		5.95		2.09		3.44		1.70	

***, **, * - significant at α=0.001, α=0.01 and α=0.05; n.s. - non-significant

Table 5. Vine germination dynamics (%) of grafted cuttings - impact of the factors

Source of variance	Signifi cance	Impact of the factors (%)
Year (A)	n.s.	1.29
Herbicide (B)	**	34.29
Interaction of the factors (A x B)	n.s.	3.55
Error	-.	29.87

***, **, * - significant at α=0.001, α=0.01 and α=0.05; n.s. - non-significant.

The tolerance of each plant species to a particular herbicide is within certain limits. When the dose exceeds the recommended limits and especially when the herbicide treatment is applied under unfavorable weather conditions, negative effects may occur. The results of the visual readings in points of phytotoxicity on EWRS scale, thirty days after the treatment of the experimental plots, in the variant with Alcans Sync Tech soil herbicide, a chlorotic halo was observed on the first leaves of the grafted vine cuttings (Figure 1).

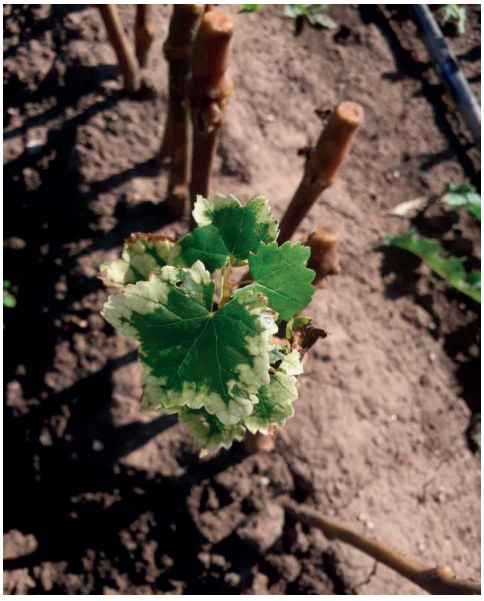


Figure 1. Phytotoxicity of Alcans Sync Tech soil herbicide, 30 days after the treatment

The observed symptoms subsided after the 60th day of treatment with the herbicide and did not affect the further development of the vine cuttings (Figure 2). The observed chlorotic halo was due to the action of clomazone - the active substance, which under certain environmental stress conditions, inhibited the biosynthesis of photosynthetic pigments.

Dual Gold 960EK and Stomp New 330EK soil herbicides did not cause phytotoxicity during the entire growing season of the cuttings.

The grafted cuttings were planted under the same conditions in the vine rooting nursery (soil, climate, agrotechnical), but the differences in the preliminary emerging determined to a large extent the changes in the growth of the shoots and the effect of herbicides on them.



Figure 2. Phytotoxicity of Alcans Sync Tech soil herbicide, 60 days after the treatment

Table 6 shows the data about the length of the shoots, during the different ten days, which were a reflection of the dynamics in the growth speed of the grafted vine cuttings. In all variants, an increase in speed was observed, from mid - July to the beginning of August, as with the approaching of the phase of wood maturation, the growth of the shoots stopped. The increase in the speed with which the shoots grew in the middle of the growing season was determined by the development of the root system of the cuttings and the larger leaf area. The length of the main shoot remained close to that of the control variant until early August, after which their fast growth was observed, as in the variant with Dual Gold 960EK, the main shoot had the smallest length, compared to the other variants. Thirty days after the use of the herbicide products, the length of the main shoot in the treated variants varied from 6.07 cm (Stomp New 330EK) to 7.75 cm (Alcans Sync Tech). A

minimal increase in length, reported for the variants compared to the control was found on the 50th day of the treatment with Alcans Sync Tech herbicide. At the end of the vegetation period all the treated variants had almost the same length of the main shoot, which was greater than that of the control. The last readings of that parameter were on the 70th day after the planting and treatment the vine cuttings, the values of which showed that growth had been already stopped at the end of September.

Table 6. Growth dynamics of the main shoot (cm), on average for the period 2018-2021

Days / Variants	30-th day	Difference	40-th day	Difference	50-th day	Difference	60-th day	Difference	70-th day	Difference
Control	6.37	-	14.65	-	23.53	-	26.98	-	40.31	-
Stomp New 330 EC	6.07n.s.	0.30	14.76n.s.	0.11	25.34n.s.	1.81	31.15n.s.	4.17	45.40n.s.	5.08
Dual Gold 960 EC	7.40n.s.	1.02	14.85n.s.	0.20	24.72n.s.	1.19	28.64n.s.	1.66	42.73n.s.	2.41
Alcans Sync Tech	7.75n.s.	1.37	14.89n.s.	0.24	24.43n.s.	0.91	30.97n.s.	3.99	44.03n.s.	3.71
LSD										
α=0.05	2.98	-	7.48	-	13.07	-	27.60	-	31.21	-
α=0.01	4.18		10.49		18.34		38.69		43.76	
α=0.001	5.91		14.89		25.93		54.70		61.86	

***, **, * - significant at α=0.001, α=0.01 and α=0.05; n.s. - non-significant.

The dispersion analysis of the data about the both indicators (year, herbicide), determined the influence of the herbicide (factor B) on the length of the main shoot, where the power of impact was 35.68%. During the period of study, the year (factor A) and the interaction between the two factors (A x B) showed an insignificant effect on the length. The unexplained influence due to random factors was 25.67% (Table 7).

Table 7. Growth dynamics (cm) of the main shoot - impact of the factors

Source of variance	Significance	Impact of the factors (%)
Year (A)	n.s.	1.39
Herbicide (B)	n.s.	35.68
Interaction of the factors (A x B)	n.s.	4.38
Error	-	25.67

***, **, * - significant at α=0.001, α=0.01 and α=0.05; n.s. - non-significant.

For the entire period of the experiment, the herbicide (factor B) had an effect on the dynamics of emergence and the growth of the main shoot of the grafted vine cuttings, while the year (factor A) and the interaction of the factors (A x B) did not have a significant effect on their speed of development. The growth and development of vine cuttings to a large extent were connected with the similarity of the main active substances (pendimethalin, s-metolachlor and clomazone) in the three tested products. The obtained results confirmed the study of Prodanova-Marinova & Staneva, 2019; Prodanova-Marinova et al., 2021), who reported that pendimethalin and s-metolachlor did not suppress the emerging and growth of the main shoot of grafted vine cuttings. Similar results were also reported by Chelebiev (1981) and Prodanova (2013) regarding the effectiveness of the herbicide products on the weeds in vineyards.

Photosynthetic pigments are the most important component of the photosynthetic apparatus, through which the absorption of light quanta and the transformation of their energy into chemical energy is carried out. The ratio of *chlorophyll **a*** to *chlorophyll **b*** in the above-ground parts is widely used as an indicator of response to light intensity and also as an early indicator of aging. The ratio of chlorophylls to carotenoids is considered a sensitive marker that differentiates natural timely aging and aging due to environmental stress factors (Misra et al., 2012). The obtained results are presented in Table 8.

Average over the period of study, the herbicides had different effects on the synthesis of photosynthetic pigments during the growing season of the rooted vine cuttings. In the course of the treatment with Dual Gold 960EK and Alcans Sync Tech soil herbicides, a slight decrease in the content of *chlorophyll **a*** and *chlorophyll **b***, compared to the control was found. The content of *chlorophyll **a*** and ***b***, in the photosynthetic apparatus of the vine cuttings, in the variant with Stomp New 330EK was the same as in the control variant. The differences in the values were insignificant and did not show a clear trend of a negative effect of the herbicides.

According to Humbeck et al. (1996) and Miersch et al. (2000), changes in the content of chlorophyll pigments after exposure to soil herbicides is typical of abiotic stress, in which the content of *chlorophyll **a*** is initially much more reduced, compared to *chlorophyll **b***. These changes in the chlorophyll content are related to the degradation of a greater part of the reaction centers of the both photosystems.

The content of *carotenoids **c*** in the leaves of vine cuttings varied from 0.09 mg g^{-1} to 0.13 mg g^{-1}. All differences were insignificant and not statistically significant.

The highest value, according to the ratio of yellow to green pigments (*chlorophyll **a**+**b*** to *carotenoids **c***), was reported by the variant with Stomp New 330EK – 3.09 mg g^{-1}, and the lowest by the variant with Dual Gold 960EK – 3.07 mg g^{-1}. Lower values in the ratio of green to yellow pigments are associated with increased protection against photooxidation of chlorophyll. According to Koyama (1991) and Demming-Adams & Adams (1996), one of the most important functions of *carotenoids **c*** in the xanthophyll cycle is the prevention of the destruction of chlorophyll molecules under the action of active oxygen forms, the amount of which increases in cells under adverse effects.

Table 8. Content of pigments (*chlorophyll **a***, ***b*** and *carotenoids **c***) in the leaves of grafted cuttings of Siyana vine variety, mg 100 g^{-1}, average for 2018-2021

Variants	Photosynthetic pigments				
	a	*b*	*c*	*a: b*	*(a+b): c*
Control	0.08 a	0.03	0.12 a	2.98 a	0.87 a
Stomp New 330EK	0.08 a	0.03	0.13 a	3.09 a	1.12 a
Dual Gold 960EK	0.07 a	0.02	0.09 a	3.07 a	0.80 a
Alcans Sync Tech	0.07 a	0.02	0.10 a	3.08 a	1.10 a
α<0.05	0.023	0.008	0.042	1.00	0.269

Means of the same letters are not significantly different at α < 0.05.

CONCLUSIONS

Stomp New 330EK, Dual Gold 960EK and Alcans Sync Tech herbicides were effective against annual late-spring weeds, and less effective against *Convolvulus arvensis* L., *Cirsium arvense* Scop., and *Sonchus arvensis* L. Dual Gold 960EK and Stomp New 330EK herbicides did not cause the appearance of phytotoxicity during the growing season of the vine cuttings. Alcans Sync Tech caused the appearance of a chlorotic halo on the first leaves of the grafted cuttings, which was overcome and did not affect their development.

Stomp New 330EK and Alcans Sync Tech provided good control of annual weeds in the vine rooting nursery, as the treated vine cuttings had the highest intensity of the main shoot growth.

The herbicides tested did not suppress bud formation and did not reduce the vigor of the main shoot. At the end of the growing season, the length of the main shoot of the treated variants exceeded that of the control.

The herbicides tested did not negatively affect the synthesis of photosynthetic pigments. Despite of the slight fluctuations in the values of the ratio chlorophylls to carotenoids, there was no evidence of premature aging due to stress factors.

REFERENCES

Andr, J., Hejnák, V., Jursík, M. & Fendrychová, V. (2014). Effects of application terms of three soil active herbicides on herbicide effi cacy and reproductive ability for weeds in maize. *Plant Soil and Environment*, *60*(10), 452-458.

Boychev, A. (1980). Studies on the water and nutrition regime of weeds in the vineyards. *Horticultural and viticultural science*, *2,* 98-103.

Chelebiev, M. & Encheva H. (2002). Chemical weed control in vineyards. –In: *"100 years Institute of Viticulture and Winemaking"*, Anniversary scientific session with international participation, Pleven, 220-227.

Chelebiev, M. **(**1981). The application of herbicides in vine rootstocks. *Viticulture and Winemaking*, *5*, 15-18.

Christopher, Ch., Hager, A., Tranel, P., Davis, A., Martin, N. & Williams, M. (2021). Future efficacy of pre-emergence herbicides in corn (*Zea mays*) is threatened by more variable weather. *Pest Management Science*, *1*, 1−7.

Correia, N., Daniel, B. & Leite, M. (2012). Intercropping corn and kudzu in a rotation system with soybean. *Acta Scientiarum*, *34*(3), 309-315.

Demming-Adams, B. & Adams, W.W. (1996). Xanthophyll cycle and light stress in nature: uniform response to excess direct sunlight among higher plant species. *Planta*, *198*, 460-470.

Dimitrov D. & Prodanova-Marinova, N. (2019). Influence of some herbicides on the content of esters, higher alcohols, aldehydes and terpenes in red wines of the Cabernet Sauvignon. *Food Science and Technology*, *20*(4), 800-808.

Dimitrov, D. (1990). Soil and climatic conditions in Obraztsov Chiflik, In: *Anniversary. Scientific session "85 years of ESC" Model Farm*, Ruse, *1*, 26-36.

Ganeva, Z. (2016). Discovering Statistics using IBM SPSS Statistics. Elestra, 265−427 (Bg).

Gitti, D., Arf, O., Vilela, R., Potugal, J., Kaneko, F. & Rodrigues, R. (2012). Epocas de semeadura de Crotalaria em consorcio com milho. *Revista Brasileira de Milho e Sorgo*, *11*(2), 156-168.

Humbeck, K., Quast, S. & Krupinska, K. (1996). Functional and molecular changes in the photosynthetic apparatus during senescence of flag leaves from field-grown barley plants. *Plant Cell Environ*, *19*(3), 337-344.

Ionescu, N. & Popescu, D.M. (2024), Results with weeds competition and their control in soybean crop. *Scientific Papers. Series A. Agronomy*, LXVII(1), 437-444.

Ivanova, I. & Rankova, Z. (2014). Study of vegetative habits of apricot seedling rootstocks under the influence of some soil herbicides. *Plant science*, *51*(2-3), 38-40.

Koyama, Y. (1991). Structure and function of carotenoids in photosynthetic system. *Photochemistry and photobiology*, *9*, 265-280.

Lakin, G. (1990). Biometry. Higher School, Moscow.

Lange, A., Lider, L., Fischer, B. & Agamalian, H. (1970). Herbicide – Variety studies of Ioung grapevines. *American Journal of Enology and Viticulture*, *21*(2), 85-93.

Leonie, W.L., Krähmer, H., Santel, H.J., Claupein, W. & Gerhards, R. (2014). Thiencarbazone-methyl efficacy, absorption, translocation, and metabolism in vining. *Weed science*, *62*(3), 512–519.

Marble, S. C., Chandler, A., & Saha, D. (2016). Postemergence control of Pilea microphylla (Artillery Weed). *Florida State Hortic*, *129*, 243–245.

Matei, G., Popa L.D., Isticioaia, S.F., Teliban, G.C. & Vladut, V.N. (2024). Study regarding the weed control in grain sorghum crop. *Scientific Papers. Series A. Agronomy*, *LXVII* (1), 527-534.

Miersch, I., Heise, J., Zelmer, I. & Humbeck, K. (2000). Differential Degradation of the Photosynthetic Apparatus During Leaf Senescence in Barley (*Hordeum vulgare* L.). *Plant Biology*, *2*(6), 618-623.

Misra, A.N., Misra, M & Singh, R. (2012). Chlorophyll fluorescence in plant biology. *Biophysics*, *7*, 171-192.

Nosratti, I., Sabeti, P., Chaghamirzaee G. & Heidari, H. (2020). Weed problems, challenges and opportunities in Iran. *Crop Protection, 134*, 1-10

Prodanova-Marinova, N. & Staneva, I. (2018). Some weed species competitiveness in a vine nursery. *Proceedings of National scientific conference with international participation „Ecology and Health“*, Plovdiv, 170-174.

Prodanova-Marinova, N. & Staneva, I. (2019). Influence of Gardoprim plus Gold on the competitive relations in the grapevine nursery. *Journal of Mountain Agriculture on the Balkans*, *22*(1), 302-314.

Prodanova-Marinova, N. (2012). Study on the efficiency and selectivity of soil herbicides in vine nursery. PhD thesis, AU-Plovdiv.

Prodanova-Marinova, N. (2013). Damage from herbicides in the vineyards. *Viticulture and Winemaking*, *1*, 46-49.

Prodanova-Marinova, N. (2015). Biological efficacy of some herbicides in vine nursery. *Journal of Mountain Agriculture on the Balkans*, *18*(4), 702-713.

Prodanova-Marinova, N., Belberova, Y. & Tsvetanov, E. (2021). Effects of some herbicides on the leaf apparatus of young vine (*Vitis vinifera* L.). *Bulgarian Journal of Agricultural Science*, *27*(2), 357–363.

Rankova, Z. & Tityanov, M. (2014). Effect of some soil herbicides on the vegetative habits of almond seedlings *Prunus dulcis* L. *Plant Science*, *51*(2-3), 45–48.

Rankova, Z. & Tityanov, M. (2017). Effect of some soil herbicides on the vegetative habits of peach seedling rootstocks in a nursery. *Journal of Mountain Agriculture on the Balkans*, *20*(4), 290-298.

Risina, M. (2007). Impact of the Common Agricultural Policy on EU on the development of viticulture. *Economy and rural management economy*, *52*, 9-16.

Sabev, L. & Stanev, S. (1963). Climatic regions in Bulgaria and their climate, Sofia.

Sarpe N., Radulescu, I. & Poienaru, S. (2007). Research with classical herbicides and new herbicides (Merlin duo and Gardoprim Plus Gold) applied on the chernozem from fudules fetesti and the bown soil – luvic from stefanesti arges. *Lucrări ştiinţifice*, *9*(50), 307-312.

Schildberger, B., Hanak, K. & Regner, F. (2007). Untersuchung von Herbizidschäden im österreichischen Weinbau. *Gesunde Pflanzen*, *591*(1), 23-28.

Staneva, I. & Rankova, Z. (2017). Competition for mineral nutrients between cultural plants and weeds in a nursery. *Journal of Mountain Agriculture on the Balkans*, *20*(4), 299–307.

Todorov, I. (2005). Production of Vine Planting Material. Dionis, Pleven

Tsvetanov, E., Prodanova-Marinova, N., Encheva, H., Dimitrova, V. & Iliev, A. (2014). Technological Investigations for Improvement of Grapevine Propagation Material Production in Bulgaria, II part. Testing of Agritechnical Practices in Vine Nursery. *Turkish Journal of Agricultural and Natural Sciences*, *1*, 1280-1287.

Venkataraman, S. & Krishnan, A. (1992). Crops and Weather. Publication and Information Division of ICAR, New Delhi.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

RESEARCH ON THE TECHNOLOGICAL CHARACTERISTICS OF SELECTED CLONAL ELITES FROM TWO POPULATIONS OF TABLE GRAPE VARIETIES UNDER THE CONDITIONS OF THE MURFATLAR VINEYARD

Anamaria TĂNASE[1], Aurora RANCA[1], Ana NEGRARU[1], Traian Ștefan COSMA[1], Ayar-Sergiu ENE[1], Ionica DINA[1], Traian Ciprian STROE[2]

[1]Research Station for Viticulture and Oenology Murfatlar, 2 Calea București, Murfatlar, Constanţa, Romania
[2]Ovidius University, Faculty of Natural and Agricultural Sciences, 124 Mamaia Blvd, Constanţa, Romania

Corresponding author email: tanasea@statiuneamurfatlar.ro

Abstract

Between 2019 and 2024, a study was conducted in Research Development Station for Viticulture and Winemaking Murfatlar on clonal selections of two table grape varieties: 'Afuz Ali' and 'Coarna neagra'. Five clonal elites from each variety were comparatively analyzed, evaluating their technological qualities relative to the standard variant. During the research, the clonal elite 'Afuz Ali 72/3/16' consistently outperformed the control and the other four studied elites, recording high and quality yields with an average of 42 tons/ha, compared to 27 tons/ha for the control variety. Similarly, the clonal elite 'Coarna neagra 14/5/22' demonstrated a consistently higher level than the control and the other four elites, with high and quality yields, averaging 38 tons/ha compared to 26 tons/ha for the control variety. The clonal selections recommended for certification and propagation in the Dobrogea area and regions with similar climates are 'Afuz Ali 72/3/16' and 'Coarna neagra 14/5/22', which exhibit superior quality over the control in terms of bunch appearance, pulp consistency, aroma, and typicity.

Key words*: clonal selection, productivity, quality, organoleptic evaluation.*

INTRODUCTION

Table grape varieties are highly valued by consumers, being a valuable source of bioactive compounds that contribute to health through their antioxidant properties, anti-inflammatory action, and ability to support endothelial function (Manach et al., 2013).

To increase table grape production, it is necessary to focus on several aspects and measures, such as expanding and cultivating new areas, applying good agricultural practices, using irrigation, implementing effective protection programs, and more. In addition to these measures, grape quality and productivity can be enhanced by using varieties with high genetic potential and selecting suitable clones (Maicas et al., 2020).

Clonal selection is considered one of the most effective methods for the genetic improvement of grapevines, based on the selection of clones and cellular modifications of the varieties, which are accompanied by an increase in vegetative mass (Salillari & Hoxha, 2001). Through clonal selection, both the production and commercial qualities of the varieties can be optimized.

Among the most important parameters in choosing a clone are: the shape and size of the grape and berry, the mechanical structure of the grape and berries, grape quality, and disease resistance (Rühl et al., 2004).

According to Winkler & Cook (1974), high-quality table grapes combine average berry size, uniformity, characteristic color, and a texture that reflects the specific taste of the variety. Today, in vineyards where table grapes are cultivated with irrigation systems, yields of over 25 tons/ha are easily obtained (Rotim & Gašpar, 2016), leading to an increase in yield between 20% and 47%, depending on annual conditions (Kryeziu, 2017; 2024).

MATERIALS AND METHODS

The research was conducted over six years (2019-2024) and involved studying clonal selections from populations of two table grape varieties: 'Afuz Ali' and 'Coarna neagra'.
The experimental plots are located on land with a slope of 2-3%. The soil type in this viticultural area is typical calcareous chernozem, and the soil has a loamy texture. The rows are oriented from east to west, the planting distance is 2.2 x 1.1 m, ensuring a density of 4132 vines per hectare. The rootstock used is Berlandieri x Riparia, Telecky Openheim SO4-4 selection. Drip irrigation systems were used only in 2024, as the system was established in 2023.
During this period five clonal elites from each variety were comparatively analyzed, evaluating their technological qualities in comparison to the standard variant (the initial varieties).
For the 'Afuz Ali' variety, the following five clonal elites were studied: 'Afuz Ali 15/8/4', 'Afuz Ali 46/9/12', 'Afuz Ali 72/3/16', 'Afuz Ali 28/5/3' and 'Afuz Ali 64/5/2'.
In the case of the 'Coarna neagra' variety, the following clonal elites were studied: 'Coarna neagra 9/7/4', 'Coarna neagra 14/5/22', 'Coarna neagra 17/5/3', 'Coarna neagra 32/1/6' and 'Coarna neagra 24/7/8'.
The objective of the research is to identify which clones of the analyzed varieties offer the best performance based on the studied parameters. The results obtained should clarify the production characteristics of each clone under the specific cultivation conditions of Dobrogea, specifically within the Murfatlar viticultural center. These data will support the selection of the most suitable clones to complete the varietal conveyor of the area, while also contributing to the improvement of the studied grape varieties.
The observations and measurements conducted focused on the production potential of the studied varieties and selections and the quality of the yield (average cluster weight, weight of 100 berries, and volume of 100 berries).
All these observations and measurements were determined considering the climatic conditions (temperature and precipitation) during the study years.
The chemical composition of the must was determined by measuring sugar content and acidity. For the organoleptic evaluation, a scale from 5 to 10 points was applied. Following the assessments and tests by breeders and ISTIS inspectors, the samples of each variant were evaluated through taste, visual, and olfactory senses based on external appearance, consistency, taste, typicity, and variety originality.

RESULTS AND DISCUSSIONS

Analyzing the average monthly temperatures from 2019 to 2024 compared to the multi-year average (minimum of 20 years), according to Figure 1, it can be observed that the average monthly temperatures have increased above normal values, with very hot summers and mild winters.

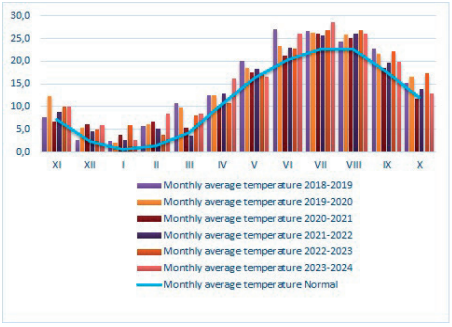


Figure 1. Analysis of the recorded temperatures during the studied period

Regarding the precipitation amounts recorded during the study period, compared to the multi-year average, according to Figure 2, they showed an uneven distribution. The years 2021, 2022, and 2023 were drought years, while in 2024, significant amounts of precipitation during the ripening and harvesting period had negative effects on the crops.

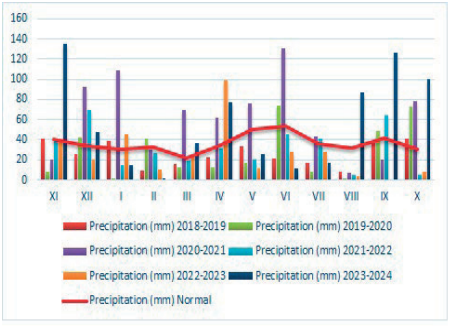


Figure 2. Analysis of the recorded precipitation during the studied period

Analyzing the data on average cluster weight, weight of 100 berries, and volume of 100 berries during the study period (2019-2024), as presented in Figure 3, it can be observed that for the 'Afuz Ali' variety, the elite that showed a distinctly significant difference compared to the control and the other studied elites is 'Afuz Ali 72/3/16'. This selection averaged 547 g per cluster compared to 440 g for the control, 535 g per 100 berries compared to 389 g for the control, and 562 in volume per 100 berries compared to 353 in volume for the control. For the 'Coarna neagra' variety, the elite that registered a distinctly significant difference compared to the control and the other studied elites is 'Coarna neagra 14/5/22'. This clonal elite averaged 466 g per cluster compared to 335 g for the control, 380 g per 100 berries compared to 229 g for the control, and 416 in volume per 100 berries compared to 206 in volume for the control, as shown in Figure 4.

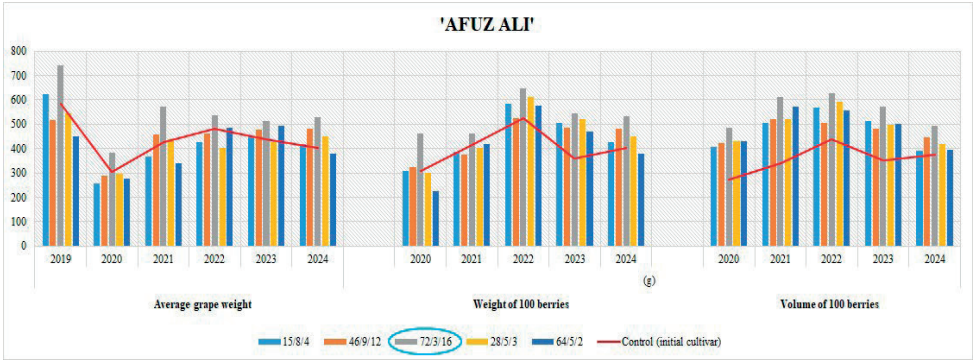


Figure 3. The quality of the yield for the 'Afuz Ali' variety

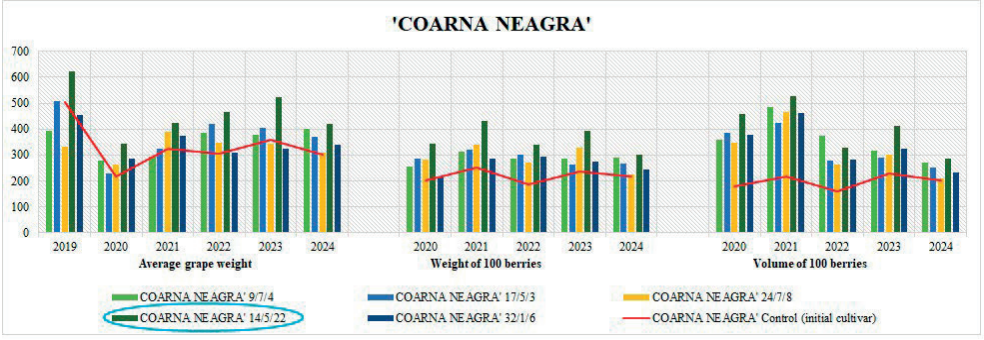


Figure 4. The quality of the yield for the 'Coarna neagra' variety

According to Figure 5, regarding average yields (tons/ha), for the 'Afuz Ali' variety, the elite 72/3/16 recorded a distinctly significant difference compared to the control, with an average yield of 42 tons/ha, compared to 27 tons/ha recorded by the control.

According to Figure 6, regarding average yields (tons/ha), for the 'Coarna neagra' variety, the elite 14/5/22 recorded a distinctly significant difference compared to the control, with an average yield of 38 tons/ha, compared to 31 tons/ha recorded by the control.

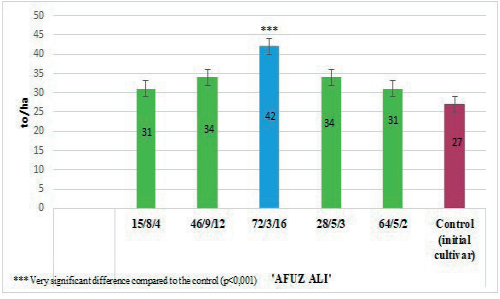


Figure 5. Average yields (tons/ha) recorded during the study period compared to the 'Afuz Ali' control variety

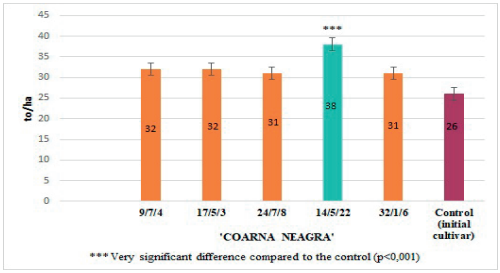


Figure 6. Average yields (tons/ha) recorded during the study period compared to the 'Coarna neagra' control variety

The chemical composition of the grapes varies depending on the variety, degree of maturity, climatic and ecological conditions, agricultural practices used, plant health, and other factors. Analyzing the data from Table 1, for the 'Afuz Ali' variety, the clonal elite 'Afuz Ali 72/3/16' recorded the largest difference compared to the control, with +11.79 g/L of sugars and a standard deviation of 16.69; in terms of acidity, this elite recorded the smallest difference of -0.38 g/l H_2SO_4 with a standard deviation of 1.56. For the 'Coarna neagra' variety, the clonal elite 'Coarna neagra 14/5/22' recorded the largest difference compared to the control, with +46.51 g/L of sugars and a standard deviation of 13.49; in terms of acidity, this elite recorded a difference of -0.48 g/l H_2SO_4, with a standard deviation of 1.21 (Table 1).

Table 1. Sugar content and acidity of the studied elites compared to the control cultivars

Cultivar	Clonal elites	Sugar g/L						Ave-rage	Diffe-renes (±)	Standard deviation	Acidity g/L H_2SO_4						Ave-rage	Diffe-rences (±)	Standard deviation
		2019	2020	2021	2022	2023	2024				2019	2020	2021	2022	2023	2024			
'AFUZ ALI'	15/8/4	142.0	160.4	190.1	156.7	150.3	142	156.92	-5.66	17.90	5.1	5.0	4.2	2.23	3.2	2.3	3.67	-0.53	1.29
	46/9/12	144.6	148.5	187.3	160.3	148.1	163	158.63	-3.95	15.84	5.0	5.2	3.7	2.28	3.0	2.6	3.63	-0.57	1.24
	72/3/16	158.3	164.0	205.1	170.4	168.4	180	174.37	+11.79	16.69	6.2	5.3	3.5	2.43	2.6	2.9	3.82	-0.38	1.56
	28/5/3	143.3	152.4	192.0	164.1	145.2	153	158.33	-4.25	18.05	6.0	4.8	4.0	2.34	3.1	2.4	3.77	-0.43	1.45
	64/5/2	150.3	151.2	191.5	168.2	147.5	129	156.28	-6.3	21.29	4.2	4.7	4.1	2.40	2.2	2.0	3.27	-0.93	1.19
	Control (initial cultivar)	**150.4**	**155.8**	**194.8**	**161.9**	**176.6**	**136**	**162.58**	**0**	**20.67**	**6.4**	**5.8**	**3.9**	**3.75**	**3.0**	**2.2**	**4.20**	**0**	**1.62**
'COARNA NEAGRĂ'	9/7/4	163.0	158.4	191.7	158.4	154.4	175	166.82	+30.05	14.12	4.6	5.1	5.4	2.31	2.5	3.2	3.85	-0.27	1.35
	17/5/3	160.1	160.1	198.4	150.6	148.3	162	163.25	+26.48	18.11	6.8	4.6	3.8	2.16	2.9	3.0	3.88	-0.24	1.66
	24/7/8	150.2	164.2	194.5	160.7	150.1	136	159.28	+22.51	19.88	7.2	6.2	3.9	2.30	2.2	2.5	4.05	-0.07	2.17
	14/5/22	187.2	186.4	203.2	162.2	176.7	184	183.28	+46.51	13.49	5.4	4.8	2.7	2.33	3.2	3.4	3.64	-0.48	1.21
	32/1/6	149.5	160.9	194.2	156.8	140.2	149	158.43	+21.66	18.92	6.5	5.3	3.7	2.25	2.2	2.8	3.79	-0.33	1.76
	Control (initial cultivar)	**140.4**	**139.8**	**152.4**	**121.6**	**135.4**	**131**	**136.77**	**0**	**10.31**	**6.0**	**5.5**	**4.0**	**3.80**	**3.0**	**2.4**	**4.12**	**0**	**1.40**

According to Table 2, in the case of the 'Afuz Ali' variety, the clonal elite 'Afuz Ali' 72/3/16 had an average gluco-acidimetric index value of 52, exceeding the control value, which indicates that the grapes were sweet, with low acidity, making them suitable for direct consumption.

In the case of the 'Coarna neagra' variety, the clonal elite 'Coarna neagra 14/5/22' had an average gluco-acidimetric index value of 55, also exceeding the control value, signifying that the grapes were sweet, with low acidity, and suitable for direct consumption.

Table 2. The gluco-acidimetric index

Cultivar	Clonal elites	The gluco-acidimetric index						Average	Differences (±)
		2019	2020	2021	2022	2023	2024		
'AFUZ ALI'	15/8/4	28	32	45	70	47	62	47	+3
	46/9/12	29	29	51	70	49	63	48	+4
	72/3/16	26	31	59	70	65	62	52	+8
	28/5/3	24	32	48	70	47	64	47	+3
	64/5/2	36	32	47	70	67	65	53	+9
	Control (initial cultivar)	**24**	**27**	**50**	**43**	**59**	**62**	**44**	**0**
'COARNA NEAGRA'	9/7/4	35	31	36	69	62	55	48	+12
	17/5/3	24	35	52	70	51	54	48	+12
	24/7/8	21	26	50	70	68	54	48	+12
	14/5/22	35	39	75	70	55	54	55	+19
	32/1/6	23	30	52	70	64	53	49	+13
	Control (initial cultivar)	**23**	**25**	**38**	**32**	**45**	**55**	**36**	**0**

Organic acids bring benefits regarding the taste of grapes and play an important role in enhancing their flavor characteristics.
The balance between sweetness and acidity is a key factor in determining the quality of consumer acceptance (Topalovic et al., 2010). As a result of the evaluations and testing conducted by the breeders at SCDVV Murfatlar and the ISTIS inspector, using a rating scale from 5 to 10, the samples of each variant were assessed through taste, visual, and olfactory senses, based on the exterior appearance, consistency, taste, and typicity, that is, the originality of the variety.
Thus, the selection with the best properties, recommended for approval and multiplication, is 'Afuz Ali 72/3/16', which has superior quality compared to the control, in terms of exterior appearance, consistency, aroma, and typicity (Table 3 and Figure 7).

Table 3. Organoleptic properties of the grapes from the 'Afuz Ali' variety

Characters	Afuz Ali control	15/8/4	46/9/12	72/3/16	28/5/3	64/5/2
Media (2019 – 2024)						
External look	6	5	5	7	6	6
Consistency	6	6	6	8	6	6
Flavour	6	6	6	7	6	6
Typicality	7	7	7	8	7	7
Clasification	poor quality	poor quality	poor quality	superior quality	poor quality	poor quality

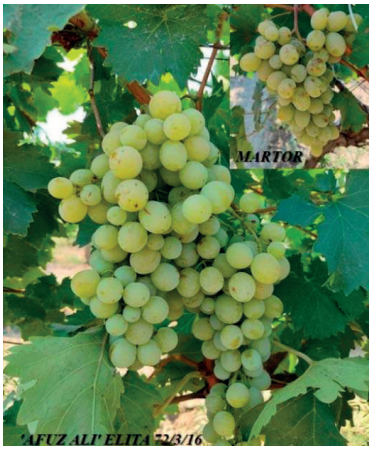


Figure 7. The bunch size and berry dimensions in the case of the clonal elite 'Afuz Ali 72/3/16', compared to the control

The selection with the best properties, recommended for approval and multiplication, is 'Coarna neagra 14/5/22', which has excellent quality compared to the control, in terms of exterior appearance, consistency, aroma, and typicity (Table 4 and Figure 8).

Table 4. Organoleptic properties of the grapes from the 'Coarna neagra' variety

Characters	Coarna neagra control	9/7/4	17/5/3	24/7/8	14/5/22	32/1/6
Media (2019-2024)						
External look	6	6	7	7	8	7
Consistency	7	7	7	6	8	7
Flavour	6	6	6	6	7	6
Typicality	7	8	7	7	8	7
Clasification	poor quality	poor quality	poor quality	poor quality	excellent	poor quality



Figure 8. The bunch size and berry dimensions in the case of the clonal elite 'Coarna neagra 14/5/22', compared to the control

CONCLUSIONS

Throughout the study, the clonal elite 'Afuz Ali 72/3/16' consistently maintained a superior level compared to the control and the other four elites studied. On average, it had 547 g per grape compared to 440 g for the control, 535 g for the weight of 100 berries, compared to 389 g for the control, and 562 for the volume of 100 berries compared to 353 for the control. The recorded yields were high and of good quality, averaging 42 t/ha compared to 27 t/ha for the control variety.
The clonal selection with the best properties, recommended for approval and multiplication in the Dobrogea region and other regions with a similar climate, is 'Afuz Ali 72/3/16', which has superior quality compared to the control in terms of exterior appearance, consistency, aroma, and typicity.

In the same period, for the 'Coarna neagra' variety, the clonal elite 'Coarna neagra 14/5/22' consistently maintained a superior level compared to the control and the other four elites studied. On average, it had 466 g per grape compared to 335 g for the control, 380 g for the weight of 100 berries, compared to 229 g for the control, and 416 for the volume of 100 berries compared to 206 for the control. The recorded yields were high and of good quality, averaging 38 t/ha compared to 26 t/ha for the control variety.

The clonal selection with the best properties, recommended for approval and multiplication in the Dobrogea region and other regions with a similar climate, is 'Coarna neagra 14/5/22', which has superior quality compared to the control in terms of exterior appearance, consistency, aroma, and typicity.

REFERENCES

Kryeziu S., Bozinovic Z., Boskov K., & Prculovski Z. (2017). Technological characteristics of some clones of table grape to Cardinal variety. *2nd International Conference Biotechnology in Agriculture. Albanian Journal of Agricultural Science*, 301-305.

Kryeziu S., Boskov K., Prculovski Z., & Zogaj M. (2024). Characteristics between clones and traditional variety of Cardinal table grape. *Journal of Agricultural, Food and Environmental Sciences, 78*(1), 93-102.

Maicas, S., & Mateo, J. J. (2020). Sustainability of Wine Production, *Sustainability 12*, 559. doi:10.3390/ su12020559, www.mdpi.com/journal/sustainability

Manach, C., Scalbert, A., Mazzone, F.M, Turci, V., Basile, T., & Antonacci, D. (2013). Valutazione della capacita antiossidante dei prodotti trasformati ottenuti da uva da tavola. *The XXXVI-th World Congress of Vine and Wine June*, Bucharest.

Rotim, N., & Gašpar, M. (2016). Federalni agromediteranski zavod Mostar, Biskupa Čule 10, 88 000 Mostar, Bosna i Hercegovina.

Rühl, E. H., Konrad, B., Lindner, B., & Bleser, E. (2004). Quality criteria and targets for clonal selection in grapevine. *First International Symposium on Grapevine Growing, Commerce and Research, ISHS*, Belgium*, Acta Horticulturae*, 652, 29-33.

Salillari, A., & Hoxha S. (2001). *Salillari A, Hoxha S: Genetics* (in Albanian), 709.

Topalović, A., & Milukovic-Petkovsek, M. (2010). Changes in sugar, organic acids and phenolics of grape berries of cultivar Cardinal during ripening. *Journal of Food, Agriculture and Environment*, *8*(3 & 4), 223-227.

Winkler, A.J., & Cook, J.A. (1974). *General Viticulture. 4th Edition*, University of California Press, Berkley, 74.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

CHEMICAL WEED CONTROL IN FRUIT-BEARING TABLE GRAPE VINEYARDS (*VITIS VINIFERA* L.)

Iliyan ZHELYAZKOV

Agricultural University of Plovdiv, 12 Mendeleev Blvd, Plovdiv, Bulgaria

Corresponding author email: zheliazkov@mail.bg

Abstract

Weeds are competitive with cultivated plants, as for vines as well. They make harvesting, manual and mechanized operations, etc. difficult. The purpose of the present study, conducted in the area of the Varben village, Bulgaria, was to investigate the efficacy of herbicides and herbicide combinations on weed density and on productivity and yield elements in a vineyard, cultivar "Bolgar". Variants of the experiment were: 1. Untreated control; 2. Economic control; 3. Two-time application of Ecopart Turbo (26.5 g/l pyraflufen-ethyl) - 0.08 L ha^{-1}, without removing the young shoots from the stem; 4. Two-time targeted application of Ecopart Turbo - 0.08 L ha^{-1}, with removing of the young shoots from the stem; 5. Double application of Roundup Classic Pro (360 g/l glyphosate) - 0.40 L ha^{-1} + Ecopart Turbo - 0.03 L ha^{-1}. The highest yield in the economic control - 11.89 t ha^{-1} was reported, which represents a 54% higher yield than that of the untreated control. In the variants with herbicides, the highest yield - 11.69 t ha^{-1} in treatment 5 (Roundup Classic Pro - 0.40 L ha^{-1} + Ecopart Turbo - 0.03 L ha^{-1}) was recorded.

Key words*: grapes, weeds, herbicides, yields.*

INTRODUCTION

By 1980, Bulgaria was in the top three in the export of table grapes after Italy and the USA. According to recent market research, the leading countries in the production of table grapes such as Afghanistan, Algeria, Bulgaria, Hungary, France, Italy and Romania have had a negative market share in the production and sale of table grapes in recent years (Seccia et al., 2015).

The production and sale of table grapes is linked to obtaining a product with a good and attractive appearance, but at the same time combined with good taste characteristics, which are determined by the balance of sugars and titratable acids. Grape production in 2023 was 147,804 tons, 9% less than the previous year.

The Southeast region produced 42% of the grapes, and the South Central region 36% (www.mzh.government.bg).

Main issue in grape productions is the uncontrolled weed flora.

Grape growers regulate competition for water and nutrients between the vines and inter-row vegetation by tilling, mulching and/or herbicide application (Winter et al., 2018).

Vior and Cârciu (2011) conduct a study which provides an information on the composition of weed flora in vineyards located in Romania. The majority of weeds consisted of monocotyledonous perennials - *Agropyron repens* and *Cynodon dactylon* in particular.

A number of researchers are conducting trials for chemical weed control around the globe.

A research conducted by Stoyanova et al. (2024) evaluates the influence of three soil herbicides (Stomp New 330 EK, Dual Gold 960EK and Alcans Sync Tech) both on the root system and the growth of grafted rooted vines. Indicators such as diameter of the internode and the length of the internodes of the shoots, length and mass of the ripe shoots and the standard planting material yield reveal that the usage of those herbicides do no cause any negative effects, compared with the untreated control.

Similar experiment was performed in the Institute of Viticulture and Enology, Pleven. Grapevine from variety Misket Kaylashki, grafted on rootstock Berlandieri x Riparia SO_4 was treated by the herbicides Venzar 80 WP, Goal 2, Devrinol 4 F, Dual Gold 960 EC, Lumax 538 SC and Stomp 33 EC at different rates. As an outcome any of the herbicides was found to cause detrimental effect on the grapevine root system and mature wood formation (Prodanova-Marinova et al., 2014).

In a study, Prodanova-Marinova (2024) assessed the effects of treating grapevine Cabernet Sauvignon variety ILV 1/11 clone, grafted on Berlandieri x Riparia selection with herbicide mixture of Targa Super 5 EK at 0.300 l/da and Pledge 50 VP at a rate of 0.040 kg/da. The tank-mix was found to control all the weeds in the plot without affecting negatively the development of the grapevine.

With regard to the profitability of herbicide application in the process of grafted rooted vines production a research was carried out by Prodanova-Marinova (2017). Treatments of the rooted cuttings of grape variety Merlot consisted of Dual Gold 960 SC (3 L ha^{-1}), Gardoprim plus Gold (4 L ha^{-1}), Wing P (4 L ha^{-1}) and untreated control. The results of the study ascertained that herbicide application with Wing P increases significantly the net income and decline the prime cost of the standard grafted vine by 15,3%.

Analysis of the impact of Gardoprim plus Gold and Lumax 536 SC applied at different rates during the first months after planting Cabernet Sauvignon grapevine was recently done. No bud's germination suppression was found. Moreover, the study reveals that the shoot length at the end of the vegetation and the mass of the mature annual growth increased in the treated variants (Prodanova-Marinova et al., 2019).

Besides the abovementioned the herbicidal treatments may have a negative influence on the vines. Zaller et al. (2018) claim that herbicides applied in vineyards may disrupt both grapevine's nutrition and soil organisms. A significant recession of root mycorrhization, nutrient composition in grapevine roots, leaves, grape juice and xylem sap after applying flazasulfuron, glufosinate and glyphosate within-row was observed.

Bigot et al. (2007) report that flumioxazin used as pre-emergent herbicide in vineyards reduce the production of the net photosynthesis. Other authors claim that affecting the grapevine roots by flumioxazin may bring about serious consequences such as water status and nitrogenous metabolites disturbance. Those physiological changes may be detrimental for the vineyard (Saladin et al., 2003).

The aim of this study is to investigate the efficacy of some herbicides and herbicide combinations on weed density, as well as on the elements of productivity and yield in a fruit-bearing vineyard of the "Bolgar" variety.

MATERIALS AND METHODS

The experiment was carried out in the period 2020-2021 in a table grape vineyard (5 years old), in the land of the Varben village, Brezovo municipality, Plovdiv region, Bulgaria (42°25'01.1"N 24°57'39.1"E).

In both experimental years, no significant deviations were observed in terms of temperature compared to the average for the region. The total precipitation in 2020, for the period April-October, was 340 mm, and in 2021, for the same period, 360 mm.

The experiment was set up using the block method, in three replications, with random placement of the variants. The size of one replication was 15 m^2 (3 inter-rows x 5 vines x 1.3 between them - 19.5 m * 0.75 area). Five treatments (T) were evaluated as follows:

1. Untreated control (K_1); 2. Economic control (K_2); 3. Two-time application of Ecopart Turbo (26.5 g/l pyraflufen-ethyl) - 0.08 L ha^{-1}, without removing the young shoots from the stem; 4. Two-time targeted application of Ecopart Turbo - 0.08 L ha^{-1}, with removing of the young shoots from the stem; 5. Double application of Roundup Classic Pro (360 g/l glyphosate) - 0.40 L ha^{-1} + Ecopart Turbo - 0.03 L ha^{-1}.

For the purpose of the study, the variety "Bolgar" was used, grafted on SO4 rootstock (Berlandieri x Riparia, selection Oppenheim 4) with a planting distance of 2.80 x 1.30 m, representing 2750 plants per hectare. The formation of the vines is medium-stemmed, with a stem height of 0.80 m, with a pruning system of 4 fruiting canes, located on two parallel supporting bodies with 10-12 buds, and 2 scions of 2 buds, left at the base.

The herbicides were applied in BBCH 68 vine development phase (after the end of flowering), respectively 25.05.2020 and 27.05.2021, for the first treatments, and for the second treatment 25.06.2020 and 27.06.2021, phase BBCH 73. The application was performed by using a backpack sprayer. The volume of the working solution was 300 L ha^{-1}.

Weed counting was performed three times (for all variants): 1. Immediately before the trial was established. 2. Thirty days after that (coinciding

the application of the second rates of herbicides.
3. Thirty days after the application of the second rates of herbicides. Weed counts were performed using the quantitative method in permanent plots - number of weeds per 1 m^2.
The following indicators were evaluatedt:
- The efficacy of the herbicides and herbicide combinations on the weed species composition and density;
- Grape yield (t ha^{-1})
- Influence of the tested variants on the and the grape quality - Sugar content, % (determined with the "Dujardin" mustometer by measuring the relative weight of the must, and depending on the temperature, the sugar content was determined) and Total acid content, g/dm^3 (determined as 10 cm^3 of grape must is titrated with 0.1 N NaOH solution. The titration is carried out in the presence of bromothymol blue indicator to pH 7.0).
The statistical analysis of some of the collected data (Table grape yields; Sugar and Total acids contents) was performed by by Duncan's Multiple Range test ($p < 0.05$).

RESULTS AND DISCUSSIONS

During the years of the study (2020 and 2021), a total of 14 weed species were registered, of which 12 were annual and 2 were perennial.
The annual weeds were represented by late spring species, of which 10 are dicotyledonous: *Abutilon theophrasti* L., *Xanthium strumarium* L., *Chenopodium album* L., *Conyza Canadensis* L., *Amaranthus retroflexus* L., *Persicaria lapathifolia* L., *Amarantus blitodes* W., *Datura stramonium* L., *Portulaca olerracea* L., *Solanum nigrum* L., and two monocotyledonous species: *Setaria viridis* L. and *Digitaria sanquinalis* L. Perennial weeds were represented by *Cynodon dactylon* Pers and *Convolvulus arvensis* L.
The percent of the weed infestation on average for the studied period is presented in Figure 1.

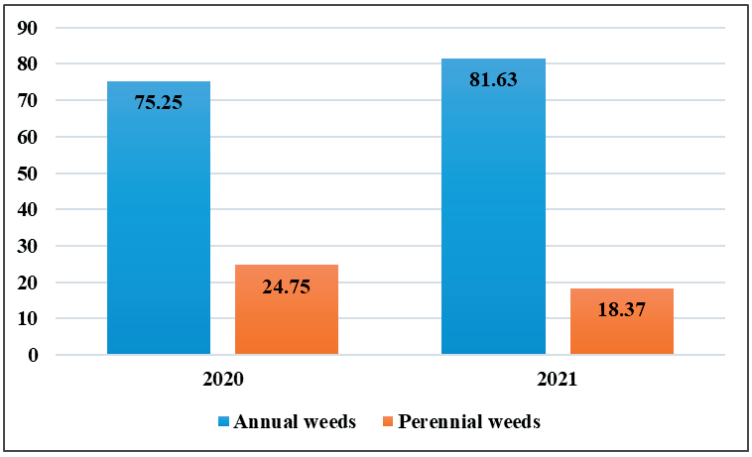


Figure 1. Total weed density per 1 m^2 before treatments, as % distribution

Analysis of the data for the two experimental years shows an almost equal ratio of annual to perennial species, with an almost constant species composition of weeds. Annual species predominate, with their density in the individual variants varying from 22 to 34 species per m^2 for 2020 and from 27 to 43 species per m^2 for 2021. This group of weed plants forms 75.25% and 81.63% of the weed association in the vineyard, respectively. The density of perennial weeds is in the range of 3 to 6 specimens per m^2 and 3 to 5 specimens per m^2 or 24.75 and 18.37% of the total weed density for 2020 and 2021. In quantitative terms, 2021 surpasses 2020, with the reported growth being 14.27%, mainly due to a high infestation with annual species.
The data on the influence of the different treatments on the weeds density for the two experimental years are shown in Table 1. The following interpretations can be made from the presented results. In the two years of the study, the highest efficiency, compared to the untreated control (Variant 1), was reported in Variant 5, respectively 53.10% for 2020 and 45.15% for

2021. The reason for this is the combined application of two active substances (from Ecopart Turbo - pyraflufen-ethyl and Roundup Classic Pro - glyphosate). The presence of herbicides with different modes of action - a foliar contact herbicide with primary action against broadleaf weeds and a systemic total herbicide, impose this system as the most effective. It is confirmed by the findings of Barbieri et al. (2022) that stated herbicidal tank mixing can optimize and widen the weed control spectrum. The efficacy of the application system was enhanced with successive re-treatment (30 days after the first application) - 63.98% for 2020 and 60.86% for 2021. Variant 2 (Economic control) ranked second with 40.30% and 30.01%, respectively, with high efficacy at the first reporting (54.38%) and lower (49.73%) at the second reporting, for 2020 and 2021 respectively. Hand hoeing is the oldest method of weed control in perennial crops, and the results obtained confirm the high effect of its application. Options 4 and 3 follow. With the highest weed density, as expected, was the untreated control.

Table 1. Efficacy of the treatments against the weeds, %.

Treatment	Biological group of weeds	2020				
		1st report	2nd report	% - K1	3rd report	% - K1
T 1 (K1)	Perennial – rhizome	5	6	100%	7	100%
	Perennial – root sprouting	3	5	100%	6	100%
	Annual – late spring	30	64	100%	72	100%
	Total	**38**	**74**	100%	**84**	100%
T 2	Perennial – rhizome	6	5	12.28%	6	14.93%
	Perennial – root sprouting	5	6	-14.00%	4	29.82%
	Annual – late spring	31	34	47.09%	29	60.00%
	Total	**42**	**44**	40.30%	**39**	54.38%
T 3	Perennial – rhizome	7	8	-35.09%	8	-14.93%
	Perennial – root sprouting	3	3	40.00%	3	47.37%
	Annual – late spring	34	39	38.74%	35	51.11%
	Total	**43**	**50**	33.15%	**46**	45.62%
T 4	Perennial – rhizome	4	5	12.28%	6	14.93%
	Perennial – root sprouting	3	4	20.00%	5	12.28%
	Annual – late spring	24	39	38.11%	33	54.31%
	Total	**31**	**48**	34.91%	**44**	48.34%
T 5	Perennial – rhizome	6	2	59.65%	1	80.60%
	Perennial – root sprouting	5	3	40.00%	3	47.37%
	Annual – late spring	22	30	53.54%	26	63.75%
	Total	**33**	**35**	53.10%	**30**	63.98%
Treatment	**Biological group of weeds**	**2021**				
		1st report	**2nd report**	**% - K1**	**3rd report**	**% - K1**
T 1 (K1)	Perennial – rhizome	4	4	100%	5	100%
	Perennial – root sprouting	3	5	100%	6	100%
	Annual – late spring	36	68	100%	82	100%
	Total	**42**	**77**	100%	**94**	100%
T 2	Perennial – rhizome	4	4	9.76%	4	16.33%
	Perennial – root sprouting	5	6	-19.23%	4	33.87%
	Annual – late spring	39	44	35.00%	39	52.91%
	Total	**48**	**54**	30.01%	**47**	49.73%
T 3	Perennial – rhizome	5	5	-31.71%	5	-10.20%
	Perennial – root sprouting	3	3	40.38%	3	50.00%
	Annual – late spring	43	45	34.56%	41	50.49%
	Total	**51**	**53**	31.44%	**49**	47.27%
T 4	Perennial – rhizome	3	4	9.76%	4	16.33%
	Perennial – root sprouting	3	4	21.15%	5	16.13%
	Annual – late spring	30	48	29.56%	42	48.67%
	Total	**36**	**56**	27.94%	**52**	44.81%
T 5	Perennial – rhizome	4	2	60.98%	1	77.55%
	Perennial – root sprouting	5	3	40.38%	3	50.00%
	Annual – late spring	27	38	44.56%	32	60.68%
	Total	**36**	**42**	45.15%	**37**	60.86%

In the grapevine production, climate change can affect yields (Ghiglieno et al., 2023). Herbicide application may play a vital role in bringing about the good development of grapevines. There is some evidence that weeds may affect grape yield up to 32% depending on their species and density (Pala, 2020). This is primarily because weeds compete the cultivated plants for nutrients and water and cause significant yield decrease (Travlos et al., 2018). In order to manage weeds successfully, it is vital to be aware of the variety of weed species present (Rassele et al., 2022).

The unfavourable climatic conditions may influence the grape quality and yield (Stanus et al., 2024). In the current trial, the analysis of variance showed that the variation in the yields of the table grape variety "Bolgar" were determined both by the herbicides and herbicide combinations, and by meteorological conditions (Table 2).

The highest average grape yield for the Economic control (Variant 2) was recorded - 11.89 t ha^{-1} that reaches 54% increase in comparison to the untreated control (7.70 t ha^{-1}). From the treatments where herbicides were applied, the highest yield from the treatment with the double application of Roundup Classic Pro + Ecopart Turbo (Variant 5) was found - 11.69 t ha^{-1}, that reaches 52% increase in comparison to the untreated control.

For the other two treatments the average table grape yield was 10.94 and 11.09 t ha^{-1} respectively for Variant 3 (Two-time application of Ecopart Turbo, without removing the young shoots from the stem) and Variant 4 (Two-time targeted application of Ecopart Turbo, with removing of the young shoots from the stem).

Table 2. Grape yields, t ha^{-1}

Variants	2020		2021		Average for the period	
	t ha^{-1}	% of yield increase compared to K_1	t ha^{-1}	% of yield increase compared to K_1	t ha^{-1}	% of yield increase compared to K_1
T 1. K_1	7.54 c	100%	7.85 c	100%	7.70	100%
T 2.	11.74 a	156%	12.04 a	160%	11.89	154%
T 3.	10.84 b	144%	11.04 b	146%	10.94	142%
T 4.	10.99 b	146%	11.19 b	148%	11.09	144%
T 5.	11.59 a	154%	11.79 ab	156%	11.69	152%

Means with different letters are with proved differences according to Duncan's Multiple Range test ($p < 0.05$).

From the analyses of the grape juice was found that in consumer maturity, the sugar content in all variants of the experiment were in normal values characteristic of the "Bolgar" variety. During the two years of the study, intensive sugar accumulation was found in all variants, with the average values for the period of harvest of the production ranging from 17.70% to 18.40% (Figure 2). The sugar content was slightly lower for the variants where herbicides were applied.

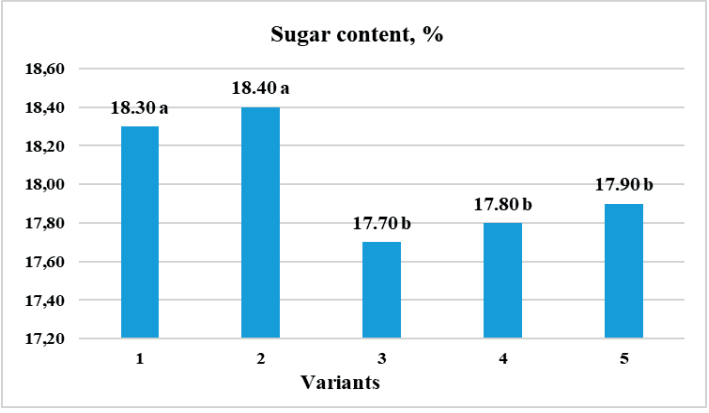


Figure 2. Sugar content in the grapes of "Bolgar" variety (%), average for the period 2020-2021
Means with different letters are with proved differences according to Duncan's Multiple Range test ($p < 0.05$)

The amount of titratable acids in the grape juice is within the limits characteristic of the variety (Figure 3). The average values for the two years of the study are in the range of 3.62 to 4.34 g/dm^3. The lowest titratable acids were reported for treatments 3 and 4.

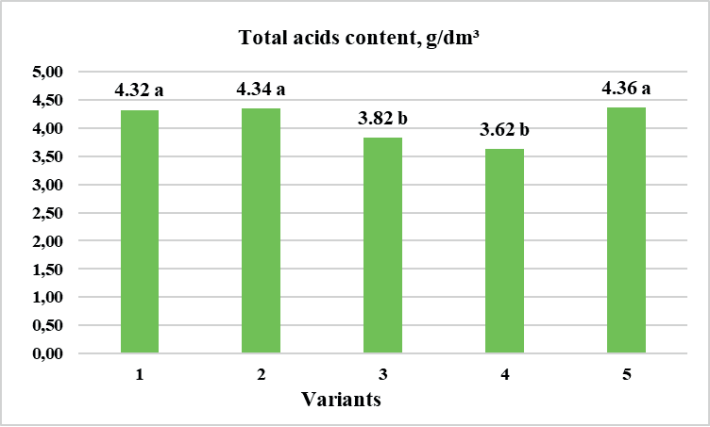


Figure 3. Total acid content in the grapes of "Bolgar" variety (g/dm³), average for the period 2020-2021
Means with different letters are with proved differences according to Duncan's Multiple Range test ($p < 0.05$)

During the study damages on the grapes in all variants in which Ecopard Turbo was applied were registered. The damages were observed on all green parts of the grapevine plant - leaves, stalks, shoots, but the most significant were the damages on the grapes. On the growing young shoots, on the forming vines, the damages were associated with longitudinal splitting, accompanied by a change in color. The death of plants with such damages was not observed. On the remaining green parts, the damages were expressed in the manifestation of small necrotic spots, with a characteristic slightly concave shape and black color. Subsequently, these disorders of the vine tissues caused, in places, partial distortions of their vegetative parts. On the grapes of the white table grape variety "Bolgar", this led to an unsightly appearance, as a result of which a large part of them were rejected and unqualified (Pictures 1 and 2).



Pictures 1 and 2. Toxicity caused by Ecopard Turbo evaporation

CONCLUSIONS

From the three studied variants involving the herbicide Ecopart Turbo, the one with the highest weed control efficacy was variant 5 (Double application of Roundup Classic Pro - 0.40 L ha^{-1} + Ecopart Turbo - 0.03 L ha^{-1}.) at vines development stage BBCH 68, for the first treatment, and for the second treatment - phase BBCH 73 (thirty days later).

The alone two-time application of Ecopart Turbo (variants 3 and 4) showed an unsatisfactory weed control efficacy against the present weed species in the experimental areas. The efficacy, on average for the two years of the experiment, was 16.4-16.77% lower when compared to the efficacy reported for variant 5.

The highest yield was reported in variant 2. The average yield for the two years of the experiment was 11.89 t ha^{-1}, representing 54% higher than the yield obtained in the untreated control.

For the variants with herbicidal applications, the highest yield of 11.69 t ha^{-1}, or 52% higher than the control, in variant 5 was recorded.

The lower yields for the variants with herbicidal applications are explained by the negative effect of the commercial product of Ecopart Turbo on the vines and the grape production, and the lower weed control efficacy as well.

The sugars and titratable acids were slightly influenced by the treatments in the study.

ACKNOWLEDGEMENTS

The trial was financially supported by Project 17-12 at the Center of Research, Technology Transfer and Protection of Intellectual Property Rights at the Agricultural University of Plovdiv, Bulgaria.

REFERENCES

Barbieri, G., Young, B., Dayan, F., Streibig, J., Takano, H., Merotto Junior, A., & Avila, L. (2022). Herbicide mixtures: interactions and modeling. *Advances in Weed Science, 40*(Spec1):e020220051. https://doi.org/10.51694/AdvWeedSci/2022;40:seventy-five011

Bigot, A., Fontaine, F., Clément, C., & Gaveau, N. (2007). Effect of the herbicide flumioxazin on photosynthetic performance of grapevine (*Vitis vinifera* L.). *Chemosphere 67*(6), 1243-1251, https://doi.org/10.1016/ j.chemosphere.2006.10.079

Ghiglieno, I., Carlin, S., Cola, G., Vrhovsek, U., Valenti, L., Garcia-Aloy, M., & Mattivi, F. (2023). Impact of meteorological conditions, canopy shading and leaf removal on yield, must quality, and norisoprenoid compounds content in Franciacorta sparkling wine. *Front. Plant Sci. 14*:1125560. https://doi.org/10.3389/fpls.2023.1125560

https://www.mzh.government.bg/media/filer_public/2024/05/14/ra439-vinewine2023.pdf

Luiz Rassele, R., Freitas, S., Colombo, J., Krause, M., Barth, H., & Barth, H. (2022). Phytosociological survey of weeds in the grapevine. *Bioscience Journal, 38*, e38093, 1-11. https://doi.org/10.14393/BJ-v38n0a2022-53677

Pala, F. (2020). The effect of different weed management on weeds and grape yield in vineyards. Fresenius Environ. *Bull*, 29, 766-772.

Prodanova-Marinova, N. (2024). Biological Efficiency of Herbicides in a Vineyard. *Journal of Mountain Agriculture on the Balkans, 27*(5), 316–332.

Prodanova-Marinova, N., Dimitrova, D., Balashkov, E. (2017). Economic analysis of the results from the application of weed control chemicals in vine nursery. *Journal of Mountain Agriculture on the Balkans*, *20*(3), 257-266.

Prodanova-Marinova, N., Dimitrova, M., Zhelyazkov, I. (2014). Influence of Some Soil Herbicides on the Yield of Grafted Rooted Grapevines from Variety Misket Kaylashki and Their Biometric Characteristics. *Plant Science, 51*(2-3), 53-56. ISSN 0568-465X

Prodanova-Marinova, N., Tsvetanov, E., & Iliev, A. (2019). Comparative testing of herbicides in newly planted vineyard. *Bulgarian Journal of Agricultural Science, 25*(1), 119-116.

Saladin, G., Magné, C., & Clément, C. (2003). Stress reactions in *Vitis vinifera* L. following soil application of the herbicide flumioxazin. *Chemosphere*, 53(3), 199-206, https://doi.org/10.1016/S0045-6535(03)00515-0

Seccia, A., Santeramo, G., & Nardone, G. (2015). Trade competitiveness in table grapes: *A global view. Outlook on agriculture, 44*. 10.5367/oa.2015.0205

Stanus (Rujoiu), M., Dobrei A., Nistor E., Cristea, T., Tac (Faur) A., Dobrei, A. (2024). The local grapevine varieties - a source of typicity, authenticity, and adaptability, within the framework of sustainable viticultural technologies. *Scientific Papers. Series B, Horticulture, LXVIII* (2), 361-372.

Stoyanova, S., Dyakova, G., & Mincheva, R. (2024). Effect of Soil Herbicides on the Growth and Development of Grafted Rooted Vines of the Dessert Siyana Variety. *Journal of Mountain Agriculture on the Balkans, 27*(5), 388–402.

Travlos, I. S., Bilalis, D. J., Katsenios, N., & De Prado, R. (2018). Sustainable weed control in vineyards. *Weed Control,* 526-542. CRC Press.

Vior, M., & Cârciu, G. (2011). Research on effective herbicides in combat weeds in the West country vineyards. *Journal of Horticulture, Forestry and Biotechnology, 15*(2), 231-237.

Winter, S., Bauer, T., Strauss, P., Kratschmer, S., Paredes, D., Popescu, D., Landa. B., Guzmán, G., Gómez, J., Guernion, M., Zaller, J., & Batáry, P. (2018). Effects of vegetation management intensity on biodiversity and ecosystem services in vineyards: A meta-analysis.

J Appl Ecol., 55, 2484-2495. https://doi.org/10.1111/1365-2664.13124

Zaller, J., Cantelmo, C., Dos Santos, G., Muther, S., Gruber, E., Pallua, P., Mandl, K., Friedrich, B., Hofstetter, I., Schmuckenschlager, B., & Faber F. (2018). Herbicides in vineyards reduce grapevine root mycorrhization and alter soil microorganisms and the nutrient composition in grapevine roots, leaves, xylem sap and grape juice. *Environ Sci Pollut Res, 25*, 23215-23226. https://doi.org/10.1007/s11356-018-2422-3


EX·TERRA·AVRVM

VEGETABLE GROWING


EX·TERRA·AVRVM

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

RESEARCH ON FEED BACK REACTIONS OF PLANTS SUBJECTED TO EXTERNAL FREQUENCY FIELDS FROM AN EPIGENETICAL POINT OF VIEW

Maria ADOCHIȚEI, Adrian CHIRA, Daniel NAGY, Constantin PĂUN, Ligia ION

University of Agronomic Sciences and Veterinary Medicine of Bucharest,
59 Mărăști Blvd, 1st District, Bucharest, Romania

Corresponding author email: ionnagyligia@yahoo.fr

Abstract

The first epigenetic modification found was DNA methylation. Several DNA modifications, such as hydroxymethylation and carboxylation, as well as many post-translational histone modifications that epigenetically shape cell identity, have been discovered to date. DNA methylation is the most important epigenetic mechanism that has been intensively investigated. There are many conventional techniques to analyze the approximate or exact methylation content of DNA. The present research aimed to study the influence of the electromagnetic field of the Teslatron device (therapy device with high electrostatic potential) and the light fields of the Bioptron (performs light treatment) on some plants in order to see if this influence changes the quantity and quality of the active substances from the treated plants, compared to the control group. Genomic DNA isolations were then performed, which was quantified at the nanodrop. It was observed that there are changes in the amount and quality of DNA influenced with the Teslatron biofield compared to the control.

Key words*: teslatron, genomic DNA, epigenetic, nanodrop.*

INTRODUCTION

Organic farming is a scientific activity based on the progress of life science; however it is backed by the balance between man and nature. The processes that make up organic farming and their correlations are still being done imperfectly, due to the insufficient or lack of applied research (Elliott, 2011).

Current trends have led to a proliferation of research on the impacts, trade-offs, and ramifications of agricultural management of rural areas in relation to the set of social and ecological goods and services that society demands from a green, sustainable agriculture (Barrett et al., 2011; Brussaard et al., 2010). Most of the results that have emerged to date have demonstrated the magnitude and severity of the impacts of agriculture on ecological systems, as well as the challenge of designing management paradigms and strategies to satisfy ecosystem services in the context of limited resources and widespread ecosystem degradation (Estrada-Carmona et al., 2014). There is a sustained interest in developing a wide variety of paradigms and strategies for agricultural landscape analysis, planning, and management to address some of these challenges (Nelson et al. 2009; O'Farrell et al., 2010).

The destructive nature of humanity has threatened both the planet and its own prospects for survival (Krausmann et al., 2008; Kapoor, 2001). Human activities generate wastes, such as CO_2, faster than the biosphere can metabolize them (Huang et al., 2009).

In response, the capacity of natural ecosystems to provide the necessary life support systems for humanity is likely to decline in the coming decades (Krausmann et al., 2008; Kitzes et al., 2015; Pulselli et al., 2008).

Statistics have shown how the average per capita consumption of ecosystem goods and services has increased over the past 50 years, leading to a continuous increase in the human Ecological Footprint (EF) (Danovaro et al., 2014).

Studies have shown that the amount of biological capacity available per person has decreased as population growth outpaces the growth of production and ecosystem yields worldwide (Krausmann et al., 2008; Danovaro et al., 2014).

The consequence is increasing ecological deficits for nations around the world.

In contrast, alternative pro-environmental paradigms have been developed to mitigate the crisis and environmental degradation. It is known that natural resources and environmental problems arise at the intersection of complex natural and social systems (Pentreath, 2004). However, conventional environmental management paradigms continue to follow disciplinary lines to address the challenges. Increasingly effective solutions have been proposed to support the resolution of environmental problems but require increased integration of social and natural sciences, new governance approaches and a new culture for environmental management. An articulated framework is required to generate such features in an environmental management approach (Virapongse et al., 2016).

The modern strategy, which is called *eco-development* (intermediate perspective), aims to synthesize the areas of overlap and create a new vision, a new philosophy for the development of human societies. Eco-development recognizes the need for economic growth, as well as the need for this growth to be of a qualitatively very different nature from that which has been pursued in conventional economic development (Tenam-Zemach et al., 2014; Elliott et al., 2012). Most of the development activity proposed by Eco-development is a form of management of the fundamental relationship between society and nature. The use of "development" implies an explicit reorientation and an improvement in the level of integration of social, ecological and economic concerns.

The essence of the eco-development paradigm is to restructure the relationship between society and nature into a "positive-sum game" by reorganizing human activities so that they are synergistic with ecosystem processes and services (Colby, 1991). The process of environmental degradation and depletion of natural resources has been attributed to human behavior and traditional agricultural production techniques which are referred to as the science of human ecology. Human ecology can be defined as a complex of studies of the structure and change in the maintenance organizations or resource pools that support human populations in dynamic and constrained environments (Buttel et al., 2002).

One of the priority objectives in agroecological research is to prevent the degradation of natural resources in agriculture and to analyze sustainability. Constantly assessing the degree of environmental degradation in agriculture is therefore essential for countries dependent on agriculture (Sabiha et al., 2016). Establishing sustainability indicators is a constant concern in environmental science, management and environmental policy. Many strategies, formal management systems and environmental performance assessment techniques have been adopted that can be applied to any farm. Among these, ISO 14001, Life Cycle Assessment (Sh Karami et al., 2015), Environmental Impact Assessment (Rezaei-Moghaddam et al., 2008) and EF (Wackernagel et al., 1994; Fatemi et al., 2018) could all be applied to agricultural processes. Thus, indicators and indices are of increasing importance in environmental assessment, monitoring and sustainable development issues. These indicators and indices can be used for a wide variety of purposes, such as to assess current conditions, predict trends, compare situations, evaluate policy implementation and monitor ecological degradation (Pennino et al., 2017). Strategies such as the use of modern technologies and devices that generate life-sustaining frequencies as well as the use of LED lighting with different wavelengths are just some of the many attempts to solve the problem of environmental degradation, reducing toxic residues in the context of organic agriculture. This paper outlines the results of the influence of frequency generators and lights of different wavelengths on the germination and growth phenomena for some horticultural plants.

MATERIALS AND METHODS

The plant material was represented by seeds of coriander, fennel and sage plants that were germinated on 22.11.2022, in the following quantities:

- 9 Petri dishes for the experiment, for treatment with the TeslaTron device;
- 9 Petri dishes for treatment with the Biotron device and;
- 3 Petri dishes for the control group.

The seeds were placed on a layer of paper and watered every day, in a room where the ambient temperature was between 18-20^{0}C, with natural light.

The experiment began on 11/22/2022 and ended on 12/11/2022. It was conducted over a period of 20 days.
The seeds were divided:

- 9 Petri dishes with 10 seeds each of the three species, as follows:
- 3 Petri dishes of 10 coriander seeds each;
- 3 Petri dishes with 10 seeds each of the fennel species;
- 3 Petri dishes with 10 sage seeds each, for each type of device.

The treatment consisted of frequencies generated by the Teslatron device or the Bioptron device on the Petri dishes. 30 minutes of 9000V was applied and another 30 minutes were continued on the intelligent IF program in the treatment with the Teslatron device, for 20 days. The Teslatron is a device that generates frequencies with high electrostatic potential (Figure 1).
The Bioptron device generates light from the entire ROGVAIV spectrum with specific wavelengths that induce different effects on living organisms. The aim of the research was to determine which is the frequency and optimal wavelength that efficiently influences the germination and growth processes of plants. Thus, for the seeds treated with the Bioptron device, 10 minutes of red light and 10 minutes of orange light were applied per day, consecutively for 20 days.



Figure 1. The four experimental plots using Teslatron

Aspects of fennel and sage cultivation are shown in Figure 2.



Figure 2. Aspects of culture of fennel and sage plants

The results were statistically expressed using the Anova program.

RESULTS AND DISCUSSIONS

Results regarding the germination of plant seeds treated with the Teslatron device

Sage

Seeds germinated on 22.11.2022, and the first germination was on 30.11.2022; on 01.12.2022 there were 2 germinated seeds; on 05.12.2022 a total of 8 germinated seeds, on 10.12.2022 there were 11 germinated seeds.

Fennel

Seeds germinated on 22.11.2022, and the first germination was on 30.11.2022; on 01.12.2022 there were 2 germinated seeds; on 05.12.2022 a total of 7 germinated seeds, on 11.12.2022 a total of 13 germinated seeds.

Coriander

Seeds germinated on 22.11.2022, on 05.12.2022 there was no germinated seed, the first germination on 09.12.2022 and on 11.12.2022, 6 germinated seeds were recorded (Tables 1 and 2).

Table 1. Results concerning the number of germinated seeds of sage, fennel and coriander

Date/No. of seeds germinated	Sage seeds no. 30	Fennel seeds no. 30	Coriander seeds no. 30
30.11.2022	1	1	0
01.12.2022	2	2	1
05.12.2022	5	4	1
09.12.2022	5	4	4
10.12.2022	5	4	4
11.12.2022	6	5	5
Total germinated	19	20	15

Table 2. ANOVA: Results of statistical Single Factor analysis of number of seeds

Groups	Count	Sum	Average	Variances
Sage seeds no. 30	6	22	3.666667	15.06667
Fennel seeds no. 30	7	26	3.714286	17.57143
Coriander seeds no. 30	4	12	3	4

It was found that fennel seeds responded better to Teslatron treatment compared to sage and coriander seeds. Fennel seeds 13 out of 30 and sage seeds 11 out of 30 seeds (Figure 3).

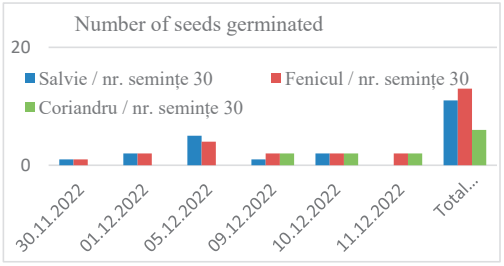


Figure 3. Graphical representation of the germination period of seeds treated with the Teslatron device

Since F (0.053505) < F critical (3.738892), we can reject the null hypothesis (H_0) and accept the alternative hypothesis (H_1) and conclude that the result varies depending on the type of seeds and the treatment with the Teslatron device.
In this example: Null hypothesis (H_0): The results do not vary depending on the type of seed ($\mu_1 = \mu_2 = \mu_3$)
Alternative hypothesis (H_1). The result varies depending on the type of seeds and the treatment with the Teslatron device (Table 3).

Table 3. ANOVA: Summary of the statistical results

Source of Variation	SS	MS	P value	F critical
Between Groups	1.47	0.73	0.94	3.73
Within Groups	192.7	13.76		
Total	194			

Results of plant seeds treated with the Bioptron device (Table 4)

Sage

Seeds germinated on 22.11.2022, and the first germination was on 30.11.2022; on 01.12.2022 there were 3 germinated seeds; on 05.12.2022 a total of 9 germinated seeds, on 11.12.2022 there were 17 germinated seeds out of a total of 30 seeds.

Fennel

Seeds germinated on 22.11.2022, and the first germination was on 30.11.2022; on 01.12.2022 there was 1 germinated seed; on 05.12.2022 a total of 1 germinated seed, on 11.12.2022 a total of 5 germinated seeds out of a total of 30 seeds.

Coriander

The seeds were germinated on 11/22/2022, on 12/05/2022 there was no germinated seed, and on 12/11/2022 we had one germinated seed.

Table 4. Plant seeds germinated on 22.11.2022 and treated with the Bioptron device

Date/No. of seeds germinated	Sage/no. seeds 30	Fennel/no. seeds 30	Coriander/no. seeds 30
30.11.2022	1	0	0
01.12.2022	2	1	0
05.12.2022	9	2	1
09.12.2022	2	2	1
10.12.2022	2	0	1
11.12.2022	1	0	1
Total germinated	17	5	4

It was found that sage seeds responded better to Bioptron treatment compared to fennel and coriander seeds. Sage seeds germinated 17 out of 30, fennel 5 out of 30.

Table 5. ANOVA: Results of statistical Single Factor analysis of number of seeds

Groups	Count	Sum	Average	Variances
Sage/no. seeds 30	5	34	6.8	42.2
Fennel/no. seeds 30	3	10	3.333	4.333
Coriander/no. seeds 30	2	2	1	0

Since F (1.083396) < F critical (4.737414), we can reject the null hypothesis (H_0) and accept the alternative hypothesis (H_1) and conclude that the result varies depending on the type of seeds treated and the device with which the treatment was performed, in this case the Bioptron device (Table 5).
In this example:
Null hypothesis (H_0): The results do not vary depending on the seed variety ($\mu_1 = \mu_2 = \mu_3$)

Alternative hypothesis (H_1): The result varies depending on the variety of seeds and the treatment with the Bioptron device.

Results of seeds from the control group

Sage

Seeds germinated on 22.11.2022, and the first germination was on 02.12.2022; on 05.12.2022 a total of 5 seeds germinated, on 10.12.2022 there were 5 seeds germinated out of a total of 10 seeds.

Fennel

Seeds germinated on 22.11.2022, and the first germination was on 01.12.2022, there was 1 germinated seed; on 05.12.2022 a total of 4 germinated seeds, on 11.12.2022 a total of 4 germinated seeds out of a total of 10 seeds.

Coriander

The seeds were germinated on 11/22/2022, on 12/05/2022 there were no germinated seeds, and on 12/11/2022 we had no germinated seeds (Table 6).

Table 6. Results regarding plant seeds germinated on 22.11.2022 control group

Date/No. of seeds germinated	Sage/no. seeds 10	Fennel/no. seeds 10	Coriander/no. seeds 10
30.11.2022	0	0	0
02.12.2022	1	1	0
05.12.2022	4	3	1
09.12.2022	4	3	1
10.12.2022	4	3	1
11.12.2022			
Total germinated	5	4	0

In the control group, it was found that the sage seeds germinated with a difference of one seed compared to the fennel seeds, these being 5 sage seeds and 4 fennel seeds out of a total of 10 seeds of each (Tables 7 and 8).

Table 7. ANOVA: Results of statistical Single Factor analysis of number of seeds

Groups	Count	Sum	Aver age	Vari - ances
Sage/no. seeds 10	3	10	3.33	4,333
Fennel/no. seeds 10	3	8	2.66	2,333
Coriander/ no. seeds 10	0	0	0	0

Table 8. ANOVA: Summary of the statistical results

Source of variation	SS	MS	P value	F critical
Between Groups	0.66	0.33	0.92	9.55
Within Groups	13.33	4.44		
Total	14			

Since F (0.075) < F critical (9.552094), we can reject the null hypothesis (H_0) and accept the alternative hypothesis (H_1) and conclude that the result varies depending on the type of seeds, not being subjected to any treatment with devices.

Null hypothesis (H_0): The results do not vary depending on the type of seeds ($\mu_1 = \mu_2 = \mu_3$)

Alternative hypothesis (H_1): The result varies depending on the type of seed.

CONCLUSIONS

It was found that fennel seeds responded better to the Teslatron treatment compared to sage and coriander seeds. Fennel seeds 13 out of 30 and sage seeds 11 out of 30 seeds. It was found that sage seeds responded better to the Bioptron treatment compared to fennel and coriander seeds. Sage seeds germinated 17 out of 30, fennel 5 out of 30. In the control group, it was found that the sage seeds germinated with a difference of one seed compared to the fennel seeds, these being 5 sage seeds and 4 fennel seeds out of a total of 10 seeds of each. In the treatment with the biofungicide to eliminate mold, the first results of mold elimination were visible after the first 3-4 days. The treatment was carried out over a period of 7 days, until its elimination.

REFERENCES

Barrett CB, Travis AJ, Dasgupta P. (2011). On biodiversity conservation and poverty traps. *Proceedings of the National Academy of Sciences of the United States of America*.; 108, 13907–1392.

Brussaard L, Caron P, Campbell B, Lipper L, Mainka S, Rabbinge R. (2010). Reconciling biodiversity conservation and food security: *Scientific challenges for a new agriculture. Current Opinion in Environmental Sustainability*; 2, 34–42.

Buttel FH, Humphery CR (2002). Sociological theory and the natural environment, in: Dunlap RE, Michelson W. (Eds.), *Handbook of Environmental Sociology. Greenwood Press*, USA; 33–69.

Colby ME. (1991). Environmental management in development: The evolution of paradigms. *Ecological Economics; 3*(3), 193–213.

Danovaro R, Snelgrove PVR, Tyler P. (2014). Challenging the paradigms of deep-sea ecology. *Trends in Ecology & Evolution.; 29 (*8), 465–475.

Elliott M, Whitfield AK. (2011). Challenging paradigms in estuarine ecology and management. *Estuarine, Coastal and Shelf Science; 94* (4), 306–314.

Estrada-Carmona N, Hart A, DeClerck FAJ, Harvey CA, Milder JC. (2014). Integrated landscape management for agriculture, rural livelihoods, and ecosystem conservation: An assessment of experience from Latin America and the Caribbean. *Landscape and Urban Planning*; 129, 1–11.

Fatemi M, Rezaei-Moghaddam K, Wackernagel M, Shennan C. (2018). Sustainability of environmental management in Iran: *An ecological footprint analysis. Iran Agricultural Research.;* 37 (2), 53–68.

Huang J, Tichit M, Poulot M, Darly S, Li S, Petit C, et al. (2015). Comparative review of multifunctionality and ecosystem services in sustainable agriculture. *Journal of Environmental Management*; 149, 138–147.

Kapoor I. (2001). Towards participatory environmental management. *Journal of Environmental Management*; 63, 269–279.

Kitzes J, Moran D, Galli A, Wada Y, Wackernagel M. (2009). Interpretation and Application of the Ecological Footprint: A Reply to Fiala (2008). *Ecological Economics; 68*(4), 929–930.

Krausmann F, Erb KH, Gingrich S, Lauk C, Haberi H. (2008). Global patterns of socioeconomic biomass flows in the year 2000: A comprehensive assessment of supply, consumption and constraints. *Ecological Economics*; *65*(3), 471–487.

Nelson E, Mendoza G, Regetz J, Polasky S, Tallis H, Cameron DR. (2009). Modeling multiple ecosystem services, biodiversity conservation, commodity production, and tradeoffs at landscape scales. *Frontiers in Ecology and the Environment, 7*(1), 4 – 11.

O'Farrell PJ, Anderson PM. (2010). Sustainable multifunctional landscapes: A review to implementation. *Current Opinion in Environmental Sustainability* ; 2, 59–65.

Pentreath RJ. (2004). Ethics, genetics and dynamics: An emerging systematic approach to radiation protection of the environment. *Journal of Environmental Radioactivity*; 74, 19–30.

Pennino MG, Bellido JM, Conesa D, Coll M, Tortosa-Ausina E. (2017). The analysis of convergence in ecological indicators: An application to the Mediterranean fisheries. *Ecological Indicators*; 78, 449–457.

Pulselli RM, Simoncicni E, Ridolfi R, Bastianoni S. (2008). Specific energy of cement and concrete: An energy-based appraisal of building materials and their transport. *Ecological Indicators*; *8*(5), 647–656.

Rezaei-Moghaddam K, Karami E. (2008). Developing a green agricultural extension theory. *International Journal of Sustainable Development and Planning*; *3*(3), 242–256.

Sabiha NE, Salim R, Rahman S, Rola-Rubzen NF (2016). Measuring environmental sustainability in agriculture: A composite environmental impact index approach. *Journal of Environmental Management*; 166, 84–93.

Sh Karami, Karami E Zand-Parsa Sh. (2015). Environmental and economic appraisal of agricultural water desalination use in South Iran: A comparative study of tomato production. *Journal of Applied Water Engineering and Research*; 2, 1–12.

Tenam-Zemach M, Kirchgessner M, Pecore J, Lai L, Hecht S. (2014). Development of an innovative method for analyzing the presence of environmental sustainability themes and an ecological paradigm in science content standards. *Studies in Educational Evaluation*; 41, 133–142.

Virapongse A, Brooks S, Metcalf EC, Zedalis M, Gosz J, Kliskey A, et al.(2016) A social-ecological systems approach for environmental management. *Journal of Environmental Management*; 178, 83–91.

Wackernagel M. Ecological Footprint and Appropriate Carrying Capacity: (1994) A Tool for Planning Toward Sustainability. PhD D Dissertation, *School of Community and Regional Planning, University of British Columbia, Toronto, Canada*.

.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

EVALUATION OF THE INTEGRATED EFFECTS OF SOIL MANAGEMENT AND FERTILIZATION STRATEGIES ON THE GROWTH, YIELD AND QUALITY OF SWEET PEPPER (*CAPSICUM ANNUUM* L.) CULTIVATED IN POLYETHYLENE GREENHOUSES

Dan-Ioan AVASILOAIEI[1], Claudia BĂLĂIȚĂ[1], Carmina BENCHEA[1], Gabriel-Alin IOSOB[1], Emilian MIREA[2], Mariana CALARA[1]

[1]Vegetable Research and Development Station Bacău, 220 Calea Bârladului Street, 600388, Bacău, Romania
[2]Vegetable Research and Development Station Buzău, 23 Mesteacănului Street, 120024, Buzău, Romania

Corresponding author email: calaramariana@gmail.com

Abstract

This study investigates the impact of various soil management practices and fertilization treatments on the growth, yield and quality of `DarianaBac`sweet pepper (Capsicum annuum L.) cultivated in polyethylene greenhouses. Experimental variants were defined by two distinct soil preparation methods: plowed soil and rotary-tilled soil, coupled with five different fertilization treatments, including Albit + Turboroot, Biochar + Woodvinegar + Cropmax, Nutriplant + Resid, Orgevit + Kerafol and untreated controls. Comprehensive assessments were conducted across several key parameters: plant biometrics (plant height, number of branches, stem collar diameter, flower count per plant), physiological and stress-related responses (anthocyanin and chlorophyll content), productive parameters (fruit count per plant, average fruit weight, yield per plant), fruit morphology (fruit width, length, and form index) and yield quality indicators (carotenoid and lycopene content, total soluble solids, and dry matter content of fruits). Statistical analysis revealed significant differences in plant development and yield outcomes influenced by both soil treatment and fertilization regime.

Key words*: ploughing tillage, rototiller, soil enrichment practices, crop management, controlled environment.*

INTRODUCTION

The sweet pepper (*Capsicum annuum* L. ssp. *annuum*), a thermophilic species within the Solanaceae family, holds global prominence for its high productivity, pest and disease resilience and versatility in fresh consumption, culinary applications and industrial processing (Brezeanu et al., 2022; Bălăiță et al., 2024). Renowned for its abundant bioactive compounds, including ascorbic acid, carotenoids, tocopherols and phenolic compounds, this crop provides potent antioxidative and health-promoting benefits, mitigating oxidative stress and reducing the risk of chronic diseases such as cardiovascular disorders and cancer (Brezeanu et al., 2022; Stoleru et al., 2023). The compositional quality and phytochemical richness of sweet peppers are profoundly influenced by genetic, environmental and agronomic factors, with sustainable cultivation practices proven to enhance nutrient content while minimizing ecological impact (Stoleru et al., 2023). Contemporary research prioritizes integrating genotype optimization with eco-conscious agricultural practices to elevate the nutritional, economic and environmental value of sweet peppers in a sustainable food system (Brezeanu et al., 2022; Stoleru et al., 2023; Elhawary et al., 2024). In controlled environments such as polyethylene greenhouses, these variables acquire heightened importance, given the need to balance resource efficiency, environmental sustainability and economic viability (Maraveas, 2019).

To meet the growing demand for premium-quality produce while ensuring sustainable agricultural practices, an integrated approach that optimizes soil management and fertilization strategies is essential (Batabyal, 2017). These two critical factors directly influence plant growth, resource use efficiency and the nutritional and phytochemical profiles of the harvested produce (Cabrera-De la Fuente et al., 2018; Heimler et al., 2017), particularly in controlled environments such as polyethylene

greenhouses (Ahmed et al., 2024; Zhao et al., 2024).

Soil management practices, such as plowing and rototilling, are fundamental to creating a conducive growth medium for crops (Mefferd, 2019). These practices influence soil aeration, water infiltration, root penetration and nutrient availability, which are vital for enhancing plant vigor and productivity (Mitchell et al., 2019). Plowing ensures deeper soil turnover, promoting nutrient redistribution (Jin et al., 2020), while rototilling focuses on surface soil refinement and microbial activity enhancement (Torotwa et al., 2023). The choice of soil management practice can significantly alter the soil's physical and biochemical properties (Kobierski et al., 2020), altering plant performance and the uptake of nutrients (Lv et al., 2023).

Similarly, fertilization management practices play an equally pivotal role in determining the physiological and biochemical quality of sweet pepper fruits (EL-Mogy et al., 2024). Fertilization not only supplies essential nutrients for optimal plant growth but also modulates the biosynthesis of bioactive compounds such as carotenoids, ascorbic acid and phenolics, which are key determinants of fruit quality (Muscolo et al., 2020; Stagnari et al., 2022).

This research seeks to unravel the synergistic effects of soil preparation techniques and fertilization regimes on growth, yield and quality attributes of sweet pepper, including the accumulation of health-promoting compounds. By examining the interplay between these two pivotal factors, the study offers critical insights into optimizing production systems in greenhouse environments. The findings are anticipated to contribute to advancing sustainnable horticultural practices that prioritize soil health, efficient resource use and high-quality crop production, addressing both consumer demand and environmental sustainability imperatives.

MATERIALS AND METHODS

Location and Experimental Setup

The experiment was conducted in 2024 at the Vegetable Research and Development Station Bacau, Romania.

The trial was carried out in an Almeria-type polyethylene-covered greenhouse with controlled environmental factors (Figure 1).



Figure 1. Geographical Site and Experimental Design

Crop Description

`Darianabac` cultivar of *Capsicum annuum* L. served as the focal point of this research, an early-maturing, medium-vigor variety with a vegetative period of 120 days until the first harvest. The plant habit is characterized by 50% erect and 50% horizontal growth, while the fruit exhibits a yellow hue with greenish tones at physiological maturity, weighing an average of 80-120 g.

Sowing and Transplanting

Sowing: 2nd decade of February

The seeds were sown in Rekyva Remix 1 horticultural peat, a substrate specially designed for seed germination. The substrate features small and/or medium fractions with the following characteristics: pH 5.5-6.5, electrical conductivity 0.5-1 mS/cm, and a chlorine-free NPK content ≤ 1 kg/m³.

Transplanting: 3rd decade of February

The seedlings were transplanted into 70-cell alveolar trays. The substrate used was Rekyva Remix 2 horticultural peat, designed for seedling cultivation, characterized by medium and/or large fractions with pH 5.5-6.5, electrical conductivity 1-2 mS/cm, and a chlorine-free NPK content ≤ 2 kg/m³.

Experimental Variants

The experimental variants, detailed in Table 1, were implemented using a mirrored design under two distinct soil management systems:

Plowed soil (30 cm depth), with variants labeled as P1R1, P1R2, P1R3, ..., P4R2, etc.

Rototilled soil (incorporated with a rototiller), with variants labeled as RT1R1, RT1R2, RT1R3, ..., RT4R2, etc.

Table 1. Experimental variants. Fertilization regimens: composition and application schedules

Crop/Cultivar	Soil management practice	Fertilization scheme	Application dates and no of treatments
`DarianaBac` sweet pepper	plowed soil	**P1** - Albit (0.01%) + Turboroot (0.5%)	I - 3rd decade of May; II - 2nd decade of June; III - 1st decade of July;
		P2 - Biochar (10 g/plant) + Wood Vinegar (0.5%) + Cropmax (0.25%)	
		P3 - Nutriplant 20:20:20 (0.015%) + Resid (2 g/plant)	
		P4 - Orgevit (10 g/plant) + Kerafol (0.25%)	
		P5 - Control (untreated)	
	rototilled soil	**RT1** - Albit (0.01%) + Turboroot (0.5%)	
		RT2 - Biochar (10 g/plant) + Wood Vinegar (0.5%) + Cropmax (0.25%)	
		RT3 - Nutriplant 20:20:20 (0.015%) + Resid (2 g/plant)	
		RT4 - Orgevit (10 g/plant) + Kerafol (0.25%)	
		RT5 - Control (untreated)	



Figure 2. Aspects regarding the application of fertilizing substances and biostimulatory compounds

Each experimental variant was replicated three times, with each replication including five plants.

Cultivation and Irrigation

Plant spacing: 25 cm between plants within a row and 70 cm between rows, resulting 57.143 plants/ha.

Irrigation: Drip irrigation was employed using drip hoses.

Weed control: Black polyethylene mulch was applied between the rows for optimal weed control.

Vegetative and Generative Development of Sweet Pepper Plants

An extensive array of growth parameters was meticulously monitored to evaluate plant development. These parameters encompassed total plant height, stem collar diameter, number of secondary stems (branches) and flowers. At the conclusion of the growth cycle, comprehensive data on fruit yield were gathered, including metrics such as the number of fruits per plant, average fruit weight, yield per plant, fruit width, fruit height, and the fruit form index (calculated as the ratio of fruit height to width). Measurements were performed through direct counting, with linear dimensions assessed using a precision ruler, diameters measured with a calibrated caliper and weights determined using a high-accuracy electronic balance.

Physiological parameters

The quantification of chlorophyll pigments and anthocyanins was performed using two comparable devices, the CCM 200 plus and ACM 200 plus, both manufactured by OptiSciences. The data were expressed through indices designed to precisely reflect the total concentrations of chlorophyll pigments and anthocyanins, namely the Chlorophyll Concentration Index and the Anthocyanin Content Index.

Quality Assessment

The quality evaluation of sweet peppers fruits influenced by the two experimental factors, encompassed several parameters, including β-carotene and lycopene concentrations, total soluble solids (TSS) and dry matter (DM) content (Figure 3).

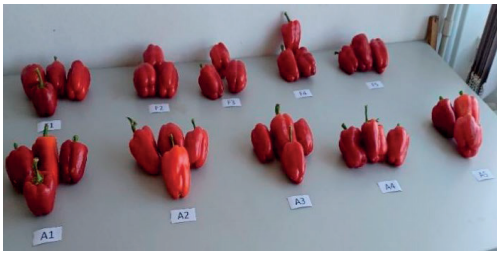


Figure 3. Processing of fruits for laboratory specimen procurement

β-carotene and lycopene quantification – extractions were conducted using petroleum ether as the solvent, with absorbance measured spectrophotometrically at 452 nm for β-carotene and 472 nm for lycopene. The respective concentrations were calculated by multiplying the absorbance readings by standardized conversion factors—19.96 for β-carotene and 14.495 for lycopene. The results were then reported in milligrams per 100 grams of fresh weight (mg·100 g^{-1} F.W.), ensuring an accurate representation of pigment concentration.

Total soluble solids (TSS) were quantified using a precision handheld refractometer, and the results were reported in degrees Brix, following the AOAC (2005) method, section 932:12.

Dry matter (DM) was determined by drying fresh, uniform samples in a forced-air oven (Biobase) maintained at a constant temperature of 103 ± 2°C for 24 hours, until achieving a stable weight. This procedure adhered strictly to the AOAC (2000) protocol, with the final results expressed as a percentage.

Statistical analysis

The analytical data obtained from the study were subjected to statistical evaluation utilizing IBM SPSS Statistics software, version 26.0. Analysis of variance (ANOVA) was employed to identify differences among group means, with subsequent pairwise comparisons performed using Tukey's post hoc test. Statistical significance was established at a confidence level of $p < 0.5$. Results are presented as mean values accompanied by their corresponding standard deviation.

RESULTS AND DISCUSSIONS

The present study assessed the influence of diverse fertilization treatments and soil management techniques on the growth and productivity of the DarianaBac sweet pepper variety under controlled greenhouse conditions.

Differences in plant height, secondary stems, stem collar diameter, flower production and fruit set 30 days after transplanting were evaluated (Table 2). While statistically significant differences (Tukey test, $p<0.05$) were identified only for flower production, observed variations in the other parameters did not reach significance, as indicated by shared letter groupings in the analysis. These findings underscore the specific sensitivity of flower production to the interplay between nutrient application and soil preparation, while other growth parameters appeared more resilient to these treatments.

Rototilled soils consistently promoted greater plant height compared to plowed soils, with the highest value recorded in rototilled soil treated with Albit and Turboroot (RT1 - 52.77 ± 8.38 cm) versus plowed soil (P1-P5, 41.77-49.00 cm). Although differences within soil management types were not statistically significant, the findings suggest that rototilling enhances root expansion and nutrient availability. Environmental factors, such as light intensity and temperature, appeared to have a more pronounced influence on plant height than soil or fertilization practices. Secondary stem production also favored rototilled soils, with RT5 (9.44 ± 1.24) and RT1 (9.11 ± 0.93) achieving the highest values. Orgevit and Kerafol treatments further supported vegetative growth, highlighting the combined role of soil structure and specific fertilizers in promoting branching (Fan et al., 2021; Bodner et al., 2021). Stem collar diameter remained consistent across treatments, suggesting structural growth is less sensitive to soil preparation and fertilization (Wolf, 2022). However, flower production showed a marked response, with rototilled soil and Nutriplant plus Resid (RT3 - 51.33 ± 7.28 flowers) achieving the highest results. These findings emphasize the role of enhanced soil aeration and targeted fertilization in optimizing flowering potential (Cho et al., 2017; Welch, 2024). Fruit set 30 days post-transplantation was highest in P4 and RT4 (Orgevit + Kerafol), yielding 3.00 ± 1.30 and 2.78 ± 2.05 fruits, respectively, compared to controls (RT5 - 2.11 ± 1.83; P5 - 1.63 ± 1.22). Fertilization treatments significantly improved fruiting, underscoring the synergy between soil management and balanced nutrient application in enhancing reproductive success (Morugán-Coronado et al., 2020; Liu et al., 2024; Brezeanu et al., 2022).

Overall, the results confirm that combining rototilling with specific fertilization strategies, such as biochar, wood vinegar, Cropmax and Orgevit, significantly improves key growth parameters and productivity.

Table 2. Morphological and reproductive characteristics of `Darianabac` sweet pepper plants

Variant	Plant height (cm)	No of secondary stems	Stem collar Ø (mm)	No of flowers/plant	No of fruits/plant (30 days after transplanting)
P1	44.20±7.98ns	7.10±1.45ns	9.39±1.32ns	32.20±9.05b	1.63±1.22ns
P2	41.77±5.78ns	7.23±1.56ns	10.29±1.25ns	32.80±5.87b	1.20±1.2ns
P3	49.00±4.41ns	7.20±1.39ns	9.82±0.93ns	44.53±6.19ab	1.77±1.56ns
P4	47.67±4.30ns	8.00±2.06ns	8.97±1.30ns	35.43±8.05ab	3.00±1.3ns
P5	47.77±6.57ns	7.43±2.01ns	9.19±1.45ns	38.53±4.8ab	1.80±1.09ns
RT1	52.77±8.38ns	9.11±0.93ns	9.99±0.97ns	45.44±10.54ab	1.11±1.27ns
RT2	49.57±4.64ns	8.22±1.86ns	10.13±1.02ns	50.33±7.75a	2.44±1.42ns
RT3	49.43±3.43ns	7.89±1.05ns	9.89±0.90ns	51.33±7.28a	2.56±1.74ns
RT4	47.43±5.08ns	8.67±2.00ns	10.08±0.85ns	50.56±8.68a	2.78±2.05ns
RT5	51.33±4.72ns	9.44±1.24ns	10.23±0.83ns	49.11±14.17ab	2.11±1.83ns

The presented values represent the arithmetic mean accompanied by the standard deviation. Lowercase letters indicate the outcomes of Tukey's post-hoc analysis at a significance threshold of $p < 0.05$, where "a" designates the highest recorded value and "ns" - non-significant differences. P1 plowed soil * Albit + Turboroot; P2 - plowed soil * Biochar + Wood Vinegar + Cropmax; P3 - plowed soil * Nutriplant 20:20:20 + Resid; P4 - plowed soil * Orgevit + Kerafol; P5 - plowed soil * unfertilized (Control); RT1 – rototilled soil * Albit + Turboroot; RT2 - rototilled soil * Biochar + Wood Vinegar + Cropmax; RT3 - rototilled soil * Nutriplant 20:20:20 + Resid; RT4 - rototilled soil * Orgevit + Kerafol; RT5 - rototilled soil * unfertilized (Control)

The study also highlighted the pivotal role of soil management and fertilization in modulating physiological parameters of sweet pepper (Figure 4).

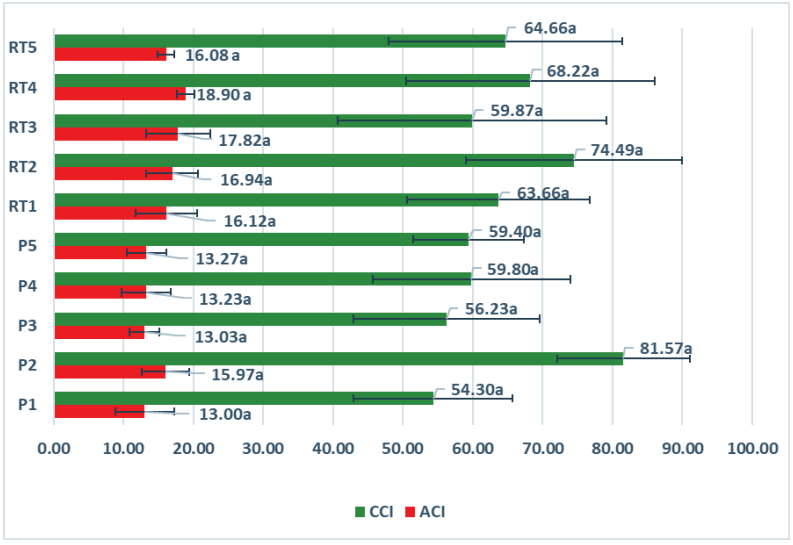


Figure 4. Concentration of anthocyanin and chlorophyll content of `Darianabac` sweet pepper leaves
Note: Same letters show no significant difference between items according to Tukey's post-hoc test; $p \leq 0.05$

The highest Chlorophyll Content Index (CCI) was recorded in plowed soil amended with biochar, wood vinegar and Cropmax (P2 - 81.57), significantly outperforming other plowed soil variants. In rototilled soil, the same fertilization scheme (RT2 - 74.49) produced the highest CCI, comparable to P2, while Orgevit and Kerafol (RT4 - 68.22) also performed well. Controls (P5 - 59.40; RT5 - 64.66) showed moderate values, demonstrating the superiority of enriched treatments in enhancing photosynthesis (Hur et al., 2024).

Anthocyanin Content Index (ACI) was highest in rototilled soil with Orgevit and Kerafol (RT4

- 18.90) and Nutriplant with Resid (RT3 - 17.82), with comparable values across most treatments, including controls (P5 - 13.27). This consistency suggests anthocyanin synthesis depends more on environmental factors than fertilization (Kutman, 2023; Kaur et al., 2023; Yan et al., 2022).

Rototilling enhanced nutrient integration, especially for biochar and wood vinegar, through improved soil aeration and microbial activity (Wang et al., 2022). However, ACI appeared largely independent of nutrient inputs, aligning with its reliance on stress-induced pathways (Araguirang & Richter, 2022; Li & Ahammed, 2023).

Thus, biochar, wood vinegar and Cropmax significantly improved chlorophyll synthesis, while anthocyanin accumulation was less responsive to fertilization. These findings highlight the interplay of soil preparation and nutrient management in optimizing physiological performance in protected cultivation systems.

The significant effects of soil preparation and fertilization on key yield parameters of sweet pepper (DarianaBac variety), including average fruit weight, fruit count per plant and total yield per plant are highlighted in Table 3.

Average fruit weight was highest in rototilled soil fertilized with biochar, wood vinegar and Cropmax (RT2, 97.33 g ± 11.88), outperforming other treatments, including P2 (95.50 g ± 22.28) and P3 (94.43 g ± 18.74). The lowest value was recorded in RT4 (79.53 g ± 14.02), treated with Orgevit and Kerafol, suggesting suboptimal interactions between this fertilization and rototilled soil. The results highlight biochar's potential to enhance soil structure and nutrient retention, while wood vinegar's organic acids further augment nutrient availability, aligning with findings by Lei et al. (2024) and Simiele et al. (2022).

Fruit count per plant (60 days post-transplant) was highest in RT2 (22.33 ± 7.57), followed by RT1 (21.33 ± 7.57) and RT4 (20.67±1.53), significantly exceeding plowed soil variants such as P1 (13.00 fruits) and P5 (9.67 fruits). Rototilled soil likely supports superior nutrient and water uptake, driving higher reproductive output.

The highest total yield per plant was observed in RT2 (2160.17 g ± 319.09), followed by RT1 (1838.63 g ± 506.8), both of which significantly outperformed plowed soil variants, such as P5 (783.39 g ± 256.75). These results underscore the pivotal role of rototilling in enhancing yield potential and optimizing large-scale production (Shah & Wu, 2019). Also, humic substances, an integral part of the treatments in P2 and RT2 variants, are recognized as the most vital constituents of soil, playing a pivotal role in its productivity (Raducanu et al., 2016). The significant differences between rototilled and plowed soil variants emphasize the crucial impact of soil preparation, while the lower yields observed in the untreated control (P5) underscore the importance of implementing targeted fertilization strategies to optimize crop production.

Table 3. Fruit yield and productivity parameters of `Darianabac` sweet pepper plants

Variant	Average fruit weight (g)	No of fruits/plant (60 days after transplanting)	Total yield/plant (g)
P1	87.10±21.07ns	13.00±4.00abc	1129.94±477.42ab
P2	95.50±22.28ns	11.33±2.89abc	1085.40±237.01ab
P3	94.43±18.74ns	11.00±3.61bc	1042.98±530.51b
P4	93.73±7.49ns	11.67±3.79abc	1111.32±329.7ab
P5	81.67±15.15ns	9.67±4.51c	783.39±256.75b
RT1	85.17±19.41ns	21.33±5.77ab	1838.63±506.8ab
RT2	97.33±11.88ns	22.33±7.57a	2160.17±319.09a
RT3	81.17±10.02ns	19.33±9.02abc	1574.11±408.33ab
RT4	79.53±14.02ns	20.67±1.53abc	1632.49±318.51ab
RT5	82.77±21.16ns	18.37±2.89abc	1517.87±253.98ab

The presented values represent the arithmetic mean accompanied by the standard deviation. Lowercase letters indicate the outcomes of Tukey's post-hoc analysis at a significance threshold of $p < 0.05$, where "a" designates the highest recorded value and "ns" - non-significant differences. P1 plowed soil * Albit + Turboroot; P2 - plowed soil * Biochar + Wood Vinegar + Cropmax; P3 - plowed soil * Nutriplant 20:20:20 + Resid; P4 - plowed soil * Orgevit + Kerafol; P5 - plowed soil * unfertilized (Control); RT1 – rototilled soil * Albit + Turboroot; RT2 - rototilled soil * Biochar + Wood Vinegar + Cropmax; RT3 - rototilled soil * Nutriplant 20:20:20 + Resid; RT4 - rototilled soil * Orgevit + Kerafol; RT5 - rototilled soil * unfertilized (Control)

The data also revealed notable variations in fruit dimensions across the different treatments (Table 4). For fruit width, plowed soil treatments, particularly P3 (66.00 mm ± 12.27), exhibited the widest fruits, followed by P2 (64.53 mm ± 5.71), P4 (64.10±4.07) and RT2 (63.23 mm ± 2.46). These results suggest that certain plowed soil treatments may be more conducive to fruit width development. In contrast, RT1 had the smallest fruit width, averaging 58.53 mm ± 7.36, which could indicate a potential limitation in nutrient availability or soil structure in this treatment. Regarding fruit length, the largest fruits were observed in P5 (80.90 mm ± 12.96), followed by RT1 (78.80 mm ± 10.47). Notably, biochar + wood vinegar + Cropmax and, respectively, Nutriplant + Resid fertilization schemes showed lower average fruit lengths in both plowed and rototilled soils. These variations highlight the differing impacts of soil preparation methods on fruit length, where plowing may promote longer fruit development. Overall, the results underscore the complex interactions between soil preparation, fertilization strategies and fruit development, with plowed treatments often outperforming rototilled ones in certain parameters.

Table 4. Morphological characteristics and fruit form indices

Variant	Fruit width (mm)	Fruit length (mm)	Fruit form index (W/L)	Fruit form index (L/W)
P1	62.97±3.28ns	72.53±14.36ns	0.88±0.10ns	1.15±0.14ns
P2	64.53±5.71ns	74.67±7.54ns	0.87±0.10ns	1.16±0.14ns
P3	66.00±12.27ns	73.10±4.67ns	0.91±0.12ns	1.12±0.15ns
P4	64.10±4.07ns	77.77±12.52ns	0.83±0.03ns	1.21±0.04ns
P5	60.33±23.75ns	80.90±12.96ns	0.75±0.24ns	1.44±0.53ns
RT1	58.53±7.36ns	78.80±10.47ns	0.74±0.06ns	1.35±0.10ns
RT2	63.23±2.46ns	73.87±6.99ns	0.86±0.07ns	1.17±0.10ns
RT3	62.03±5.22ns	72.27±8.88ns	0.86±0.05ns	1.17±0.07ns
RT4	61.27±7.30ns	69.00±24.07ns	0.90±0.14ns	1.13±0.19ns
RT5	61.40±8.91ns	75.67±15.46ns	0.84±0.24ns	1.26±0.40ns

The presented values represent the arithmetic mean accompanied by the standard deviation. Lowercase letters indicate the outcomes of Tukey's post-hoc analysis at a significance threshold of $p < 0.05$, where "ns" - non-significant differences. P1 plowed soil * Albit + Turboroot; P2 - plowed soil * Biochar + Wood Vinegar + Cropmax; P3 - plowed soil * Nutriplant 20:20:20 + Resid; P4 - plowed soil * Orgevit + Kerafol; P5 - plowed soil * unfertilized (Control); RT1 – rototilled soil * Albit + Turboroot; RT2 - rototilled soil * Biochar + Wood Vinegar + Cropmax; RT3 - rototilled soil * Nutriplant 20:20:20 + Resid; RT4 - rototilled soil * Orgevit + Kerafol; RT5 - rototilled soil * unfertilized (Control)

On the nutritional quality of sweet pepper, specifically lycopene, β-carotene, soluble solids and dry matter content, the analysis of soil management and fertilization practices revealed significant variations, highlighting the complex interactions between these factors (Table 5).

Lycopene content was highest in RT4 (6.71 mg/100 g ± 0.12), significantly surpassing other treatments, likely due to the synergistic effect of Orgevit and Kerafol. RT5 also exhibited elevated lycopene levels (6.26 mg/100g ± 0.02), suggesting the positive influence of rototilled soil with control fertilization. Conversely, plowed treatments, particularly P3 (4.23 mg/100 g ± 0.09) and P2 (4.80 mg/100 g ± 0.06), demonstrated lower lycopene levels, likely reflecting suboptimal conditions for carotenoid biosynthesis.

For β-carotene, RT4 again showed higher concentration (15.98 mg/100 g ± 0.25), but the highest one was observed in P1 (18.36 ± 0.18), indicating that nutrient optimization in these treatments enhances β-carotene production. Plowed soil variants, such as P2 (10.49 mg/ 100 g ± 0.13) and P4 (10.41±0.21), had significantly lower β-carotene levels, emphasizing the role of nutrient bioavailability in carotenoid biosynthesis (Khaliq et al., 2023).

Soluble solids content, indicative of fruit sweetness, was highest in RT5 (9.30 °Brix ± 0.26), suggesting that rototilling with the control treatment promotes sugar accumulation. P2 (6.90 °Brix ± 0.26) exhibited the lowest soluble solids content, possibly due to imbalances in nutrient uptake.

For dry matter content, both P1 (11.01% ± 0.20) and RT5 (11.44% ± 0.03) showed higher values, reflecting enhanced biomass accumulation due to improved photosynthetic activity. Conversly, P5 (7.97% ± 0.22), the untreated control in plowed soil, had the lowest dry matter content, emphasizing the necessity of fertilization in improving plant biomass (Condon, 2020).

Table 5. Nutritional and biochemical composition of `Darianabac` sweet pepper fruits

Variant	Lycopene (mg·100 g^{-1} F.W)	β-carotene (mg·100 g^{-1} F.W.)	Soluble solids (°Brix)	Dry matter (%)
P1	5.10±0.10de	18.36±0.18a	8.67±0.25ab	11.01±0.20a
P2	4.80±0.06e	10.49±0.13e	6.90±0.26b	8.41±0.04de
P3	4.23±0.09f	14.14±0.08d	7.30±0.26ab	9.79±0.15b
P4	6.43±0.09ab	10.41±0.21e	8.53±0.32ab	9.01±0.04cd
P5	5.85±0.28c	17.23±0.56b	8.07±1.77ab	7.97±0.22e
RT1	5.36±0.14d	10.54±0.23e	8.50±0.20ab	8.83±0.29cd
RT2	5.89±0.08c	14.46±0.05d	7.80±0.40ab	8.67±0.23cd
RT3	5.15±0.07de	11.11±0.43e	7.93±1.59ab	8.88±0.19cd
RT4	6.71±0.12a	15.98±0.25c	8.57±0.29ab	9.13±0.41c
RT5	6.26±0.02b	15.18±0.08cd	9.30±0.26a	11.44±0.03a

The presented values represent the arithmetic mean accompanied by the standard deviation. Lowercase letters indicate the outcomes of Tukey's post-hoc analysis at a significance threshold of $p < 0.05$, where "a" designates the highest recorded value. P1 plowed soil * Albit + Turboroot; P2 - plowed soil * Biochar + Wood Vinegar + Cropmax; P3 - plowed soil * Nutriplant 20:20:20 + Resid; P4 - plowed soil * Orgevit + Kerafol; P5 - plowed soil * unfertilized (Control); RT1 – rototilled soil * Albit + Turboroot; RT2 - rototilled soil * Biochar + Wood Vinegar + Cropmax; RT3 - rototilled soil * Nutriplant 20:20:20 + Resid; RT4 - rototilled soil * Orgevit + Kerafol; RT5 - rototilled soil * unfertilized (Control)

CONCLUSIONS

The results demonstrate that both soil preparation and fertilization treatments significantly affect the growth and productivity of sweet pepper. The highest yields, largest fruit sizes and most favorable fruit production were consistently observed in rototilled soil treatments with biochar, wood vinegar and Cropmax. These findings emphasize the importance of integrated soil management and nutrient application for optimizing crop productivity, particularly in greenhouse environments. While certain fertilization treatments in plowed soils yielded positive results, rototilled soil consistently provided the most advantageous conditions for growth and fruit production.

Both soil management practices combined with targeted fertilization, significantly enhance the nutritional quality and biomass of sweet pepper, underscoring the critical role of optimized nutrient availability in promoting carotenoid biosynthesis, sugar accumulation and overall plant vigor.

ACKNOWLEDGEMENTS

This study was conducted with the financial and logistical support of the Ministry of Agriculture and Rural Development, as part of the 6.3.20 ADER 2023-2026 Sectorial Plan initiative

REFERENCES

Ahmed, N., Zhang, B., Chachar, Z., Li, J., Xiao, G., Wang, Q., ... & Tu, P. (2024). Micronutrients and their effects on horticultural crop quality, productivity and sustainability. *Scientia Horticulturae*, *323*, 112512.

AOAC (2000) Official Methods of Analysis. 17th Edition, The Association of Official Analytical Chemists, Gaithersburg, MD, USA. Methods 925.10, 65.17, 974.24, 992.16.

AOAC. (2005). Official Methods of Analysis of AOAC International, 21st ed.; AOAC: Gaithersburg, MD, USA

Araguirang, G. E., & Richter, A. S. (2022). Activation of anthocyanin biosynthesis in high light–what is the initial signal? *New Phytologist*, *236*(6), 2037-2043

Batabyal, K. (2017). Nutrient management for improving crop, soil, and environmental quality. *Essential plant nutrients: uptake, use efficiency, and management*, 445-464.

Bălăiță, C., Ambăruș, S., Brezeanu, P. M., Calara, M., Avasiloaiei, D. I., & Drăghici, E. M. (2024). Preliminary studies on the influence of different substrates on the cultivation of peppers (*Capsicum annuum* L.). *Scientific Papers. Series B. Horticulture*, *68*(2

Bodner, G., Mentler, A., & Keiblinger, K. (2021). Plant roots for sustainable soil structure management in cropping systems. *The root systems in sustainable agricultural intensification*, 45-90.

Brezeanu, C., Brezeanu, P. M., Stoleru, V., Irimia, L. M., Lipșa, F. D., Teliban, G. C., ... & Murariu, O. C. (2022). Nutritional value of new sweet pepper genotypes grown in organic system. *Agriculture*, *12*(11), 1863.

Cabrera-De la Fuente, M., González-Morales, S., Juárez-Maldonado, A., Leija-Martínez, P., & Benavides-Mendoza, A. (2018). Plant nutrition and agronomic management to obtain crops with better nutritional and nutraceutical quality. In *Therapeutic foods* (pp. 99-140). Academic Press.

Cho, L. H., Yoon, J., & An, G. (2017). The control of flowering time by environmental factors. *The Plant Journal*, *90*(4), 708-719.

Elhawary, S. M. A., Ordóñez-Díaz, J. L., Nicolaie, F., Montenegro, J. C., Teliban, G. C., Cojocaru, A., ... & Stoleru, V. (2024). Quality Responses of Sweet Pepper Varieties Under Irrigation and Fertilization Regimes.

EL-Mogy, M. M., Adly, M. A., Shahein, M. M., Hassan, H. A., Mahmoud, S. O., & Abdeldaym, E. A. (2024). Integration of Biochar with Vermicompost and Compost Improves Agro-Physiological Properties and Nutritional Quality of Greenhouse Sweet Pepper. *Agronomy*, *14*(11), 2603.

Fan, Y., Hao, X., Carswell, A., Misselbrook, T., Ding, R., Li, S., & Kang, S. (2021). Inorganic nitrogen fertilizer and high N application rate promote N2O emission and suppress CH4 uptake in a rotational vegetable system. *Soil and Tillage Research*, *206*, 104848.

Heimler, D., Romani, A., & Ieri, F. (2017). Plant polyphenol content, soil fertilization and agricultural management: A review. *European Food Research and Technology*, *243*, 1107-1115.

Hur, G., Ahraf, M., Nadeem, M. Y., Rehman, R. S., Thwin, H. M., Shakoor, K., ... & Baozhong, Y. (2024). Exogenous Application of Wood Vinegar Improves Rice Yield and Quality by Elevating Photosynthetic Efficiency and Enhancing the Accumulation of Total Soluble Sugars. *Plant Physiology and Biochemistry*, 109306.

Iacomino, G., Idbella, M., Staropoli, A., Nanni, B., Bertoli, T., Vinale, F., & Bonanomi, G. (2024). Exploring the Potential of Wood Vinegar: Chemical Composition and Biological Effects on Crops and Pests. *Agronomy*, *14*(1), 114.

Jin, C. H. E. N., Pang, D. W., Min, J. I. N., Luo, Y. L., Li, H. Y., Yong, L. I., & Wang, Z. L. (2020). Improved soil characteristics in the deeper plough layer can increase grain yield of winter wheat. *Journal of Integrative Agriculture*, *19*(5), 1215-1226.

Kaur, S., Tiwari, V., Kumari, A., Chaudhary, E., Sharma, A., Ali, U., & Garg, M. (2023). Protective and defensive role of anthocyanins under plant abiotic and biotic stresses: An emerging application in sustainable agriculture. *Journal of Biotechnology*, *361*, 12-29.

Khaliq, H., Anwar, S., Shafiq, F., Ashraf, M., Zhang, L., Haider, I., & Khan, S. (2023). Interactive effects of soil and foliar-applied nanobiochar on growth, metabolites, and nutrient composition in Daucus carota. *Journal of Plant Growth Regulation*, *42*(6), 3715-3729.

Kobierski, M., Lemanowicz, J., Wojewódzki, P., & Kondratowicz-Maciejewska, K. (2020). The effect of organic and conventional farming systems with different tillage on soil properties and enzymatic activity. *Agronomy*, *10*(11), 1809.

Kutman, U. B. (2023). Mineral nutrition and crop quality. In *Marschner's Mineral Nutrition of Plants* (pp. 419-444). Academic Press.

Lei, Y., Xu, L., Wang, M., Sun, S., Yang, Y., & Xu, C. (2024). Effects of Biochar Application on Tomato Yield and Fruit Quality: A Meta-Analysis. *Sustainability*, *16*(15), 6397.

Li, Z., & Ahammed, G. J. (2023). Plant stress response and adaptation via anthocyanins: A review. *Plant Stress*, 100230.

Liu, C., Han, X., Chen, X., Yan, J., Lu, X., Song, B., ... & Ma, X. (2024). Inversion Tillage Combined with Organic Fertilizer Application Increased Maize Yield via Improving Soil Pore Structure and Enzymatic Activity in Haplic Chernozem. *Agronomy*, *14*(5), 927.

Lv, L., Gao, Z., Liao, K., Zhu, Q., & Zhu, J. (2023). Impact of conservation tillage on the distribution of soil nutrients with depth. *Soil and Tillage Research*, *225*, 105527.

Maraveas, C. (2019). Environmental sustainability of greenhouse covering materials. *Sustainability*, *11*(21), 6129.

Mefferd, A. (2019). *The organic no-till farming revolution: High-production methods for small-scale farmers*. New Society Publishers.

Mitchell, J. P., Reicosky, D. C., Kueneman, E. A., Fisher, J., & Beck, D. (2019). Conservation agriculture systems. *CABI Reviews*, (2019), 1-25.

Morugán-Coronado, A., Linares, C., Gómez-López, M. D., Faz, Á., & Zornoza, R. (2020). The impact of intercropping, tillage and fertilizer type on soil and crop yield in fruit orchards under Mediterranean conditions: A meta-analysis of field studies. *Agricultural Systems*, *178*, 102736.

Muscolo, A., Papalia, T., Mallamaci, C., Carabetta, S., Di Sanzo, R., & Russo, M. (2020). Effect of organic fertilizers on selected health beneficial bioactive compounds and aroma profile of red Topepo sweet pepper. *Foods*, *9*(9), 1323.

Raducanu D., Stefanescu I, Ifrim I, Patriciu O.I., Rați V. I., Prisecaru M., Stamate M., Lazar I. (2016). Effect of different sanitization treatments on soil characteristics, *Environmental Engineering and Management Journal*, Vol 15, No. 3, p. 624-634, ISSN: 1582-9596

Shah, F., & Wu, W. (2019). Soil and crop management strategies to ensure higher crop productivity within sustainable environments. *Sustainability*, *11*(5), 1485.

Simiele, M., Argentino, O., Baronti, S., Scippa, G. S., Chiatante, D., Terzaghi, M., & Montagnoli, A. (2022). Biochar enhances plant growth, fruit yield, and antioxidant content of cherry tomato (Solanum lycopersicum L.) in a soilless substrate. *Agriculture*, *12*(8), 1135.

Stagnari, F., Ficcadenti, N., Manetta, A. C., Platani, C., Dattoli, M. A., & Galieni, A. (2022). Modulation of light and nitrogen for quality-traits improvement: A case study of altino sweet pepper. *Horticulturae*, *8*(6), 499.

Stoleru, V., Mangalagiu, I., Amăriucăi-Mantu, D., Teliban, G. C., Cojocaru, A., Rusu, O. R., ... & Jităreanu, G. (2023). Enhancing the nutritional value of sweet pepper through sustainable fertilization management. *Frontiers in Nutrition*, *10*, 1264999.

Torotwa, I., Ding, Q., Awuah, E., & He, R. (2023). Biomimetic tool design improves tillage efficiency, seedbed quality, and straw incorporation during rototilling in conservation farming. *Journal of Agricultural Engineering*, *54*(1).

Wang, Z., Pan, X., Kuang, S., Chen, C., Wang, X., Xu, J., ... & Wang, X. (2022). Amelioration of coastal salt-affected soils with biochar, acid modified biochar and wood vinegar: enhanced nutrient availability and bacterial community modulation. *International Journal of Environmental Research and Public Health*, *19*(12), 7282.

Welch, R. M. (2024). Importance of seed mineral nutrient reserves in crop growth and development. In *Mineral Nutrition of Crops* (pp. 205-226). CRC Press.

Wolf, S. (2022). Cell wall signaling in plant development and defense. *Annual Review of Plant Biology*, *73*(1), 323-353.

Yan, W., Li, J., Lin, X., Wang, L., Yang, X., Xia, X., ... & Ke, Q. (2022). Changes in plant anthocyanin levels in response to abiotic stresses: a meta-analysis. *Plant Biotechnology Reports*, *16*(5), 497-508.

Zhao, X., Peng, J., Zhang, L., Yang, X., Qiu, Y., Cai, C., ... & Wang, Z. (2024). Optimizing the quality of horticultural crop: insights into pre-harvest practices in controlled environment agriculture. *Frontiers in Plant Science*, *15*, 1427471.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

MATHEMATICAL MODELING IN THE APPLICATION OF BIOFERTILIZERS IN BELL PEPPER CROPS

Claudia BĂLĂIȚĂ[1, 4], Mariana CALARA[1], Ionuț STOICA[2], Ana-Maria GEORGESCU[3], Dumitra RĂDUCANU[2], Elena Maria DRĂGHICI[4]

[1]Vegetable Research and Development Station Bacău, 220 Calea Bârladului Street, Bacău, Romania
[2]"Vasile Alecsandri" University of Bacău, Faculty of Science, Biology, 157 Calea Mărășești Street, Bacău, Romania
[3]"Vasile Alecsandri" University of Bacău, Faculty of Engineering, Department of Chemical and Food Engineering, 157 Calea Mărășești Street, Bacău, Romania
[4]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăști Blvd, District 1, Bucharest, Romania

Corresponding author emails: dora.raducanu@ub.ro, draghiciem@yahoo.com

Abstract

Mathematical modeling is a useful tool for assessing the potential risks in pepper crops. It has been demonstrated that biofertilizers can improve plant growth and development by mobilizing nutrients from the soil. The objective of this study is to analyze a mathematical model to assess the stimulatory capacity of a biofertilizer on the germination of bell pepper seeds following their wetting in solutions of varying concentrations of the product. Furthermore, the study employs collembola (Folsomia candida L.) to assess the ecotoxicity of the utilized product, thereby providing a multifaceted examination of soil health in relation to plant development and evolution under the influence of diverse substances. The population dynamics of F. candida serve as a barometer for this relationship, offering insights into the interactive dynamics between soil health, plant growth, and the impact of chemical substances.

Key words*: mathematical modeling; seed germination; biofertilizers; Folsomia candida; Capsicum annuum L.*

INTRODUCTION

In the context of Romanian agriculture, the cultivation of bell peppers (*Capsicum annuum* L.) holds particular significance, particularly within the southern regions of the country. This prominence can be attributed to the optimal soil and climatic conditions that prevail in these areas, resulting in a high demand from consumers and food processors (Barcanu-Tudor & Drăghici, 2018).

Peppers are a highly suitable crop for sunny, fertile, and irrigated vegetable land in our country, where they are predominantly cultivated in the field (Barcanu-Tudor & Drăghici, 2018). In response to the high demand and interest in year-round consumption, the area under peppers has expanded in recent years to include protected areas (greenhouses), as well as intensive and industrial systems.

Pepper crops demonstrate a reduced susceptibility to diseases and pests; however, they exhibit an increased vulnerability to soil contamination, which can impede germination (Iosob et al., 2019). The impact of this inhibition is amplified in soils that are already contaminated. The risk factors for pepper crops include the following: seedling depreciation (inappropriate age and density), the presence of diseases and/or pests, delayed cultivation due to the implementation of inappropriate technological measures, and climatic factors (Iosob et al., 2023).

The objective of this study is to assess the stimulatory capacity of a biofertilizer on the germination of pepper seeds, whilst concomitantly utilising collembola (*Folsomia candida*) to detect the ecotoxicity of the product. Germination rate and seed vigour are pivotal factors in determining the efficiency of seedling production, which is crucial for successful crop establishment (Larson et al., 2020). The time required for germination can vary significantly between different *C. annuum* L. seeds, with

some completing the process within a few days, while others requiring up to 21 days (Samarah et al., 2016). The seeds of bell pepper exhibit sensitivity to elevated temperatures and salt stress, which result in diminished seed physiological potential at temperatures of 20°C and 35°C and augmented NaCl concentrations (de Assis et al., 2025). Pepper seeds contain substantial amounts of both free and bound polyphenols, which undergo changes during the germination process. These compounds are utilised by the seeds to scavenge free radicals. These compounds may also function as signal compounds or be bound to plant defence, participating in induced systemic resistance (Ureche et al., 2021).

Biofertilizers are defined as substances containing naturally occurring biocompounds and micro-organisms. When applied to the seed or plant surface or soil, biofertilizers promote plant growth and development by increasing the supply or availability of primary nutrients to the host plant. The utilization of biofertilizers exerts a positive impact on both the environment and the crop by increasing plant productivity (Dobrin et al., 2019; Avasiloaiei et al., 2024).

These biofertilizers may comprise micro- or macro-organisms, plant residues, enzymes, or substances that promote plant growth by fixing atmospheric nitrogen, solubilizing phosphorus, or producing growth-promoting substances. They function as an environmentally friendly alternative to chemical fertilizers and play an important role in sustainable agriculture (Avasiloaiei et al., 2023).

Groundfix® (Figure 1) is a root biofertilizer that has been demonstrated to mobilize phosphorus and potassium from insoluble compounds, fix nitrogen, and increase the efficiency of mineral fertilizer use.



Figure 1. Groundfix® biofertilizer

The compound contains cells of the bacteria *Bacillus subtilis*, *Bacillus megaterium* var. *phosphaticum*, *Azotobacter chroococcum, Enterobacter* spp., *Paenibacillus polymyxa,* as well as beneficial bacteria (lactic acid bacteria, enzyme producers), vitamins, amino acids, and other physiologically active substances.

The OECD has issued a recommendation that all products utilized in agricultural settings, including biofertilizers, be subjected to a rigorous evaluation of their toxicity. This evaluation aims to ascertain their impact on the environment, particularly with regard to their effect on soil, plants, and even groundwater. In the context of organic agriculture, it has become imperative to undertake an ecotoxicological risk assessment of these substances on soil.

The utilization of bioindicators, such as the *F. candida*, as outlined in OECD 232, serves as a crucial element in the assessment of the ecotoxicity of products employed in pepper cultivation. This approach ensures the safety and efficacy of biofertilizers, while also offering a cost-effective alternative to more traditional methods. The benefits of this approach are evident, as it leads to a marked enhancement in the health of the soil, which, in turn, positively impacts the vitality of the plants and the effectiveness of the substances used.

MATERIALS AND METHODS

In this study, we utilized seeds of the bell pepper variety Dariana Bac, obtained from Vegetable Research and Development Station Bacau, as the biological material. This variety is characterized by an early maturation period, with a vegetation period of 120 days leading to the initial harvest. The plant exhibits average vigor and a 50% erect and 50% horizontal growth pattern.

The application of biofertilizers to seeds prior to planting has been demonstrated to facilitate the establishment of beneficial microorganisms during the early stages of plant development (Kuts et al., 2024, brochure Groundfix®)

The Groundfix® biofertilizer was applied to the pepper seeds in accordance with the mathematical model of type 2^3.

Factorial design

In this paper, the influence of two parameters for the germination of Dariana Bac bell pepper (*Capsicum annuum* L.) was investigated, which are expressed in terms of amount of fertilizer

(X_1) and wetting duration (X_2) upon the five response functions. The response functions investigated were: initial germination (Y_1), final germination (Y_2), mean germination time (Y_3), mean germination rate (Y_4) and germination speed (Y_5). The variation ranges are summarized in Table 1.

Table1. Parameters that influence the germination of bell pepper and their variation domain

Parameters (x_i)	Reduced variable	Minimal level (X_i^{min})	Median level (X_i^{med})	Maximal level (X_i^{max})	ΔX_i
Amount of fertilizer [mL]	x_1	2.5	3.75	5	1.25
Wetting duration [h]	x_2	1	2.5	4	1.5

The ecotoxicity of Groundfix® biofertiliser was the subject of an evaluation which employed the collembola, *F. candida*, as the test organism. This evaluation was conducted in accordance with the OECD 232 protocol.

F. candida is a common soil-dwelling organism found across the globe, playing a pivotal role in the decomposition process and nutrient cycling within terrestrial ecosystems (Calara et al., 2020; Martins et al., 2023).

Adult individuals of *F. candida*, maintained in the laboratory on activated carbon medium and fed yeast, were exposed to soil samples containing biofertilizer at the established concentrations in plastic containers (Figure 2) with lids and kept in the Sanyo chamber at constant temperature and humidity for the duration of the experiment.



Figure 2. Preparation of soil samples

In preparation for the experiment, soil samples were collected in plastic containers and stored in the Sanyo chamber set at 20-22°C. Prior to the introduction of the collembola, the soil samples were sterilized and saturated with distilled water. Following the addition of the biofertilizer, the samples were aerated every 48 hours and subjected to foliar feeding. The biofertilizer solutions employed in the experiment adhered to the maximum degradation time of the compound, not exceeding 4 hours until the moment of use, wetting the samples at a frequency of 24-48 hours, each 5 ml. The duration of the experiment was 28 days, during which the survival and reproduction of the collembola were evaluated.

RESULTS AND DISCUSSIONS

Design of experiments

The five response functions investigated were: initial and final germinations, mean germination time, mean germination rate and germination speed, which values are presented in Table 1. The reduced values of the variables are presented in parenthesis. Three other tests were also realized in the central point of the domain (0, 0) to calculate the significance of the program. The obtained values are shown in Table 2.

Table 2. Various germination parameters

Sample	Amount of fertilizer [mL]	Wetting duration [h]	Initial germination [mm]	Final germination [mm]	Mean germination time [h]	Mean germination rate [mm]	Germination speed [%]
	x_1	x_2	Y_1	Y_2	Y_3	Y_4	Y_5
1	2.5 (-1)	1 (-1)	64	90	5.52	0.18	18.25
2	2.5 (-1)	2.5 (0)	24	73.33	6.76	0.15	15.2
3	2.5 (-1)	4 (+1)	44.67	56	5.16	0.19	19.38
4	3.75 (0)	1 (-1)	36	56.67	5.42	0.18	18.44
5	3.75 (0)	2.5 (0)	33.33 (34.00; 34.56; 34.33)	76.00 (75; 77; 78)	5.89 (5.75; 5.92; 5.89)	0.17(0.17; 0.17; 0.18)	17.01 (17.05; 17.03; 17.01)
6	3.75 (0)	4 (+1)	20	49.33	6.57	0.16	15.53
7	5 (+1)	1 (-1)	72	95.33	5.25	0.19	19.05
8	5 (+1)	2.5 (0)	26.7	61.33	7.71	0.13	13.05
9	5 (+1)	4 (+1)	30.67	82.67	6.29	0.16	16.13

Elaboration of the mathematical model

The particular form of response function for factorial program of 3^2 types is:

$$Y = a_o + a_1 \cdot x_1 + a_2 \cdot x_2 + a_{12} \cdot x_1 \cdot x_2 + a_{11} \cdot x_1^2 + a_{22} \cdot x_2^2 \quad (1)$$

In Table 3 are presented the coefficients values of polynomial mathematical models, which were calculated according to the literature specifications (Lazic, 2004).

Table 3. Values of the polynomial coefficients

Coefficients	Coefficient value for Y_1	Coefficient value for Y_2	Coefficient value for Y_3	Coefficient value for Y_4	Coefficient value for Y_5
a_0	18.75	59.71	6.68	0.152	15.18
a_1	-0.55	3.33	0.30	-0.007	-0.76
a_2	-12.78	-9	0.31	-0.008	-0.78
a_{12}	-3.67	3.56	0.23	-0.007	-0.67
a_{11}	13.90	15.78	0.15	-0.002	-0.15
a_{22}	16.55	1.44	-1.08	0.027	2.71

According to the polynomial coefficients, the mathematical models which describe the response functions of the optimizing criterion are:

$$Y_1 = 18.75 - 0.55 \cdot x_1 - 12.78 \cdot x_2 - 3.67 \cdot x_1 \cdot x_2 + 13.90 \cdot x_1^2 + 16.55 \cdot x_2^2 \quad (2)$$

$$Y_2 = 59.71 + 3.33 \cdot x_1 - 9 \cdot x_2 + 3.56 \cdot x_1 \cdot x_2 + 15.78 \cdot x_1^2 + 1.44 \cdot x_2^2 \quad (3)$$

$$Y_3 = 6.68 + 0.3 \cdot x_1 + 0.31 \cdot x_2 + 0.23 \cdot x_1 \cdot x_2 + 0.15 \cdot x_1^2 - 1.08 \cdot x_2^2 \quad (4)$$

$$Y_4 = 0.152 - 0.007 \cdot x_1 - 0.008 \cdot x_2 - 0.007 \cdot x_1 \cdot x_2 - 0.002 \cdot x_1^2 + 0.027 \cdot x_2^2 \quad (5)$$

$$Y_5 = 15.18 - 0.76 \cdot x_1 - 0.78 \cdot x_2 - 0.67 \cdot x_1 \cdot x_2 - 0.15 \cdot x_1^2 + 2.71 \cdot x_2^2 \quad (6)$$

Determination of the coefficients' significance

In order to determine the significance of the polynomial coefficients of the five response functions, the *t - student* test was used. The average values of the response function and the average values of the measurement errors were calculated according to the algorithm presented in the literature (Georgescu et al., 2013; Georgescu et al., 2017; Georgescu et al., 2023). The *t-student* test results for the five response functions are presented in Table 4.

Table 4. *T*-student test results

t_j	t_0	t_1	t_2	t_{12}	t_{11}	t_{22}
Values for Y_1	104.04	-3.05	-70.91	-20.35	77.11	91.81
Values for Y_2	918.52	51.28	-138.46	54.70	242.73	22.22
Values for Y_3	102.82	4.62	4.69	3.58	2.37	-16.68
Values for Y_4	2.34	-0.11	-0.12	-0.1	-0.02	0.41
Values for Y_5	233.63	-11.78	-12.06	-10.37	-2.31	41.75

According to *t-student* test results, the individual coefficient (a_1) has to be eliminated in the case of mathematical model which describes Y_1 response function and in the case of mathematical model of Y_5, the quadratic term coefficient (a_{22}) has to be eliminated.

The effects simulation in the optimization study involves analyzing both the absolute value and the sign of each coefficient individually.

In the case of the first response function, the a_o value indicates that the optimal initial germination is close to 18.75 mm. Although this value is not the maximum value obtained in the experimental program, it can be concluded that the optimal initial germination is not the one with the highest value. The individual coefficients (a_1 and a_2) are negative, so the x_1 and x_2 variables have an unfavorable individual effect on the germination process. The individual effect of the x_{12} term is negative, so the x_1 and x_2 variables have an unfavorable effect on the germination process. Analyzing the quadratic term coefficients (a_{11} and a_{22}) which values are positive, it can be said that the response function (initial germination) is characterized by a minimum in relation to variables x_1 and x_2, having approximately the same curvature in relation to variable x_1 and variable x_2, because they have close values.

In the case of the second response function, the value of a_0 (59.71) indicates that we have an optimal final germination at a value close to this value. The coefficient for simple effects a_1 is positive, so that variable x_1 has an individual favorable action to the process. On the other hand, the coefficient for simple effects a_2 being negative, variable x_2 has an unfavorable influence on the process. Since the interaction coefficient a_{12} is positive (3.56), it can be concluded that the variables x_1 and x_2, through their interaction, have a favorable effect on the process. This effect has approximately the same intensity as the individual action of the variable x_1. Since the quadratic coefficients a_{11} and a_{22} have the same sign, it results that the response function is characterized by a minimum, having greater curvature in relation to the variable x_1, compared to the variable x_2.

Regarding the simulation of the effects in the case of the response function Y_3 (mean germination time), the value of a_0 indicates that we have an optimal mean germination time at a value close to 6.68 h. The coefficients for simple effects (a_1 and a_2) being positive, it can be said that the amount of fertilizer and the wetting duration have individual favorable actions to the process. The interaction coefficient a_{12} is positive (0.23), so the variables x_1 and x_2,

through their interaction, have a favorable effect on the process. This effect has approximately the same intensity as the individual action of the variable x_1 or of variable x_2, since the values are close. Analyzing the quadratic term coefficients (a_{11} and a_{22}) which values are positive, respectively negative, it can be said that the response function (mean germination time) is characterized by a minimum in relation to variable x_1 and by a maximum in relation to variable x_2. These coefficients having opposite signs, it results that the response function is characterized by an inflection point, the graphic representation being in the form of a saddle.

In the case of the fourth response function, the value of a_o indicates that we have an optimal mean germination rate at a value close to 0.152 h. The coefficients for simple effects (a_1 and a_2) being negative, it can be said that the amount of fertilizer and the wetting duration have individual unfavorable actions to the process. The interaction coefficient a_{12} is negative (-0.007), so the variables x_1 and x_2, through their interaction, have an unfavorable effect on the process. This effect has approximately the same intensity as the individual action of the variable x_1 or of variable x_2, since the values are approximately the same. Analyzing the quadratic term coefficients (a_{11} and a_{22}) which values are negative, respectively positive, it can be said that the response function (mean germination rate) is characterized by a maximum in relation to variable x_1 and by a minimum in relation to variable x_2. These coefficients having opposite signs, it results that the response function is characterized by an inflection point, the graphic representation being in the form of a saddle.

In the case of the last response function (germination speed), the value of a_0 indicates that we have an optimal mean germination rate at a value close to 0.152 h. The coefficients for simple effects (a_1 and a_2) being negative, it can be said that the amount of fertilizer and the wetting duration have individual unfavorable actions to the process. The interaction coefficient a_{12} is negative (-0.007), so the variables x_1 and x_2, through their interaction, have an unfavorable effect on the process. This effect has approximately the same intensity as the individual action of the variable x_1 or of variable x_2, since the values are approximately the same. Analyzing the quadratic term coefficients (a_{11} and a_{22}) which values are negative, respectively positive, it can be said that the response function (mean germination rate) is characterized by a maximum in relation to variable x_1 and by a minimum in relation to variable x_2. These coefficients having opposite signs, it results that the response function is characterized by an inflection point, the graphic representation being in the form of a saddle. For response functions obtained, the partial derivatives of first order were calculated, in rapport with each variable.

In the case of the first response function (Y_1), by equating the partial derivatives of first order with 0, the linear system resulted was resolved. The optimal point searched was (0.052; 0.392), represented in dimensionless coordinates. It can be seen, that the optimal values for x_1 and x_2 are within the limits of the domain (-1, 1) which initially were supposed. The real values of the optimal conditions for bell pepper germination process were obtained for an amount of fertilizer of 3.82 mL and a wetting duration of 3.09 h.

In the case of the second response function (final germination), the optimal point searched was (0.532; 3.78), represented in dimensionless coordinates. It can be seen, that the optimal value for x_1 is within the limits of the domain (-1, 1) which initially were supposed, but in the case of optimal value of x_2 it isn't. The real values of the optimal conditions for bell pepper germination process were obtained for an amount of fertilizer of 4.42 mL and a wetting duration of 8.17 h. The results obtained in mathematical modeling are in accordance with literature (Ciofu et al., 2019) because the seeds can be damaged during a longer soaking time. In the case of the third response function (Y_3), the optimal point searched was (-1.02; 0.034), represented in dimensionless coordinates. It can be seen, that the optimal values for x_1 and x_2 reflect the gentle action of the Groundfix® biofertilizer. The real values of the optimal conditions for bell pepper germination process were: 2.48 mL and 2.55 h.

In the case of mean germination time response function (Y_4), the optimal point searched was (-1.63; -0.06), represented in dimensionless coordinates. It can be seen, that the optimal values for x1 and x2 reflect the gentle action of the Groundfix® biofertiliser. The real values of

the optimal conditions for bell pepper germination process were: 1.71 mL and 2.41 h. In the case of germination speed, the optimal point searched was (-1.16; -0.61), represented in dimensionless coordinates. It can be seen, that the optimal values for x_1 and x_2 reflect again the gentle action of the Groundfix® biofertiliser. The real values of the optimal conditions for bell pepper germination process were: 2.3 mL and 1.59 h.

In this study, the initial and final germinations, mean germination time, average germination rate and germination speed, were established according to the two factors, amount of fertilizer and wetting duration for the germination of Dariana Bac bell pepper, dependence that can be illustrated using the curves presented in Figures 3-7.

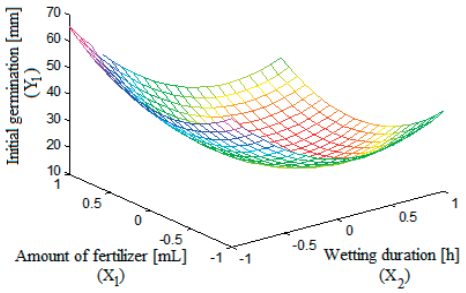


Figure 3. Influence of amount of fertilizer (X_1) and wetting duration (X_2) on initial germination (Y_1)

$$Y_1 = 18.75 - 12.78 \cdot x_2 - 3.67 \cdot x_1 \cdot x_2 + 13.9 \cdot x_1^2 + 16.55 \cdot x_2^2$$

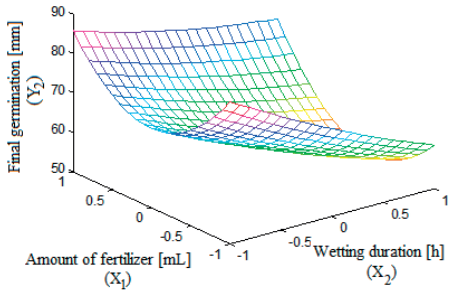


Figure 4. Influence of amount of fertilizer (X_1) and wetting duration (X_2) on final germination (Y_2)

$$Y_2 = 59.71 + 3.33 \cdot x_1 - 9 \cdot x_2 + 3.56 \cdot x_1 \cdot x_2 + 15.78 \cdot x_1^2 + 1.44 \cdot x_2^2$$

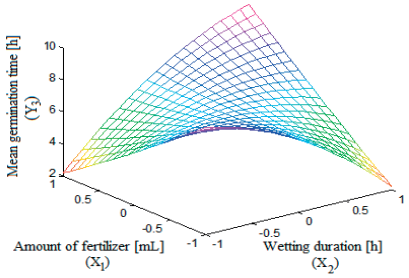


Figure 5. Influence of amount of fertilizer (X_1) and wetting duration (X_2) on mean germination time (Y_3)

$$Y_3 = 6.68 + 0.3 \cdot x_1 + 0.31 \cdot x_2 + 0.23 \cdot x_1 \cdot x_2 + 0.15 \cdot x_1^2 - 1.08 \cdot x_2^2$$

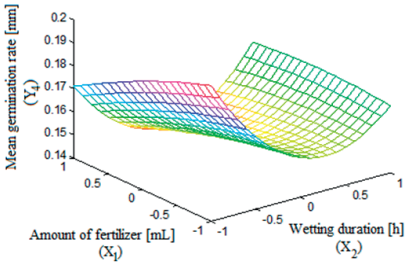


Figure 6. Influence of amount of fertilizer (X_1) and wetting duration (X_2) on mean germination rate (Y_4)

$$Y_4 = 0.152 - 0.007 \cdot x_1 - 0.008 \cdot x_2 - 0.007 \cdot x_1 \cdot x_2 - 0.002 \cdot x_1^2 + 0.027 \cdot x_2^2$$

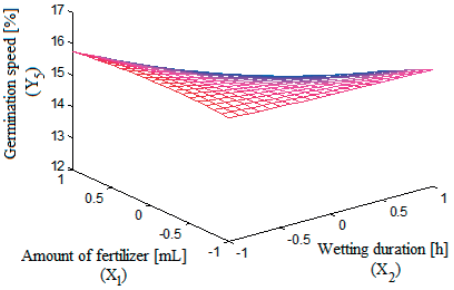


Figure 7. Influence of amount of fertilizer (X_1) and wetting duration (X_2) on germination speed (Y_5)

$$Y_5 = 15.18 - 0.76 \cdot x_1 - 0.78 \cdot x_2 - 0.67 \cdot x_1 \cdot x_2 - 0.15 \cdot x_1^2$$

Mathematical modeling highlighted the optimal results of the parameters analyzed in the relationship between bell pepper and the Groundfix® biofertiliser.

The utilization of *F. candida* to evaluate the ecotoxicity of Groundfix® biofertiliser in pepper crops is indicative of the health status of the soil in relation to the plant and the fertilizers employed. The alterations in the collembola population substantiate this assertion. The findings of our study demonstrated a favourable survival rate of colembolla following the incorporation of 3.8 mL/L of biofertilizer. From the number of experimental variants analysed (Figure 8), the number of adults of *F. candida* suffered a significant decrease at 5 mL/L Groundfix® concentration (P7, P8, P9).

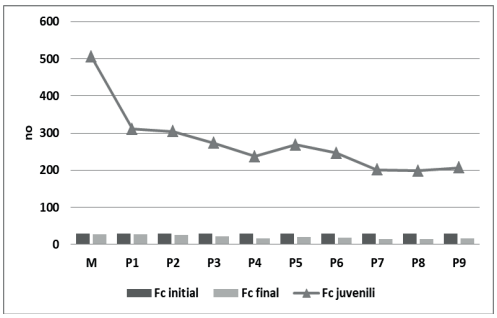


Figure 8. Dynamics of the number of *Folsomia candida* to biofertilizer action

The number of juveniles reflects a low level of toxicity of the product compared to the control: 41.40% at a concentration of 2.5 mL/L (P1, P2, P3), 50.43% at a concentration of 3.8 mL/L (P4, P5, P6) and 60% at a concentration of 5 mL/L (P7, P8, P9) biofertilizer.

CONCLUSIONS

The optimized values obtained from the experimental design are in agreement with the values of parameters initially explored, with the exception of final germination of bell pepper. Following mathematical calculations, a germination time value of approximately 8 h was obtained for a quantity of almost 5 mL of biofertilizer (Y_2 response function), which is in accordance with the maximum quantity recommended by the manufacturer for use.

The fact that the final germination and the average germination rate are within the limits imposed by the factorial program (2.5-5 mL biofertilizer), shows that the bell pepper seeds used in this research have good germination capacity.

From the analysis of the data obtained from the modeling and those observed from the behavior of *Folsomia* in relation to the biofertilizer Groundfix®, its efficiency in bell pepper cultivation since germination is justified.

As a perspective, we recommend the using of mathematical modeling also for other categories of substances allowed in organic agriculture.

ACKNOWLEDGEMENTS

This research work was carried out with the support of Projects ADER 6.3.6, ADER 6.3.20 and for the doctoral thesis entitled "Contributions to the improvement of technological links in the cultivation of peppers in protected spaces on various substrates".

REFERENCES

Avasiloaiei, D. I., Brezeanu, C., Bălăiță, C., Antal-Tremurici, A., & Calara, M. (2024, May). Evaluating the efficiency of using biostimulants for sweet pepper seedlings production (Capsicum annuum L. var. grossum) in an organic system. In *EHC2024: International Symposium on Sustainable Vegetable Production from Seed to Health Booster Sources 1416* (pp. 149-156).

Avasiloaiei, D. I., Calara, M., Brezeanu, M. P., & Cristea, T. O. (2023). Review on the sustainability of some regenerative agriculture practices for organic vegetable growing. *Scientific Papers. Series B. Horticulture,* 67(2).

Barcanu-Tudor, E., Draghici, E. M. (2018). New bred cultivars of bell pepper obtained at VRDS BUZAU, *Scientific Papers-Series B-Horticulture* 62, 445-449. Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286 -1580, ISSN-L 2285-5653

Calara, M., Benchea, C., Raţi, I. V., & Raducanu, D. (2020). Ecotoxicity study on the influence of glyphosate upon the *T. estivum* L. Delabrad 2 cultivar (OECD 208) and F. candida (OECD 232). *Scientific Studies & Research. Series Biology/Studii si Cercetari Stiintifice. Seria Biologie*, *29*(2).

Ciofu Ruxandra., Stan N., Popescu V., Chilom Pelaghia., Apahidean S. Al., Horgoș A., Berar V., Lauer K. F., Atanasiu N. (2004). *Tratat de legumicultură.* Editura Ceres, Bucureşti

de Assis, V. C. S. S., de Oliveira, J. A., Arruda, N., Benett, C. G. S., & Benett, K. S. S. (2025). Physiological potential of bell pepper seeds under heat and salt stress. *Revista de Agricultura Neotropical*, *12*(1).

Dobrin, A., Nedelus, A., Bujor, O., Mot, A., Zugravu, M., & Badulescu, L. (2019). Nutritional quality parameters of the fresh red tomato varieties cultivated in organic system. *Scientific Papers. Series B. Horticulture*, *63*(1), 439-443.

Georgescu, A., Brabie, G., Nistor, I., Nardou, F., Penot, C, (2013). Utilization of experimental design for

specific surface area optimization of a pillared bentonite. *Food and Environment Safety Journal,* 12(4); http://fens.usv.ro/index.php/FENS/ article/ view/179.

Georgescu A.-M., Muntianu G., Nistor I. D., Nardou F. (2017) - Modeling and optimization of Al-pillaring process using experimental design procedure, *Journal of Agroalimentary Processes and Technologies*, 23(4), https://www.journal-of-agroalimentary.ro.

Georgescu A.-M., Ursachi C., Ungurean C.V., Răducanu D. (2023) - Phytotoxic effects of a common laundry detergent on lettuce (*Lactuca sativa* L.) using the mathematical modelling of factorial type, *Scientific Study & Research – Biology,* ISSN 1224-919X, 2023, Volume 32, no.1, p. 37-41

Iosob, G. A., Cristea, T. O., Antal-Tremurici, A., Calara, M., & Benchea, C. (2023, September). Disease and pest management for solanaceous vegetables in the NE region of Romania. In *IX South-Eastern Europe Symposium on Vegetables and Potatoes 1391* (pp. 357-364).

Iosob, G. A., Nedeff, V., Sandu, I., Prisecaru, M., & Cristea, T. O. (2019). Study of phytotoxic effects of Cu2+ and Cd2+ on seed germination and chlorophyll pigments content to the bell pepper. *Rev. Chim. (Bucharest)*, *70*, 1416.

Kuts, O., Kokoiko, V., Semenenko, S., Semenenko I., & Romanov, V. (2024). The impact of biologically enhanced fertilisation systems on bell potato (Ipomoea batatas) productivity and quality. *Scientific Horizons*, 27(9), 64-72. doi: 10.48077/scihor9.2024.64.

Lazic Z. (2004). Design of experiments in chemical engineering. Wiley-VCH, Verlag GmbH & Co KGaA, New York, pp. 157-170.

Martins, M. R., Zanatta, M. C. K., & Pires, M. S. G. (2023). Sustainable agricultural use of sewage sludge: impacts of high Zn concentration on on *Folsomia candida*, *Enchytraeus crypticus*, *Lactuca sativa*, and *Phaseolus vulgaris*. *Environmental monitoring and assessment*, *195*(3), 359.

Samarah, N. H., Wang, H., & Welbaum, G. E. (2016). Pepper (*Capsicum annuum*) seed germination and vigour following nanochitin, chitosan or hydropriming treatments. *Seed Science and Technology*, *44*(3), 609-623.

Ureche, M. A. L., Pérez-Rodriguez, M. M., Ortiz, R., Monasterio, R. P., & Cohen, A. C. (2021). Rhizobacteria improve the germination and modify the phenolic compound profile of pepper (*Capsicum annum* L.). *Rhizosphere*, *18*, 100334.

https://www.oecd.org/chemicalsafety/risk-assessment/1948450.pdf (accessed on 15.11.2024).

https://btu-center.com/en/groundfix - brochure (accessed on 10.10.2024).

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

THE RESPONSE OF SOME CHERRY TOMATO HYBRIDS TO FOLIAR FERTILIZATION

Alexandra BECHERESCU[1], Gheorghița HOZA[2], Florin SALA[1, 3]

[1]University of Life Sciences "King Mihai I" from Timişoara, 119 Calea Aradului Street, 300645, Timișoara, Romania
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăști Blvd, District 1, Bucharest, Romania
[3]Agricultural Research and Development Station Lovrin, 200 Principală Street, Lovrin, 307250, Timiş County, Romania

Corresponding author email: florin_sala@usvt.ro

Abstract

This study evaluated the response of some cherry tomato hybrids to foliar fertilizers. The experiment was carried out in protected space conditions (solar tunnel). As biological material were used four hybrids (factor A): Arielle F1 (a1), Ravello F1 (a2), Black Cherry F1 (a3) and Vespolino F1 (a4). The foliar treatments (factor B) were: unfertilized (b1), Kerafol Evo (b2), Plantfert U (b3). From the combination of factors, 12 experimental variants resulted. Physiological indices, productivity elements and yield were analyzed in relation to the experimental variants. In unfertilized conditions (b1), the yield (Y, kg plr^{-1}) recorded differentiated values in relation to the potential of the hybrids, Y = 1.416 kg plr^{-1} (a1), Y = 1.632 kg plr^{-1} (a2), Y = 1.668 kg plr^{-1} (a3) respectively Y = 1.890 kg plr^{-1} (a4). Through foliar treatments, the performance of the hybrids increased, but the Vespolino F1 hybrid (a4) remained with the highest yield, Y = 2.538 kg plr^{-1} (b3). The foliar fertilizer Plantfert U (b3) ensured the highest increase in yield (ΔY) in all the tested hybrids. Mathematical models described yield variation in relation to physiological indices and productivity elements.

Key words: *cherry tomatoes, foliar fertilizers, mathematical model, physiological indices, productivity elements, yield.*

INTRODUCTION

Tomato (*Solanum lycopersicum* L.), *Solanaceae* Family, is a plant with high ecological plasticity, which is suitable for different crop systems, and is one of the most important plants in the vegetable category (Maboko and Du Plooy, 2017; Kai et al., 2020; Arshad et al., 2023; Aydi et al., 2023; Tsouvaltzis et al., 2023).

Tomato is an important crop plant, for fresh consumption and for industrialization, with high nutritional and economic value (Hita et al., 2007; Chapagain and Orr, 2009; Jerca and Smedescu, 2023; Nie et al., 2023; Petek et al., 2024).

Cherry tomatoes are highly popular worldwide, especially in modern markets, and attract different categories of consumers, through the variety of shapes, colors, aromas, attractive taste, content of nutrients and active principles and high flexibility to be integrated into different culinary menus (Hita et al., 2007; Kusumiyati et al., 2023; Chang et al., 2024).

Different cherry tomato genotypes were evaluated based on agronomic characters, physiological indices, productivity elements, yield, and fruit quality indices (Aguirre and Cabrera, 2012; Kusumiyati et al., 2023).

Tomato fruit quality was studied in relation to the position of the fruit on the plant and in the cluster, the harvest period, the duration and method of storage, as well as the interactions of these factors (Tsouvaltzis et al., 2023).

Cherry tomatoes involve considerably higher inputs than other crops (e.g. fertilizers, pesticides, irrigation water), which require optimization for crop profitability but also to reduce environmental risks (Guo et al., 2021; Ahmad et al., 2023).

Yield and certain quality indices were studied in cherry tomato genotypes in response to different types of organic fertilization (Murtic et al., 2018; Irfanulden Abdulhadı Qahraman et al., 2020). Duffaut et al. (2023) recorded a positive response in cherry tomatoes by fertilization with pigeon guano, under urban farming conditions.

The influence of different chemical and organic fertilizer resources, used in simple or combined fertilizations, was evaluated in cherry tomatoes, based on agronomic (vegetative) parameters, yield, and fruit quality indices (Kai et al., 2020; Stoleru et al., 2020; Tao et al., 2022; Badea et al., 2023).

Vegetative growth parameters, yield and quality indices of cherry tomatoes were quantified in relation to mixed fertilization (Rashid et al., 2022). Cherry tomatoes significantly increased yield with different nutrients applied through fertilization (Nie et al., 2023).

Agronomic characteristics of plants and fruits, yield and fruit quality indices of cherry tomatoes (popular local landraces) were evaluated in relation to different fertilization options (Nie et al., 2023).

The differential response of some cherry tomato hybrids, depending on the potential of the genotypes, was recorded in relation to the foliar application of NPK complex fertilizers (Hussein and Al-Tufaili, 2023).

The influence of fertilizers in conventional fertilization systems as well as in new, promising technological variants (e.g. associated with hydrogen gas) was evaluated in cherry tomatoes, with cost-effective effects on physiological indices, agronomic and fruit quality parameters and yield (Li et al., 2024).

This study evaluated the response of four cherry tomato hybrids, in relation to two foliar fertilizers, based on morphological parameters, productivity and yield elements, in a solar-type protected space cultivation system.

MATERIALS AND METHODS

The study was organized and conducted in the southern area of Timisoara Municipality, on a family farm, in the 2019 agricultural year. The experiment was located in a protected, solar-type space.

The study evaluated morphological parameters, productivity elements and yield of four cherry tomato hybrids, under the influence of foliar fertilization.

The biological material was represented by four cherry tomato hybrids (factor A): a1 – Arielle F1; a2 – Ravello F1; a3 – Black Cherry F1; a4 – Vespolino F1.

Foliar fertilization was provided with two fertilizers (factor B): b1 – Mt – unfertilized; b2 – Kerafol Evo (2-3 l ha^{-1}); b3 – Plantfert-U (1-2%). The combination of the two factors resulted in 12 experimental variants (a1b1, to a4b3), placed in repetitions.

The crop technology ensured uniform conditions for the experiment. For each genotype, the culture was established with 60-day-old seedlings on April 15. The planting distance was 80 cm between rows and 45 cm between plants per row. This resulted in a nutrient space of 0.36 m^2/plant, a plant density of 2.5 plants/m^2 (25,000 plants ha^{-1}). Basic fertilization was done by uniformly applying and incorporating Cropcare and Ferticare fertilizers, into the soil.

Morphological parameters, productivity elements and yield were determined for each experimental variant: plant height (PH, cm), cluster number on plants (CnP), flower number in cluster (FlnC), fecundated flower number in cluster (FFlnC), flower number on plant (FlnP), fecundated flower number on plant (FFlnP), fruits number in cluster (FrnC), mean fruit weight (FrW), fruit weight in cluster (FrCW), fruits number on plant (FrnP), yield on plant (YP, kg plt^{-1}).

The experimental results have been adequately analyzed by Anova Test, multivariate analysis, correlation analysis, and simple and quadratic regression analysis. The experimental results were analyzed in EXCEL, PAST and Wolfram Alpha (Hammer et al, 2001; Wolfram, 2020).

RESULTS AND DISCUSSIONS

The study analyzed the genetic potential of four cherry tomato hybrids, expressed in the conditions of the control variants (b1 – Ct – unfertilized), and the ability of the hybrids to capitalize on the applied fertilizations (b2, b3) through the crop technology.

The values of the morphological parameters, of the productivity and yield elements recorded presented in tabular format (Table 1).

The Anova test results confirmed the existence of variance and the statistical reliability of the experimental results ($F>Fcrit$; $p<0.001$).

The specific response of each hybrid for morphological parameters, productivity and yield elements was recorded, according to the values presented in Table 1.

Table 1. Mean values of cherry tomato parameters in relation to foliar fertilizers

Trial	Plant height	Cluster number on plant	Flower number on cluster	Fecunded flower number in cluster	Flower number on plant	Fecunded flower number on plant	Fruit number on cluster	Fruit weight	Fruit cluster weight	Fruit number on plant	Yield on plant
	PH	CnP	FlnC	FFlnC	FlnP	FFlnP	FrnC	FrW	FrWC	FrnP	YP
	(cm)	(no)						(g)		(no)	(kg)
a1b1	179.30	6.10	15.60	13.90	97.80	87.40	13.90	16.20	225.18	87.40	1.42
a1b2	184.70	6.10	16.50	15.20	103.20	95.20	15.20	18.50	281.20	95.20	1.76
a1b3	195.70	6.00	16.40	15.20	101.10	93.90	15.20	19.60	297.92	93.90	1.84
a2b1	187.40	6.40	15.20	13.40	99.30	88.20	13.40	18.50	247.90	88.20	1.63
a2b2	189.70	6.90	15.20	13.80	106.90	97.50	13.80	21.00	289.80	97.50	2.05
a2b3	190.50	6.70	15.30	14.20	105.20	98.30	14.20	22.10	313.82	98.30	2.17
a3b1	190.40	5.40	17.70	16.00	98.30	89.20	16.00	18.70	299.20	89.20	1.67
a3b2	194.80	5.70	17.80	16.40	104.20	95.90	16.40	21.90	359.16	95.90	*2.10*
a3b3	198.70	5.80	17.50	16.50	104.20	98.50	16.50	22.70	374.55	98.50	2.24
a4b1	182.80	5.80	15.80	14.70	94.20	87.50	14.70	21.60	317.52	87.50	1.89
a4b2	188.40	6.60	16.00	15.20	108.30	103.20	15.20	23.00	349.60	103.20	*2.37*
a4b3	189.70	6.50	16.10	15.50	107.60	103.60	15.50	24.50	379.75	103.60	2.54
SE	±1.58	±0.13	±0.27	±0.30	±1.27	±1.67	±0.30	±0.69	±13.99	±1.67	±0.10

The interdependence between the representative elements considered in the study, in the evaluation of the four cherry tomato hybrids, was evaluated through correlation analysis. The correlation matrix in Figure 1 resulted, which presents the interdependence level at the level of the considered parameters.

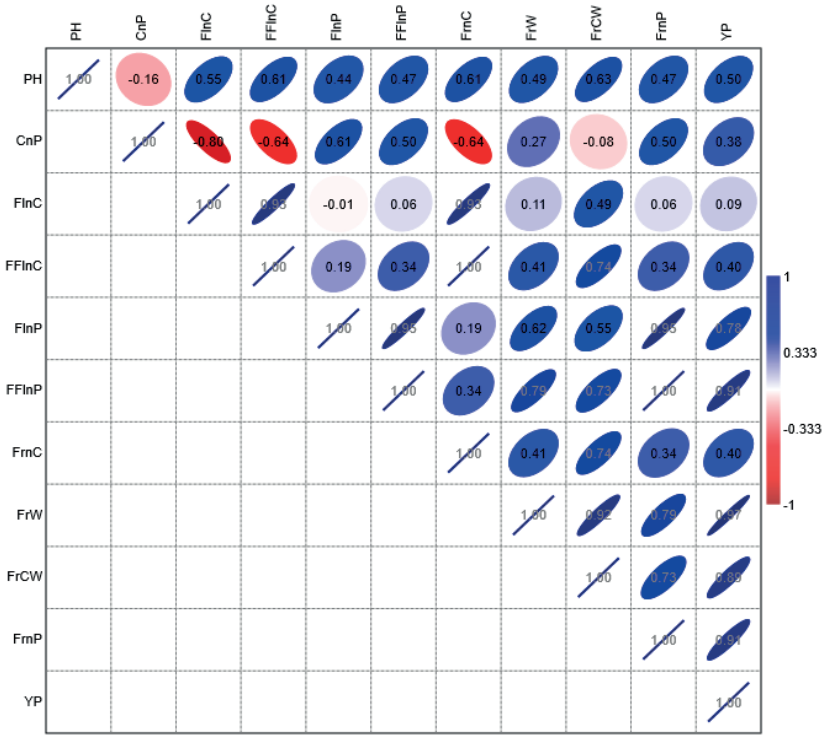


Figure 1. Correlation level between determined parameters in cherry tomatoes

Positive and negative correlations were recorded, with varying levels of intensity. Yield (YP) showed moderate level of correlation with FlnP (r = 0.78), strong correlation with FrCW (r = 0.89), and very strong correlation with FFlnP (r = 0.91), FrnP (r = 0.91), and FrW (r = 0.97).

The multivariate analysis was performed based on morphological parameters, and on productivity and yield elements.

In relation to morphological parameters, the main components explained 97.963% of variance, according to the diagram in Figure 2. Associated with FlnC and FFlnC parameters, the variants a3b2 and a3b3 were positioned. Associated with FlnP and FFlnP parameters, the variants a4b2 and a4b3 were positioned. Opposite positioning was presented by the variants a1b1, a2b1 and a4b1.

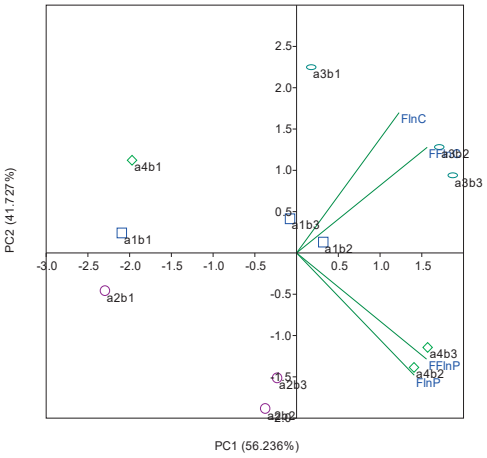


Figure 2. PCA diagram based on morphological parameters in cherry tomatoes in relation to foliar fertilization

In relation to the productivity and yield elements, the principal components explained 95.091% of variance, according to the diagram in Figure 3. Associated with FrnC and FrCW, the variants a3b2 and a3b3 were positioned. Associated with FrW, FrnP, and YP parameters, some variants were positioned (a4b2; a4b3; a2b3; a2b2). The other variants presented independent positions.

Based on the main productivity parameters (FrW, FrCW, YP) a cluster analysis was performed. The dendrogram in Figure 4 resulted (Coph.corr. = 0.772).

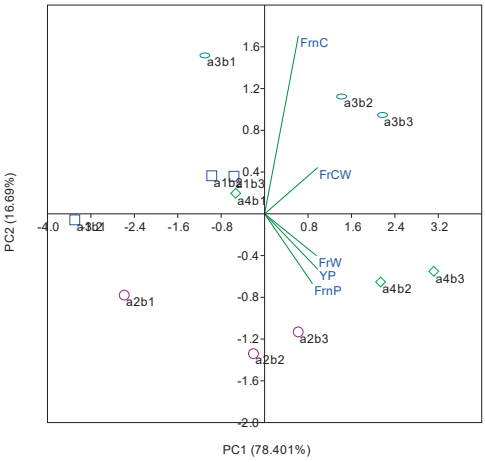


Figure 3. PCA diagram based on productivity elements and yield in cherry tomatoes in relation to foliar fertilization

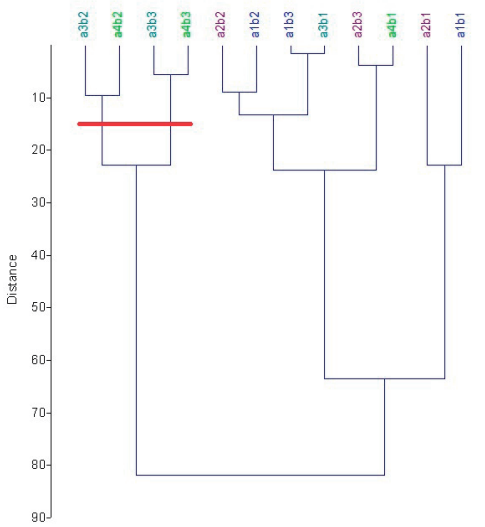


Figure 4. Clustering dendrogram of cherry tomato variants

The variants were associated in three distinct subclusters. Cluster C1 (marked with a red line in the dendrogram) included four variants with high values of the considered parameters.

The hierarchy of the variants was performed based on FrW, FrCW and YP and the ranking diagram in Figure 5 resulted.

In horticultural production systems, in tomato crops, yield is an important indicator, along with fruits quality indices.

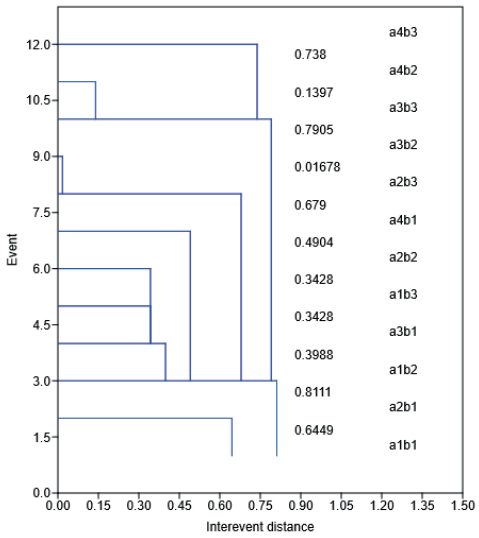


Figure 5. Ranking of variants based on productivity elements and yield

The yield is based on fruits (number, weight), and the number of fruits depends on the number of fecunded flowers. Under the study conditions, under the influence of foliar fertilization, the variation of FFlnC in relation to FlnC (variable x in the equation) was described by equation (1), with $R^2 = 0.914$, $p<0.001$ (Figure 6).

$$FFlnC = -0.3078x^2 + 11.16x - 84.89 \quad (1)$$

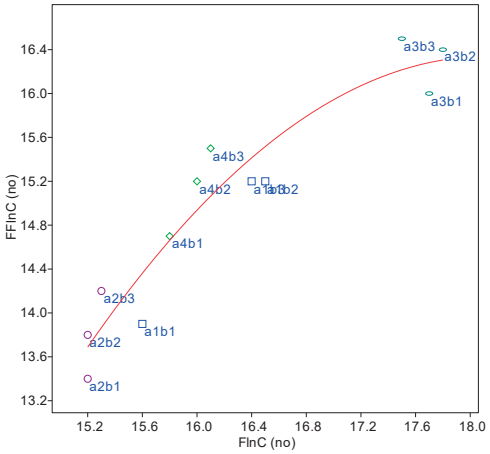


Figure 6. Variation of FFlnC in relation to FlnC in cherry tomato hybrids under the influence of foliar treatments

The yield on plant (YP) was analyzed in direct relation to the productivity elements in cherry tomato crops, to find out the level of contribution of each parameter in the formation of the yield (YP).

Equation (2) described the variation of YP in relation to FrnC, with $R^2 = 0.193$, $p=0.381$, $F=1.0755$.

$$YP = -0.0612x^2 + 1.964x - 13.66 \quad (2)$$

Equation (3), with the graphical distribution in Figure 7, described the variation of YP in relation to FrW, $R^2 = 0.952$, $p<0.001$, $F = 90.127$.

$$YP = 0.055489x^2 - 0.09005x + 1.458 \quad (3)$$

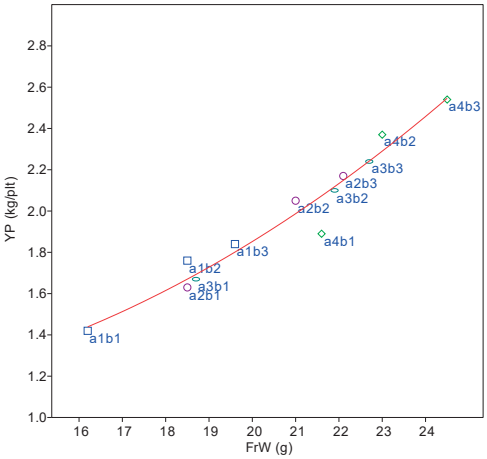


Figure 7. Graphical distribution of YP in relation to FrW in cherry tomatoes, under foliar fertilization conditions

Equation (4) described the variation of YP in relation to FrCW, $R^2 = 0.799$, $p<0.001$, $F = 17.942$.

$$YP = 1.206\,E-06x^2 + 0.005368x + 0.127 \quad (4)$$

Equation (5), has described the variation of YP in relation to FrnP, $R^2 = 0.845$, $p<0.001$, $F = 24.565$.

$$YP = 0.001552x^2 - 0.2427x + 10.99 \quad (5)$$

Regression analysis was appropriately used to describe the variation of yield (YP) in relationdepending to the morphological parameters and productivity elements, as a direct and interaction effect. The result was equation

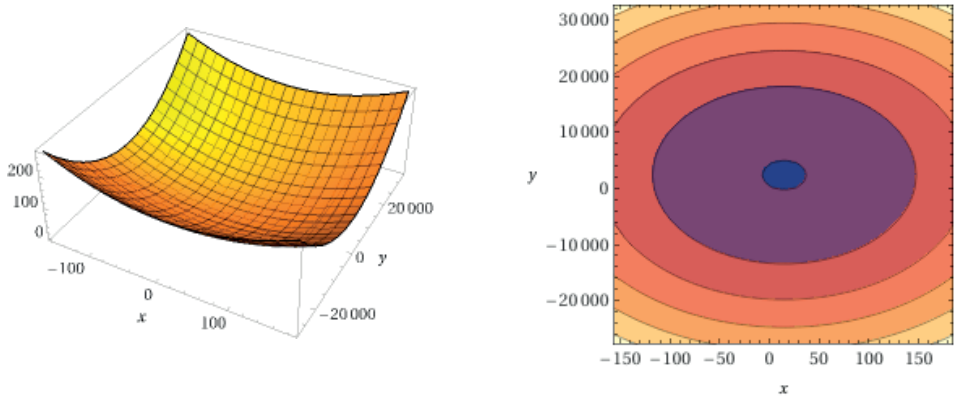
(6), as a general model, with different values of the variables x and y, depending on the parameters considered, and values of the equation coefficients, according to Table 2. The graphic representation of the YP variation, in relation to CnP and FrCW, as the most reliable result, according to the statistical parameters, is presented in Figure 8.

$$YP = ax^2 + by^2 + cx + dy + exy + f \quad (6)$$

where: YP – yield on plant (kg plt^{-1});
x, and y – the variables of equation (6), according to Table 2;
a, b, c, d, e, and f – the coefficients of the equation (6), Table 2.

Table 2. Statistical values related to equation (6)

Experimental parameters		Values of the coefficients of equation (6)						Statistical parameters			
x	y	a	b	c	d	e	f	R^2	p	F	RMSE
PH	CnP	-0.00125330	-0.19205039	0.20033474	-7.20253236	0.05193695	0.13915401	0.492	0.4225	1.1629	0.2253
CnP	FlnC	2.01339206	0.35293950	-53.5978844	-22.2644936	1.84773638	339.2739735	0.794	0.0444	4.6346	0.1434
CnP	FFlnC	0.64710798	0.10396957	-14.5541236	-5.77429743	0.48953238	84.96469880	0.871	0.0119	8.1387	0.1133
FrnC	FrW	0.00157594	0.00753871	0.15617575	-0.02870534	-0.0097621	-0.36026190	0.953	0.0006	24.8775	0.0679
CnP	FrW	-0.07169783	0.00199852	0.16103264	-0.18939024	0.03852248	1.84680725	0.967	0.0002	35.6109	0.0571
CnP	FrCW	0.00293364	0.00000020	-0.08328623	-0.00103288	0.00117308	0.42549542	0.999	<0.001	6498.76	0.0043



(a) (b)

Figure 8. Graphical distribution of yield (YP) in cherry tomatoes in relation to parameters CnP (x – axis) and FrCW (y – axis); (a) – 3D model, (b) isoquants model

The studied cherry tomato genotypes presented differentiated values for determined parameters, in relation to foliar treatments.
Regarding plant height, under unfertilized conditions (b1), the Arielle F1 genotype presented a lower value (PH = 179.3 cm), and the Black Cherry F1 genotype presented a higher value (PH = 190.4 cm). In response to the applied foliar fertilization, all variants recorded an increase in plant height, with values between PH = 184.7 cm (a1b2) and PH = 198.7 cm (a3b3).
The number of clusters on plant, in relation to the genotype, presented the value CnP = 5.4 (Black Cherry F1) and CnP = 6.4 (Ravello F1). Under the influence of foliar fertilization, the number of clusters increased to the highest value, CnP = 6.9, variant a2b2.
The number of the flowers in the cherry tomato cluster (FlnC), varied according genotypes with values of FlnC = 15.20 (a2b1) and FlnC = 17.70 (a3b1). As an effect of the applied foliar fertilizers, the number of the flowers in the cluster increased in each hybrid,, but within small limits.
Positive effects were recorded in cherry tomatoes on some physiological indices, the number and size of fruits as well as on yield in relation to mixed fertilization (Rashid et al., 2022).
The number of fertilized flowers in the cluster (FFlnC) increased under the influence of foliar fertilization, except for the Vespolino F1 (a4)

hybrid. The increase in the number of fertilized flowers, as a result of technological interventions, is important, and is reflected in the yield. In the present study, the variation of the FFlnC parameter in relation to FlnC was recorded, under conditions of $R^2 = 0.914$, $p<0.001$.

The number of fruits in the cluster (FrnC) and the number of fruits per plant (FrnP) corresponded to the parameters FFlnC and FrnC, respectively.

Productivity parameters (e.g. number of fruits per plant, fruit diameter, yield, etc.) were analyzed in cherry tomatoes in relation to the watering regime (Ahmad et al., 2023).

Fruit shape and size were important characters for evaluating some cherry tomato genotypes, such as adaptability to certain growing conditions (Gaswanto, 2021).

The average fruit weight (FrW) varied among genotypes between FrW = 16.20 g (a1b1) and FrW = 21.60 g (a4b1). Under the influence of fertilizers, an increase in fruit weight was recorded for each hybrid, with the maximum value FrW = 24.50 g (a4b4).

Average fruit weight and yield per plant were important criteria for the selection of cherry tomato genotypes (Aguirre and Cabrera, 2012).

Differential variation of fruit parameters, mineral element content and quality indices was recorded in cherry tomatoes in relation to organic and mineral fertilizers (Kai et al., 2020).

The fruit cluster weight (FrCW) at the level of the genotype potential (factor A) presented the value FrCW = 225.18 g (a1b1) and FrCW = 317.52 g (a4b1). Under the influence of foliar fertilization, the values increased for each hybrid, up to the value FrCW = 379.75 g (a4b3).

CONCLUSIONS

The tested cherry tomato hybrids showed differentiated potential for the evaluated parameters. All hybrids responded positively to the application of foliar treatments for most of the evaluated parameters.

The Ravello F1 (a2) genotype showed an advantage for the CnP parameter, compared to the other hybrids. The Black Cherry F1 (a3) genotype showed an advantage for the FlnC and FFlnC parameters, and maintained the advantage over the other hybrids under the effect of foliar treatments.

The Vespolino F1 (a4) genotype showed an advantage in the FlnP and FFlnP parameters in the case of variants a4b2 and a4b3. The Black Cherry F1 (a3) genotype showed an advantage in the FrnC parameter. The Vespolino F1 (a4) genotype showed an advantage in the FrW and FrCW parameters, followed by the Black Cherry F1 (a3) genotype.

For yield (YP), the Vespolino F1 (a4) genotype ranked first, in the case of the a4b3 variant, followed by the a4b2 variant.

The next positions were occupied by the Black Cherry F1 genotype with the a3b3 variant, and the Ravello F1 genotype with the a2b3 variant, respectively.

Among the foliar products applied, the Plantfert-U (b3) product showed better efficiency in combination with the tested hybrids.

REFERENCES

Aguirre, N.C., Cabrera, F.A.V. (2012). Evaluating the fruit production and quality of cherry tomato (*Solanum lycopersicum* var. *cerasiforme*). *Revista Facultad Nacional de Agronomia Medellín*, 65(2), 6599–6610.

Ahmad, F., Kasumiyati, K., Soleh, M.A., Khan, M.R., Sundari, R.S. (2023). Watering volume and growing design's effect on the productivity and quality of cherry tomato (*Solanum lycopersicum cerasiformae*) cultivar Ruby. *Agronomy*, 13(9), 2417.

Arshad, A., Jerca, I.O., Chan, S., Cîmpeanu, S.M., Teodorescu, R.I., Țiu, J., Bădulescu, L., Drăghici, E.M. (2023). Study regarding the influence of some climatic parameters from the greenhouse on the tomato production and fruits quality. *Scientific Papers. Series B, Horticulture*, LXVII(2), 295–306.

Aydi, S., Sassi Aydi, S., Marsit, A., El Abed, N., Rahmani, R., Bouajila, J., Merah, O., Abdelly, C. (2023). Optimizing alternative substrate for tomato production in arid zone: Lesson from growth, water relations, chlorophyll fluorescence, and photosynthesis. *Plants* (Basel), 12(7), 1457.

Badea, M.L., Călin, I., Iordăchescu, M., Lagunovschi-Luchian, V., Bădulescu, L. (2023). Foliar solutions influence on several physiological indicators in *Solanum lycopersicum* L.(*Solanaceae*) plants. *Acta Horticulturae*, 1391, 463–470.

Chang, Y., Zhang, X., Wang, C., Ma, N., Xie, J., Zhang, J. (2024). Fruit quality analysis and flavor comprehensive evaluation of cherry tomatoes of different colors. *Foods*, 13(12), 1898.

Chapagain, A.K., Orr, S. (2009). An improved water footprint methodology linking global consumption to local water resources: A case of Spanish tomatoes. *Journal Environmental Management*, 90(2009), 1219–1228.

Duffaut, C., Brondeau, F., Gasparini, J. (2023). An original and efficient fertilizer for cherry tomato plants

grown in urban agriculture: Feral pigeon guano. *Urban Agriculture & Regional Food Systems*, 8(1), e20046.

Gaswanto, R. (2021). Adaptation test of five introduced cherry tomato lines in Lembang highland. *IOP Conference Series: Earth and Environmental Science*, 883, 012066.

Guo, X.-X., Zhao, D., Zhuang, M.-H., Wang, C., Zhang, F.-S. (2021). Fertilizer and pesticide reduction in cherry tomato production to achieve multiple environmental benefits in Guangxi, China. *Science of The Total Environment*, 793, 148527.

Hammer, Ø., Harper, D.A.T., Ryan, P.D. (2001). PAST: Paleontological Statistics software package for education and data analysis. *Palaeontologia Electronica*, 4(1), 1–9.

Hita, O., Romacho, I., Soriano, M. T., Morales, I., Escobar, I., Suarez-Rey, E. M.,. Hernandez, J., Castilla, N. (2007). Comparison of two Mediterranean greenhouses 'technological packets' with cherry tomato. *Acta Horticulturae*, 747, 309–313.

Hussein, Y.M., Al-Tufaili, A.K.H. (2023). Effect of spraying with NPK fertilizer on the growth and yield of three hybrids of cherry tomato (Solanum lycopersicum var. cerasiforme). *IOP Conference Series: Earth and Environmental Science*, 1262, 042028.

Irfanulden Abdulhadı Qahraman, R., Gülşen, O., Güneş, A. (2020). Effects of different organic fertilizers on some bioactive compounds and yield of cherry tomato cultivars. *Gesunde Pflanzen*, 72, 257–264.

Jerca, I.O., Smedescu, C. (2023). A decade of change in Europe's tomato greenhouses: Insights and trends. *Scientific Papers Series Management, Economic Engineering in Agriculture and Rural Development*, 23(4), 431–436.

Kai, T. , Nishimori, S., Tamaki, M. (2020). Effect of organic and chemical fertilizer application on growth, yield, and quality of small-sized tomatoes. *Journal of Agricultural Chemistry and Environment*, 9, 121–133.

Kusumiyati, K., Ahmad, F., Khan, M.R., Soleh, M.A., Sundari, R.S. (2023). Productivity of cherry tomato cultivars as influenced by watering capacities and microclimate control designs. *The Open Agriculture Journal*, 17, 1–12.

Li, M., Zhu, G., Liu, Z., Li, L., Wang, S., Liu, Y., Lu, W., Zeng, Y., Cheng, X., Shen, W. (2024). Hydrogen fertilization with hydrogen nanobubble water improves yield and quality of cherry tomatoes compared to the conventional fertilizers. *Plants (Basel)*, 13(3), 443.

Maboko, M. M., Du Plooy, C.P. (2017). Response of hydroponically grown cherry and fresh market tomatoes to reduced nutrient concentration and foliar fertilizer application under shadenet conditions. *HortScience*, 52(4), 572–578.

Murtic, S., Oljaca, R., Smajic Murtic, M., Vranac, A., Akagic, A., Civic, H. (2018). Cherry tomato productivity as influenced by liquid organic fertilizer under different growth conditions. *Journal of Central European Agriculture*, 19(3), 503–516.

Nie, J., Li, Y.H., Yang, X., Zheng, J.R., Xie, Y.M., Shi, L.L. (2023). Effect of fertilization treatment on growth, yield, fruit quality, and nutrition accumulation of cherry tomato. *Applied Ecology and Environmental Research*, 21(5), 3849–3863.

Petek, M., Šokec, A., Šic Žlabur, J., Konopka, K., Fabek Uher, S. (2024). Content of potassium and iron in tomato products. *Scientific Papers. Series B, Horticulture*, LXVIII(2), 541–547.

Rashid, M.H.A., Rahman, M.F., Karim, M.R., Saha, R., Hossain, M.I. (2022). Improving growth, yield and quality of cherry tomato (*Solanum lycopersicum* var. *cerasiforme*) using staking and mixed fertilization. *Journal of Agriculture, Food and Environment*, 3(3), 77–85.

Stoleru, V., Inculet, S.-C., Mihalache, G., Cojocaru, A., Teliban, G.-C., Caruso, G. (2020). Yield and nutritional response of greenhouse grown tomato cultivars to sustainable fertilization and irrigation management. *Plants*, 9, 1053.

Tao, Y., Liu, T., Wu, J.Y., Wu, Z.S., Liao, D.L., Shah, F., Wu, W. (2022). Effect of combined application of chicken manure and inorganic nitrogen fertilizer on yield and quality of cherry tomato. *Agronomy*, 12, 1574.

Tsouvaltzis, P., Gkountina, S., Siomos, A.S. (2023). Quality traits and nutritional components of cherry tomato in relation to the harvesting period, storage duration and fruit position in the truss. *Plants* (Basel), 12(2), 315.

Wolfram, Research, Inc., Mathematica, Version 12.1, Champaign, IL (2020).

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

ASSESMENT OF VARIOUS CONCENTRATION OF SALICYLIC ACID IN TISSUE CULTURE *IN VITRO* SYSTEMS FOR THEIR EFFECT ON MODULATING ABIOTIC STRESS TOLERANCE MECHANISMS IN PEPPER (*CAPSICUM ANNUUM* L.) PLANTS

Tina Oana CRISTEA, Gabriel-Alin IOSOB, Creola BREZEANU, Petre Marian BREZEANU, Claudia BĂLĂIȚĂ, Mariana CALARA, Dan Ioan AVASILOAIEI

Vegetable Research and Development Station Bacău,
220 Calea Bârladului Street, Bacău, Romania

Corresponding author email: iosob.gabriel@gmail.com

Abstract

Abiotic stress, particularly in climate-sensitive crops like Capsicum annuum L., severely impacts crop productivity. Understanding the mechanisms through which plants can mitigate these stresses is key to developing more resilient crop varieties for the future. Salicylic acid is a plant hormone known for its role in regulating plant growth and enhancing resistance to abiotic and biotic stresses. Thus, this study focuses on elucidating the dose-dependent effects of exogenous SA application on physiological, biochemical, and morphogenetic responses of pepper explants cultivated in vitro. The findings revealed a dose-dependent effect of SA, where lower concentrations significantly enhanced seed germination rates, shoot initiation and elongation while promoting robust root development. It also increased the total phenolic and chlorophyll content, suggesting an activation of defense mechanisms and improved photosynthetic efficiency.

Key words*: salicylic acid, in vitro; resilient, vegetable, crops.*

INTRODUCTION

The productivity of agricultural crops is significantly affected by abiotic stress, particularly in species that are highly sensitive to environmental fluctuations, such as *Capsicum annuum* L. (Zhang et al., 2024). Stress factors like drought, salinity, and extreme temperatures can trigger adverse physiological, biochemical, and molecular changes that impede plant growth and development (Dos Santos et al., 2022). These conditions often lead to reduced biomass accumulation, impaired photosynthetic capacity, and diminished crop yields. With the increasing impact of climate change, finding effective strategies to improve plant resilience has become a major priority in agricultural and biotechnological research (Garland and Curry, 2022).

Salicylic acid (SA), a naturally occurring plant hormone, plays a crucial role in the regulation of plant responses to both biotic and abiotic stress (Liu et al., 2022). Its involvement extends to modulating defence mechanisms, enhancing the activity of antioxidant enzymes, and stimulating osmolyte production. SA participates in key metabolic pathways and signaling cascades that influence plant adaptation to adverse conditions by improving stress tolerance, photosynthetic efficiency, and secondary metabolite synthesis (Salam et al., 2023).

Previous research has highlighted the beneficial effects of exogenous SA application in counteracting stress-induced damage that occurs in the presence of salt (Quamruzzaman et al., 2001; Li and Liu, 2023), UV-B (Khare et al., 2023), ozone (Liu et al., 2021), drought (Naz et al., 2021), heat (Shaukat et al., 2022), cold (Soualiou et al., 2022; Shaukat et al., 2022) and metal stress (Bilal et al., 2023). Studies indicate that SA enhances the activity of antioxidant defense systems, including superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), which mitigate oxidative damage caused by stress (Fujita and Hasanuzzaman, 2022; Zulfiqar and Ashraf, 2021). Additionally, SA influences stomatal regulation, thereby optimizing water-use

efficiency under drought conditions. Moreover, its role in stimulating the production of secondary metabolites such as flavonoids and phenolic compounds is well-documented, with these compounds playing a protective role in reducing oxidative stress. However, the extent of SA's effectiveness depends on its concentration, with both stimulatory and inhibitory effects observed depending on the applied dose (Robertson and Li, 2021; Hasan et al., 2022).

In vitro tissue culture techniques offer a controlled environment to investigate the physiological and biochemical responses of plants to SA under stress conditions. These systems minimize external environmental variability, allowing precise analysis of SA's impact on plant growth and adaptation mechanisms. Additionally, *in vitro* cultures facilitate the study of morphogenetic responses, such as shoot and root development, callus formation, and somatic embryogenesis, which are indicative of stress resilience (Custódio et al., 2022; Wijerathna-Yapa and Hiti-Bandaralage, 2023).

This study aims to elucidate the effects of varying SA concentrations on *Capsicum annuum* L. explants cultivated under in vitro conditions. By assessing parameters such as germination rates, shoot initiation, root development, chlorophyll content, and total phenolic accumulation, we seek to identify the optimal SA concentration that enhances stress tolerance mechanisms. The outcomes of this research will deepen our understanding of SA-mediated stress adaptation and may provide valuable insights for improving the resilience of pepper plants in commercial agriculture and crop improvement programs.

The findings revealed a dose-dependent effect of SA, where lower concentrations (0.1 mM and 0.5 mM) significantly enhanced seed germination rates, shoot initiation, and elongation while promoting robust root development. These concentrations also increased the total phenolic and chlorophyll content, suggesting an activation of defense mechanisms and improved photosynthetic efficiency. Conversely, higher concentrations (1 mM SA) delayed germination and inhibited root and shoot development, indicating potential phytotoxic effects at elevated levels.

The study underscores the role of SA in modulating stress-responsive pathways, particularly through its interaction with endogenous hormonal signaling and defense-related processes. By optimizing SA concentrations, this research provides a foundation for improving pepper plant resilience to abiotic stresses in controlled environments, with potential implications for field applications and sustainable agriculture.

MATERIALS AND METHODS

For this study, seeds of pepper, 'Ionel' variety were selected as plant material. The experiments were conducted at the Vegetable Research and Development Station in Bacau, within the in vitro Tissue Culture Laboratory. The primary objective was to evaluate the impact of exogenous salicylic acid (SA) application on pepper plants' responses to abiotic stress. To ensure aseptic conditions for tissue culture experiments, the seeds underwent a surface sterilization process by immersion in a 0.1% mercuric chloride ($HgCl_2$) solution for 10 minutes, followed by thorough rinsing with sterile distilled water to eliminate any remaining sterilizing agents.

Murashige and Skoog (MS) medium (1962) were used as the basal growth medium. It was supplemented with 30 g/L sucrose as a carbon source to support plant growth. To solidify the medium, agar was added at a concentration of 8.0 g/L. Three different concentrations of SA (1 mM, 0.5 mM, and 0.1 mM) were incorporated into the medium to assess their effects on morphogenetic reaction and plant growth. The pH of the medium was adjusted to 5.8 before autoclaving.

Sterilized seeds were germinated on MS medium under optimal conditions, and germination indices were recorded to assess SA's effects. One-week-old seedlings provided apical and hypocotyl explants for tissue culture. Four treatment groups were established: control (V0, no SA), 1 mM SA (V1), 0.5 mM SA (V2), and 0.1 mM SA (V3). Each treatment variant was replicated three times to ensure result accuracy.

The tissue cultures were maintained under controlled environmental conditions, with an incubation temperature of 26±1°C. A 16-hour

photoperiod with a light intensity of 5000 lx was provided to simulate optimal conditions for photosynthesis. Subculturing was performed every 30 days to prevent overcrowding and maintain continuous growth. Daily observations were conducted to monitor morphological and physiological changes in response to the different SA concentrations.

To facilitate acclimatization, rooted plantlets were transferred to hydroponic conditions within bottles, while newly formed shoots were transplanted into fresh media for the microclonal propagation phase. Hydroponic solutions contained a Previcur 0.15%, and in order to maintain a relative humidity of 100%, the containers were covered with transparent bags. The newly formed plants were kept in an acclimatization room under a 16-hour light/8-hour dark photoperiod, with temperatures maintained between 20°C and 23°C.

After acclimatization, plants were transferred to a potting mixture consisting of sterilized sand and vermiculite (1:1) in plastic containers. They underwent a hardening phase inside a mist chamber with 80% relative humidity for two weeks before final transplantation to the greenhouse.

Chlorophyll content was assessed following the methodology described by Lichtenthaler (1987). Fresh plant samples (0.1 g) were homogenized and extracted using 80% acetone. The extract was centrifuged for 10 minutes at 3.530 rpm using a Hettich Universal Centrifuge 320 | 320 R. The supernatant was collected, and absorbance was measured at 470 nm, 647 nm, and 663 nm using a UV-VIS spectrophotometer.

The concentrations of chlorophyll a, chlorophyll b, and carotenoids were calculated using the following formulas:

- Chlorophyll a (Cla) concentration (mg/L) = 12.25 × Abs 663 nm - 2.79 × Abs 647 nm
- Chlorophyll b (Clb) concentration (mg/L) = 21.50 × Abs 647 nm - 5.10 × Abs 663 nm
- Carotenoids (Car) concentration (mg/L) = (1000 × Abs 470 nm - 1.82 × Cla - 85.02 × Clb) / 198

Tissue culture data were analyzed using ANOVA to assess the significance of SA's effects. Mean values and standard deviations were calculated for each treatment variant to facilitate comparative analysis and interpretation of results.

RESULTS AND DISCUSSIONS

Effects of salicylic acid on germination and seedling development

The application of salicylic acid (SA) at different concentrations significantly influenced the germination dynamics and early seedling development of *Capsicum annuum* L. Our results indicate that seeds cultivated on variant with 1 mM SA exhibited a pronounced delay in germination, approximately three days longer than the control group. This suggests that elevated SA concentrations interfere with dormancy release and germination initiation, potentially by modulating hormonal balance or altering cellular metabolic processes.

Following germination, seedlings exposed to 1 mM SA displayed severe root developmental anomalies, including stunted growth, calluses and, in some cases, complete inhibition of root formation. The irregular root architecture observed in these seedlings suggests that high SA concentrations may disrupt root elongation and differentiation, ultimately impairing the plant's ability to efficiently uptake water and nutrients (Figure 1).



Figure 1. Plantlets with abnormal development exhibited on variant with 1.0 mM SA

Conversely, at a lower concentration of 0.1 mM SA, germination rates remained comparable to those of the control group, indicating that this concentration does not negatively impact seed dormancy release. Moreover, the plants emerged on variant with 0.1 mM SA exhibited enhanced shoot growth and overall vigor,

highlighting the dose-dependent nature of SA's effects on early plant development (Figure 2).



Figure 2. Pepper plants regenerated on variant with 0.1 mM SA

Morphogenetic responses of explants to SA treatment

The morphogenetic response of *C. annuum* explants varied depending on both the type of explant used and the concentration of SA in the culture medium. Apical explants exhibited the highest morphogenetic potential, with rapid shoot initiation at the base of the explants. The addition of 0.1 mM SA further enhanced shoot multiplication and elongation, underscoring the positive role of this concentration in stimulating shoot formation.

In contrast, hypocotyl explants displayed a different developmental pattern, primarily forming roots rather than shoots (Figure 3).



Figure 3. Morphogenetic reaction of pepper hypocotyls under the influence of SA

However, on lower concentrations of SA, shoot formation was observed at one side of the hypocotyl segments, which subsequently developed into fully differentiated plantlets. These observations suggest that the developmental fate of hypocotyl explants is influenced by local hormonal gradients and the microenvironment created by SA supplementation.

Quantitative analysis revealed that shoot formation varied significantly across different SA concentrations. In media supplemented with 0.1 mM SA, 96.94% of explants successfully generated shoots, compared to 74.84% in the control group. The highest SA concentration (1 mM) led to a notable decline in shoot initiation, with only 67.74% of explants forming shoots. These results indicate that while moderate SA concentrations can enhance shoot proliferation, excessive levels may exert an inhibitory effect on shoot induction and development (Table 1).

Table 1. Effects of different concentrations of SA in MS medium for multiple shoot induction at pepper plants after 60 days of culture - means ± SE

Variant	% of explant showing response	Average no. of shoots
V0 - Control	74.84	19.3±1.0
V1 - 1 mM SA	67.74	8.7±0.4
V2 - 0.5 mM SA	88.04	28.1±0.3
V3 - 0.1 mM SA	96.94	35.9±1.3

Impact of SA on shoot and root elongation

SA treatment significantly influenced both shoot and root elongation, with notable differences depending on the applied concentration. The most pronounced growth stimulation was observed in explants cultured on media supplemented with 0.1 mM and 0.5 mM SA, where shoot elongation rates exceeded those of the control group. These results suggest that SA promotes the transition from meristematic to shoot-forming tissues, accelerating shoot emergence and elongation.

Similarly, root elongation was significantly enhanced in explants exposed to 0.1 mM SA, indicating a stimulatory effect on root growth. However, at 1 mM SA, root development was severely restricted, confirming the dose-dependent influence of SA on plant morphogenesis.

The comparative analysis of shoot proliferation and elongation rates across different SA concentrations underscores the critical

importance of optimizing exogenous SA application in in vitro culture. While lower concentrations (0.1-0.5 mM) enhance plant growth and vigor, higher concentrations may trigger inhibitory responses, limiting morphogenetic potential (Figure 4).

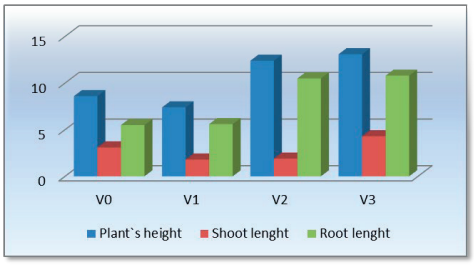


Figure 4. Biometric measurements on Brassica plants cultivated on media supplemented with different concentration of SA

Acclimatization and survival of plantlets

Following root formation, plantlets were transferred to hydroponic conditions and subjected to a hardening process to facilitate acclimatization. During the initial week, a high relative humidity (90%) was maintained, followed by gradual reductions to simulate environmental adaptation. This approach resulted in a survival rate exceeding 95%, demonstrating the effectiveness of the hardening process in ensuring successful plant establishment under hydroponic conditions.

Influence of SA on photosynthetic activity

Chlorophyll content analysis revealed a significant increase in total chlorophyll in plants treated with 0.1 mM SA compared to the control group. However, at higher SA concentrations, chlorophyll a content was either similar to or slightly lower than that of the control. These results align with previous studies (Gupta and Seth, 2021; Azeem et al., 2023), which suggest that moderate SA levels can enhance chlorophyll biosynthesis, while excessive concentrations may disrupt pigment accumulation and photosynthetic efficiency.

Overall, our findings highlight the complex regulatory role of SA in *C. annuum* development. The concentration-dependent effects observed in germination, morphogenesis, and photosynthetic pigment accumulation underscore the necessity of precise SA dosage optimization to maximize its benefits while mitigating potential inhibitory effects.

CONCLUSIONS

This study examines how varying concentrations of salicylic acid (SA) influence the growth, development, and stress tolerance of *C. annuum* under in vitro conditions by modulating physiological and biochemical responses.

The study found that high SA concentrations (1 mM) inhibited seed germination, root development, and shoot formation, while lower concentrations (0.1 mM) promoted seedling vigor and significantly enhanced shoot formation (96.94% success). These findings highlight SA's dual role in early plant development, depending on its concentration.

Low SA concentrations (0.1-0.5 mM) promoted shoot elongation, root formation, and chlorophyll accumulation, while high concentrations (1 mM) inhibited growth and reduced photosynthetic efficiency. *In vitro* derived plantlets adapted well to hydroponic conditions, achieving over 95% survival, highlighting SA's role in plant morphogenesis and greenhouse cultivation potential.

Optimizing SA concentrations is crucial to balancing its benefits and inhibitory effects. The 0.1 mM SA concentration proved most effective in enhancing growth, shoot proliferation, and stress tolerance. These findings provide insights into SA-mediated physiological responses, with practical applications for in vitro propagation and stress resilience in commercial pepper cultivation.

ACKNOWLEDGEMENTS

This work was supported by a grant from the Romanian Ministry of Agriculture and Rural Development, ADER 6.3.18./2023-2026, ADER 6.3.6, ADER 6.3.20, ADER 6.1.6 and ADR Nord East Leg(O)nest project.

REFERENCES

Adams, R.S., & Ishler, V.A. (2009). Trouble-shooting problems with low milk production. *Dairy and Animal Science*, *4*(1), 98–16. Retrieved June 3, 2018, from

Azeem, M., Sultana, R., Mahmood, A., Qasim, M., Siddiqui, Z. S., Mumtaz, S., ... & Siddiqui, M. H. (2023). Ascorbic and salicylic acids vitalized growth, biochemical responses, antioxidant enzymes, photosynthetic efficiency, and ionic regulation to alleviate salinity stress in Sorghum bicolor. *Journal of Plant Growth Regulation, 42*(8), 5266-5279.

Bilal, S., Saad Jan, S., Shahid, M., Asaf, S., Khan, A. L., Lubna, ... & AL-Harrasi, A. (2023). Novel insights into exogenous phytohormones: Central regulators in the modulation of physiological, biochemical, and molecular responses in rice under metal (loid) stress. *Metabolites, 13*(10), 1036.

Custódio, L., Charles, G., Magné, C., Barba-Espín, G., Piqueras, A., Hernández, J. A., ... & Rodrigues, M. J. (2022). Application of in vitro plant tissue culture techniques to halophyte species: A review. *Plants, 12*(1), 126.

Dos Santos, T. B., Ribas, A. F., de Souza, S. G. H., Budzinski, I. G. F., & Domingues, D. S. (2022). Physiological responses to drought, salinity, and heat stress in plants: a review. *Stresses, 2*(1), 113-135.

Fujita, M., & Hasanuzzaman, M. (2022). Approaches to enhancing antioxidant defense in plants. *Antioxidants, 11*(5), 925.

Garland, S., & Curry, H. A. (2022). Turning promise into practice: Crop biotechnology for increasing genetic diversity and climate resilience. *PLoS Biology, 20*(7), e3001716.

Gupta, S., & Seth, C. S. (2021). Salicylic acid alleviates chromium (VI) toxicity by restricting its uptake, improving photosynthesis and augmenting antioxidant defense in *Solanum lycopersicum* L. *Physiology and Molecular Biology of Plants, 27*, 2651-2664.

Hasan, M., Sajjad, M., Zafar, A., Hussain, R., Anjum, S. I., Zia, M., ... & Shu, X. (2022). Blueprinting morpho-anatomical episodes via green silver nanoparticles foliation. *Green Processing and Synthesis, 11*(1), 697-708.

Khare, S., Singh, A., Amist, N., Azim, Z., & Singh, N. B. (2023). Secondary metabolites interference on potential of *Solanum lycopersicum* grown under UV-B stress and its impact on developmental attributes of *Capsicum annuum*. *Plant Stress*, 8, 100167.

Li, X., & Liu, G. (2023). Emerging Roles of Salicylic Acid in Plant Saline Stress Tolerance. *International Journal of Molecular Sciences, 24*(4), 3388.

Lichtenthaler, H. K. (1987). Chlorophyll fluorescence signatures of leaves during the autumnal chlorophyll breakdown. *Journal of plant physiology*, *131*(1-2), 101-110.

Liu, C., Kang, H., Wang, Y., Yao, Y., Gao, Z., & Du, Y. (2021). Melatonin relieves ozone stress in grape leaves by inhibiting ethylene biosynthesis. *Frontiers in Plant Science*, 12, 702874.

Liu, J., Qiu, G., Liu, C., Li, H., Chen, X., Fu, Q., ... & Guo, B. (2022). Salicylic acid, a multifaceted hormone, combats abiotic stresses in plants. *Life, 12*(6), 886.

Naz, R., Sarfraz, A., Anwar, Z., Yasmin, H., Nosheen, A., Keyani, R., & Roberts, T. H. (2021). Combined ability of salicylic acid and spermidine to mitigate the individual and interactive effects of drought and chromium stress in maize (*Zea mays* L.). *Plant Physiology and Biochemistry*, *159*, 285-300.

Quamruzzaman, M., Manik, S. N., Shabala, S., & Zhou, M. (2021). Improving performance of salt-grown crops by exogenous application of plant growth regulators. *Biomolecules, 11*(6), 788.

Robertson, B. C., He, T., & Li, C. (2021). The genetic control of stomatal development in barley: new solutions for enhanced water-use efficiency in drought-prone environments. *Agronomy, 11*(8), 1670.

Salam, U., Ullah, S., Tang, Z. H., Elateeq, A. A., Khan, Y., Khan, J., ... & Ali, S. (2023). Plant metabolomics: an overview of the role of primary and secondary metabolites against different environmental stress factors. *Life, 13*(3), 706.

Shaukat, K., Zahra, N., Hafeez, M. B., Naseer, R., Batool, A., Batool, H., ... & Wahid, A. (2022). Role of salicylic acid–induced abiotic stress tolerance and underlying mechanisms in plants. In *Emerging plant growth regulators in agriculture* (pp. 73-98). Academic Press.

Shaukat, K., Zahra, N., Hafeez, M. B., Naseer, R., Batool, A., Batool, H., ... & Wahid, A. (2022). Role of salicylic acid–induced abiotic stress tolerance and underlying mechanisms in plants. In *Emerging plant growth regulators in agriculture* (pp. 73-98). Academic Press.

Soualiou, S., Duan, F., Li, X., & Zhou, W. (2022). Crop production under cold stress: An understanding of plant responses, acclimation processes, and management strategies. *Plant Physiology and Biochemistry*, 190, 47-61.

Wijerathna-Yapa, A., & Hiti-Bandaralage, J. (2023). Tissue culture - A sustainable approach to explore plant stresses. *Life, 13*(3), 780.

Zhang, X., Ma, X., Wang, S., Liu, S., & Shi, S. (2024). Physiological and Genetic Aspects of Resistance to Abiotic Stresses in *Capsicum* species. *Plants, 13*(21), 3013.

Zulfiqar, F., & Ashraf, M. (2021). Bioregulators: unlocking their potential role in regulation of the plant oxidative defense system. *Plant Molecular Biology*, 105, 11-41.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

CARBON FOOTPRINT OF LETTUCE GROWED WITH MINERAL AND BIOLOGICAL FERTILIZATION IN GREENHOUSES

Dimitar DIMITROV, Kostadin KOSTADINOV, Stoyan FILIPOV

Agricultural University - Plovdiv, AU, 12 Mendeleev Blvd,
4000, Plovdiv, Bulgaria

Corresponding author email: kostadinov8888@gmail.com

Abstract

The amounts of carbon emissions during the cultivation of lettuce in greenhouse conditions during the autumn-winter period were measured in a polyethylene greenhouse. Fertilised and unfertilised soils, with and without plants, were placed in hermetically sealed chambers for 24 hours under identical conditions, after which the quantitative content of CO_2 in each chamber was determined. The impact of mineral and biological fertilizers on greenhouse gas emissions was studied. To assess the contribution of soil microflora to carbon emissions in the gas chambers, a comparison was made between the CO_2 levels measured in the unfertilised and fertilized soil variants, as well as between soils with and without plants. Model calculations were performed for the absorbed CO_2 gas over a relatively equal period in each of the three research years for NPK-fertilized, biologically fertilized, and unfertilized lettuce cultivation variants. It was found that mineral fertilization when cultivating salads in greenhouse production conditions, in norms N12.5P4.17K15.83 and has lower emissions - as a share in greenhouse gases (in the amount of 90.74 g/m^3) compared to unfertilized and fertilized with organic plants.

Key words*: greenhouses gases, lettuce, carbon emission, mineral and biological fertilization.*

INTRODUCTION

The greenhouse effect, driven by the accumulation of greenhouse gases (GHGs) in the atmosphere, represents one of the most pressing environmental issues of the 21st century. The most significant impact on the greenhouse effect is exerted by methane and carbon dioxide emissions (Borisova at al., 2023). Lettuce cultivation plays a significant role in global horticultural production, necessitating sustainable management practices to minimize environmental impact while ensuring high yields. Among the key factors influencing both productivity and ecological footprint are fertilization strategies and greenhouse management techniques. The selection of organic versus mineral fertilization has been shown to affect soil microbiota, root exudation patterns, and plant defense mechanisms, ultimately shaping the overall health of lettuce crops (Windisch et al., 2021). Understanding these interactions is crucial for optimizing agricultural practices that balance sustainability and crop performance.

Furthermore, soil carbon emissions represent a critical aspect of agricultural sustainability, as greenhouse gas emissions from lettuce fields contribute to climate change concerns. Recent studies indicate that different irrigation and fertilization strategies significantly influence soil gaseous emissions, emphasizing the need for tailored approaches to mitigate environmental impact (Zhang et al., 2024). Addressing the combined effects of fertilization type and irrigation methods on lettuce cultivation is essential for developing climate-smart agricultural practices.

Previous studies have highlighted that the type of fertilization strategy used can influence these environmental impacts significantly. Fertilizer application, particularly nitrogen-based fertilizers, is a key driver of greenhouse gas emissions from agricultural systems, contributing to both direct emissions of nitrous oxide (N_2O) and indirect emissions from the production and transportation of fertilizers (Thapa, Chatterjee, & Awale, 2016). This research seeks to quantify the carbon footprint associated with two different fertilization approaches, mineral and biological, and analyze their impacts on GHG emissions during lettuce cultivation in greenhouses.

A number of authors have studied the influence of different organic fertilizers on different crops

in terms of their growth and development (Kostadinova at al., 2018; Dermendzhieva et al., 2021; Borisova et al., 2022).

Several studies have explored the impact of different fertilization techniques on the carbon footprint of greenhouse vegetable production.

The effects of reduced mineral fertilization and straw return on vegetable cultivation have been examined, concluding that integrating organic practices such as straw return can help balance greenhouse gas emissions while maintaining vegetable yield (Huang et al., 2020).

This aligns with the study of the carbon emissions of cucumber, tomato, and lettuce production in greenhouses which demonstrated that optimizing fertilizer use could significantly reduce the carbon footprint of greenhouse agriculture, particularly in systems using biological fertilizers (Pereira et al., 2021).

Mineral fertilizers, while effective in enhancing crop yield, have been associated with significant environmental costs. Conventional fertilization practices in lettuce production have an environmental burden, particularly in terms of the increased emissions of N_2O (Rahman, Alam, and Hossain, 2018). In contrast, biological fertilization, which incorporates natural soil amendments such as biofertilizers, has been suggested as a viable alternative to mitigate environmental impacts. It has been reviewed the potential of biofertilizers to reduce greenhouse gas emissions by enhancing soil microbial activity, thus promoting more sustainable crop growth (Schmalenberger and Fox, 2017).

The carbon footprint of lettuce production in greenhouses is also influenced by various operational practices, such as irrigation methods and temperature regulation. It has been explored the role of irrigation methods in influencing soil gaseous emissions in lettuce fields, emphasizing that efficient irrigation can reduce both water and nitrogen losses, thereby minimizing the overall carbon footprint of the system (Wang et al., 2024). Furthermore, has been provided a comprehensive analysis of environmental factors impacting the quality and sustainability of greenhouse-grown vegetables, suggesting that integrated management practices, including optimized fertilization, are crucial for reducing the environmental footprint (Gruda, 2005). Continuous organic and mineral fertilization and their impact on the soil microbiome and CO_2 emissions have been studied (Kuncheva et al., 2024).

The growing body of literature has shown that integrating biological fertilization methods, such as the use of biochar and other organic amendments, can mitigate the carbon footprint of greenhouse crop production (Pereira et al., 2015; Zhang et al., 2023). Biofertilizers, which harness the power of beneficial soil microbes, have been identified as a key tool in promoting sustainable agriculture by reducing reliance on mineral fertilizers (Schmalenberger & Fox, 2017). Biochar, in particular, has been shown to alter nitrogen transformations in soil, thereby reducing nitrous oxide emissions and enhancing soil carbon sequestration (Qi et al., 2020).

In terms of life cycle analysis (LCA), several studies have quantified the total environmental impact of lettuce production systems. They provide an LCA of nitrogen fertilizers, highlighting the substantial contribution of nitrogen application to global warming potential (Lättemäe, Särekanno, and Järvenpää, 2020). Similarly, LCA has been employed to evaluate the carbon emissions of fresh produce supply chains in Western Australia, including lettuce, and found that fertilizer use and transportation significantly contributed to the carbon footprint (Gunady et al., 2012).

Overall, the literature indicates that both fertilization type and greenhouse management practices play pivotal roles in determining the carbon footprint of lettuce cultivation. Reducing emissions while maintaining yield requires careful consideration of both fertilizer type and operational strategies, a balance that this research aims to explore in depth. This research focuses on evaluating the carbon footprint of lettuce grown under two contrasting fertilization strate-/gies-mineral and biological-within greenhouse systems. This study aims to evaluate the carbon footprint of lettuce grown under contrasting fertilization strategies-mineral and biological-within controlled greenhouse environments.

By integrating insights from microbiological interactions and soil gas emissions, the research seeks to provide a comprehensive understanding of sustainable lettuce production.

The findings will contribute to the broader discussion on optimizing resource use in horticulture while minimizing adverse environmental consequences.

MATERIALS AND METHODS

The experiment was conducted during the period 2021-2024 in an unheated polyethylene greenhouse at the Agricultural University of Plovdiv. The study examined the amount of greenhouse gases emitted during lettuce cultivation. It investigated the impact of mineral and organic fertilization on gas exchange within the soil-plant-atmosphere system. Two fertilization treatments were tested: mineral fertilization (NPK) and Vitaorganic, with an unfertilized control. The study was carried out with lettuce of the Maritima variety (Batavia type). The experiment was set up with four replications, each consisting of 28 plants per plot. Fertilization was applied during the final soil preparation. The organic fertilizer Vitaorganic was applied at a rate of 1500 kg/ha, while mineral fertilization was conducted using potassium sulfate, triple superphosphate, and ammonium nitrate (N – 125 kg/ha, P_2O_5 – 41.7 kg/ha, K_2O – 158.3 kg/ha). The lettuce was planted at the 4-5 leaf stage in early November, following a spacing scheme of 70 + 30 + 30 + 30/30 cm. During the vegetation period, all planned agronomic practices were carried out, and irrigation was performed using a drip system.

1. ***Influence of the type of fertilizer used on the CO2 emissions released by soil microflora***

The influence of the fertilizer used on the emission of carbon dioxide from fertilized and unfertilized soils was studied. The difference in the amounts of gas between the chambers of the variants of unfertilized, organic and mineral fertilization without plants was calculated. The data are plotted in graphs 1-3.

The values X0 shown in the graphs were calculated using the formulas:

$$X0_{soil\ NPK} = C_{\%CO_2}^{contr.soil} - C_{\%CO_2}^{NPK\ fert.soil.}$$

$$X0_{soil\ BIO} = C_{\%CO_2}^{contr.soil} - C_{\%CO_2}^{BIO\ fert.soil}$$

where:

- $X0_{soil\ NPK}$ represents the NPK-fertilized value in the graphs, and $X0_{soil\ BIO}$ represents the BIO-fertilized value.
- $C_{\%CO_2}^{contr.soil}$ is the percentage concentration of CO_2 in the control soil chamber.
- $C_{\%CO_2}^{NPK\ fert.soil}$ and $C_{\%CO_2}^{BIO\ fert.soil}$ are the percentage CO_2 concentrations in the chambers with mineral (NPK) and organic fertilization, respectively.

2. ***Influence of grown plants on CO_2 emissions***

The difference in the amounts of gas between variants with and without plants in chambers without fertilization, organic and mineral fertilization was calculated.

The values for gas exchange in the NPK variant, plotted in graphs, were calculated according to the formulas:

$$X1_{NPK\ fert.} = C_{\%CO_2}^{NPK\ fert.soil.} - C_{\%CO_2}^{NPK\ fert.soil+plant}$$

where:

- $X1_{NPK\ fert.}$ is the NPK-fertilized value in the graphs.
- $C_{\%CO_2}^{NPK\ fert.soil+plant}$ is the percentage concentration of CO_2 in the chamber with NPK-fertilized soil containing plants.

The calculations for the other variants were made similarly.

3. ***Influence of fertilization and plants on CO_2 emissions***

The difference in gas quantities between the unfertilized variant without plants and the chambers without fertilization, organic and mineral fertilization with plants was calculated.

The gas exchange values for the NPK variant, plotted in graphs, were calculated according to the formulas:

$$X3_{NPK} = (C_{\%CO_2}^{contr.soil} - C_{\%CO_2}^{NPK\ fert.+plant}) \times 10 \times 44{,}01/22{,}4,\ g/m^3$$

where:

- $X3_{NPK}$ is the NPK-fertilized value in the graphs, calculated in g/m³.
- $C_{\%CO_2}^{contr.soil}$ и $C_{\%CO_2}^{NPK\ fert.+plant}$ are the percentage concentrations in the respective chambers.
- M_{CO_2}= 44.01 g/mol is the molecular mass of CO_2.
- V_{CO_2}= 22.4 L is the molar volume of gas.

The calculations for the other treatments followed the same methodology.

Further calculations in the tables include:

$$X_{average}^{NPK} = \left[\sum_{09.02.to\ 06.04} X3_{NPK}\right]/9$$

$$X_{CO_2}^{NPK\ sum} = X_{average}^{NPK} \times 57\ days$$

where:
- $X_{average}^{NPK}$ is the average value of $X3_{NPK}$ across the measured dates.
- $X_{CO_2}^{NPK\ sum}$ is the modeled CO_2 absorption for the NPK treatment over 57 days.

The calculations for the other options are made analogously to those for the NPC.

Gas Composition Monitoring and Measurement

The gas composition was monitored dynamically by measuring CO_2 exchange. Gas composition analysis was performed using chambers measuring 0.5 × 0.5 × 0.5 m³, placed weekly over the experimental plots for 24 hours, covering four plants per chamber. The chambers were embedded 10 cm into the soil to ensure airtight conditions and prevent gas leakage.

After 24 hours, air samples were collected from each chamber. CO_2 concentration was measured every seven days over a 68-day experimental period, until the end of the harvest. The quantitative determination of CO_2 was performed using a gas chromatograph INTERSMAT IGC 112M with Porapak "Q" columns and a catharometer detector.

Microclimatic factors such as solar radiation, humidity, and air and soil temperature were monitored using a Meteobot weather station. The gas analysis results were presented in both graphical and tabular form, showing the relationship between CO_2 exchange and the interaction between lettuce plants and environmental factors.

Calculations were based on gas sample collection from chambers with different combinations of fertilization and plant presence:

1. Control: CO_2 percentage in an unfertilized, plant-free soil chamber.
2. NPK Fertilization: CO_2 percentage in an NPK-fertilized, plant-free soil chamber.
3. Organic Fertilization: CO_2 percentage in an organically fertilized, plant-free soil chamber.
4. NPK with Plants: CO_2 percentage in an NPK-fertilized soil chamber with plants.
5. Organic Fertilization with Plants: CO_2 percentage in an organically fertilized soil chamber with plants.

To estimate CO_2 emissions (g/m³) for fertilized and unfertilized treatments over 50–60 days during the final growth phase of lettuce, a mathematical model was applied. The model used values derived from the difference between the accumulated CO_2 in the control chamber (unfertilized, plant-free) and those in chambers with plants (unfertilized, NPK, and Vitaorganic treatments).

The model assumed that daily CO_2 exchange remained constant, averaging all measurements taken during the observation period. The sum of emission and absorption values was divided by the number of measurements to estimate total CO_2 exchange during the vegetation period. The average daily values were multiplied by the duration of the experiment (57 days in 2022, 57 and 56 days in 2023 and 2024, respectively).

RESULTS AND DISCUSSIONS

The results of the three-year study confirm that the dynamics of CO_2 in the soil is a complex process influenced by multiple interconnected factors, including soil temperature, moisture, solar radiation, plant cover, and type of fertilization. The values in the graphs below represent the difference in CO_2 quantity between the experimental chambers under different comparisons. Positive values indicate that the plants (or soil) in the respective variant absorb CO_2, while negative values indicate emission.

1. *Influence of Fertilizer Type on CO_2 Emissions from Soil Microflora*

The analysis of the recorded CO_2 concentration values allows for determining the impact of key agro-climatic factors on the CO_2 balance.

1.1. *Influence of Soil Temperature on CO_2 Dynamics*

The data from the study are presented in Figures 1-3. During the measurement period, the soil temperature ranged between 11ºC and 19ºC, with no significant changes in depth (Figure 1). For both fertilization variants, a general trend was observed - a decrease in CO_2 emissions with increasing soil temperature. This confirms the importance of soil temperature in relation to microbiological activity and the decomposition of organic matter, which leads to either CO_2 release or absorption. On April 4th, significant CO_2 absorption was recorded for both fertilization types compared to other measurements, at soil temperatures ranging from 17ºC to 19ºC. Since the analyzed variant

was without plants, it can be assumed that the absorption was due to increased microbiological activity.

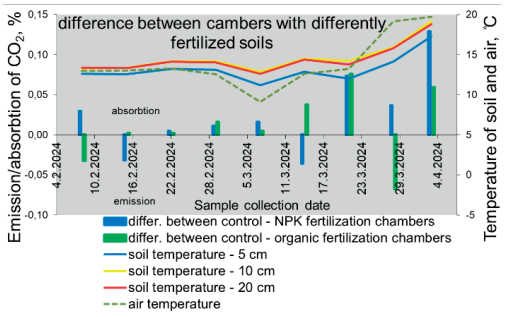


Figure 1. Influence of soil and air temperature on CO_2 emission/absorption in chambers without plants

A comparative analysis between the NPK and organic fertilization variants revealed a significant difference. CO_2 absorption at higher temperatures in the NPK chamber was twice as high as in the BIO chamber. This is likely due to the higher solubility and availability of mineral fertilizer, which increases the rate of uptake by microorganisms and plants.

On February 8th and March 28th, CO_2 absorption was observed in the NPK variant, while the BIO variant exhibited emissions. On these dates, soil temperatures were approximately the same, with a difference of about 2°C. At the same time, under similar temperature conditions, the opposite effect was observed-CO_2 emissions from NPK and absorption in the BIO-fertilized variant (February 15th, March 14th). This suggests that soil temperature alone is not the determining factor in CO_2 emissions or absorption.

Within a temperature range of 10-20°C, an inverse relationship was observed between temperature and CO_2 emissions-higher temperatures were associated with lower emissions. Despite this trend, opposing effects were recorded at similar temperatures during certain periods, highlighting the influence of additional factors.

1.2. *Influence of Soil Water Content on CO_2 Dynamics*

The water content in the upper soil layers varied significantly throughout the experiment, ranging from 3 to 32 l/m² depending on the sampling date and soil depth (Figure 2). As expected, soil moisture was higher in the middle and deeper layers (5, 10, and 20 cm).

On colder days (February 8th and March 7th), low moisture levels in the upper layer increased CO_2 emissions only in the organic fertilization variant. As moisture levels increased, soil permeability decreased, leading to lower CO_2 emissions. A similar trend was observed on March 28th-when the moisture level in the upper soil layer dropped below 21 l/m², CO_2 emissions increased in the organic fertilization variant.

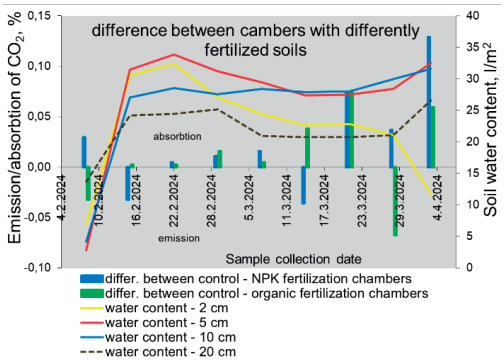


Figure 2. Influence of soil water content on CO_2 emission/absorption in chambers without plants

The data from April 3rd show a reverse trend - further decreases in soil moisture were associated with significant CO_2 absorption. Once again, this confirms that CO_2 balance is influenced by multiple overlapping variables rather than a single factor.

A comprehensive assessment of CO_2 dynamics indicates that soil moisture affects metabolic processes, with higher moisture levels enhancing microbial activity and leading to increased CO_2 emissions. The surface soil layer plays a crucial role as a barrier that regulates CO_2 release from deeper layers.

From a chemical perspective, alkaline soils tend to absorb more CO_2 compared to neutral and acidic soils. In alkaline conditions, CO_2 can form carbonates, bicarbonates, and mixed hydroxycarbonates, allowing it to remain in crystalline form.

High soil moisture (>25%) correlates with intense microbial activity and increased emissions, whereas low moisture levels (<15%) result in enhanced CO_2 absorption, particularly in the organic fertilization variant. This confirms that CO_2 balance is determined by the interaction of multiple factors rather than individual influences.

1.3. *Influence of Solar Energy on CO_2 Dynamics*
Solar energy varied between 0.023 and 0.151 kWh/m² during the measurement period (Figure 3). The data indicate that on days with higher soil temperatures and intense solar activity, CO_2 absorption increased significantly.
Notably, on days with higher solar radiation, such as March 21st and April 3rd, a substantial increase in CO_2 absorption was observed. The figures demonstrate that soil temperature rises in proportion to increasing solar activity. Since the illuminated surface is relatively small compared to the total volume of soil in the experiment, the leading factor influencing CO_2 absorption appears to be soil temperature.

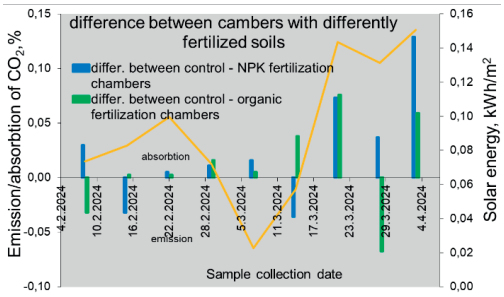


Figure 3. Influence of solar energy on CO_2 emission/absorption in chambers without plants

2. ***Influence of Cultivated Plants on CO_2 Emissions Under Organic and Mineral Fertilization, With and Without Plants*** (Figures 4, 5, 6)
For most of the experiment, the variants with plants (especially in chambers with mineral and organic fertilizers) showed a tendency to absorb CO_2, particularly on days with higher temperatures (Figure 4).

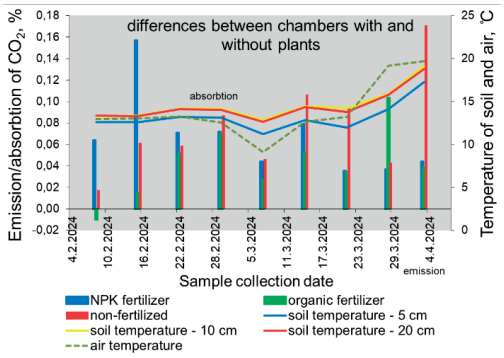


Figure 4. Influence of soil and air temperature on CO_2 emission/absorption in chambers with plants

A natural course of absorption is also observed with an increase in the leaf mass of the plants. Negative values in the data indicate that there is an additional amount of CO_2 in the chambers compared to the unfertilized soil.
On some dates, especially during the winter months and at lower temperatures, the (NPK) variant shows a significant release of CO_2, probably due to intense microbial activity associated with the decomposition of the fertilizer in the soil (Figure 5).

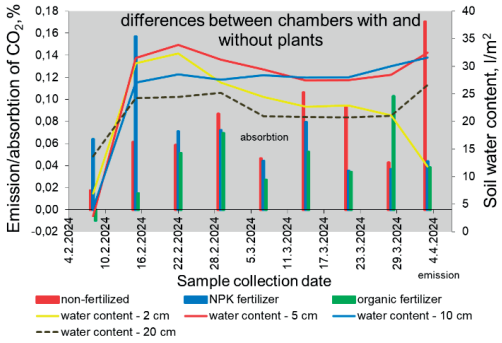


Figure 5. Influence of soil water content on CO_2 emission/absorption in chambers with and without plants

The results show that organic and mineral fertilization have different effects on the CO_2 balance. NPK fertilization leads to greater dynamics in the CO_2 values and is more dependent on meteorological conditions and the phenophase of the plants (Figure 6).

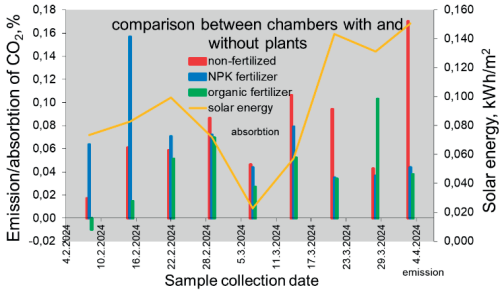


Figure 6. Influence of solar energy on CO_2 emission/absorption in chambers with and without plants

On some days (16.02.), significant CO_2 absorption is observed, which can be associated with the activity of microorganisms decomposing the fertilizer in the soil. The better availability of mineral fertilizers to soil microorganisms. The high water solubility of mineral fertilizer, its

uptake and metabolism by microorganisms is significantly accelerated compared to biofertilizer. In later dates, especially after March, there is a trend towards greater CO_2 absorption.

3. *Influence of fertilization and plants on CO_2 emissions*

The experimental results are shown in Figures 7-9. Stoichiometric calculations are based on the air volume in the chambers, the molar mass and molar volume of the gas. The CO_2 values are calculated in g/m^3. The data indicate that the more intensive growth of plants with increasing daily temperatures accelerates photosynthesis and compensates for the release of gases from the soil (Figure 7). The biofertilizer variant shows constant and stable CO_2 absorption values compared to mineral fertilizer. This can be associated with more balanced microbial activity in the soil, as well as better and more consistent decomposition of organic matter in the biofertilizer over time. As vegetation progresses, the biofertilizer variant tends to reduce CO_2 (Figure 8). This suggests a better long-term ecological effect of organic fertilizer compared to mineral fertilization. At the beginning of vegetation, the mineral fertilization (NPK) variant shows higher CO_2 absorption values. This can be explained by intense microbial activity and the initially small leaf mass of the lettuce. As vegetation progresses and photosynthesis intensifies, increased CO_2 absorption is observed. Of all the factors studied, soil temperature and solar activity have the greatest influence on emissions (Figures 7 and 9).

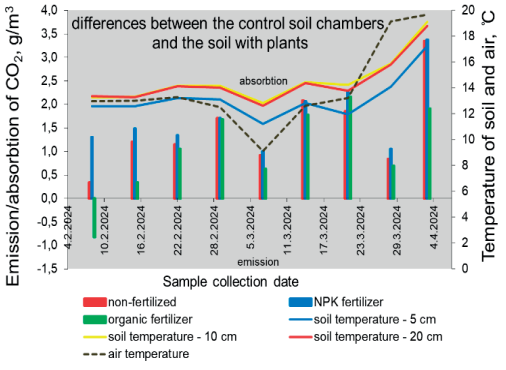


Figure 7. Influence of soil and air temperature on CO_2 emission/absorption in chambers with and without plants

It is observed that CO_2 absorption increases proportionally with increasing temperature and solar energy. The recorded results of carbon dioxide emissions show that even after planting, plants begin to absorb this gas in the variants without fertilization and those fertilized with biofertilizer. Plants from these variants manage to compensate for the CO_2 release from microorganisms in the soil. Low solar activity and temperature slow down plant photosynthesis. The increase in CO_2 emissions from the soil is facilitated by low moisture in the topsoil layer and more active microbiological metabolism (Figures 7 and 9).

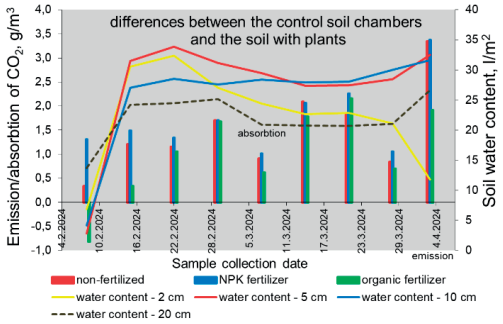


Figure 8. Influence of soil water content on CO_2 emission/absorption in chambers with and without plants

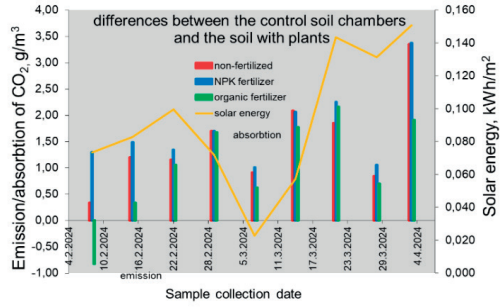


Figure 9. Influence of solar energy on CO_2 emission/absorption in chambers with and without plants

Tables 1-3 show the calculation results for the absorbed carbon dioxide from the plant chambers over a relatively equal period of time for the three research years. As sampling was performed once a week, as mentioned above, a mathematical model of averaging and estimation was used.

The values highlight that mineral fertilization (NPK) generally leads to the highest total CO_2 uptake, with values reaching 96.97 g/m^3 in 2024.

Organic fertilization shows more stable uptake trends but is weaker than NPK at high temperatures. Interestingly, the unfertilized chambers in 2023 demonstrate the highest sequestration efficiency (94.72 g/m³), possibly due to the optimal balance of microbial and plant activity under the prevailing climatic conditions.

The analysis of the data shows that in the experimental year 2022, the largest absorbed amount of CO_2, within 86.43 g/m³, was recorded with mineral fertilization (Table 1) and the least in the unfertilized variant. Trends of increased absorption with mineral fertilization were also calculated for 2024.

Table 1. Total amount of CO_2 absorbed during the cultivation period, g/m³, 2022

2022/57 days sample collection date	Non-fertilized	NPK fertilizer	Organic fertilizer
9.2.2022	0.21	0.11	0.37
15.2.2022	0.64	0.66	0.49
23.2.2022	1.88	1.69	1.69
2.3.2022	0.02	0.40	0.33
9.3.2022	0.15	0.34	0.53
16.3.2022	0.75	0.65	0.41
23.3.2022	2.02	2.09	1.99
30.3.2022	3.56	3.85	3.90
6.4.2022	3.88	3.86	3.81
average value	1.46	1.52	1.50
Total amount of CO2 absorbed during the cultivation period	82.94	86.43	85.67

This fact can be explained not by the ongoing microbiological activity in the soil, but by the increased growth resulting from mineral fertilization.

Table 2. Total amount of CO_2 absorbed during the cultivation period, g/m³, 2023

2023/57 days sample collection date	Non-fertilized	NPK fertilizer	Organic fertilizer
18.1.2023	1.90	1.63	1.71
25.1.2023	1.41	1.20	0.98
1.2.2023	1.90	1.47	1.61
8.2.2023	1.95	1.85	1.75
15.2.2023	2.16	2.02	2.02
22.2.2023	1.30	1.56	1.54
1.3.2023	0.48	0.26	0.84
8.3.2023	2.69	2.62	2.21
15.3.2023	1.17	1.17	0.84
average value	1.66	1.53	1.50
Total amount of CO_2 absorbed during the cultivation period	94.72	87.29	85.49

Table 3. Total amount of CO_2 absorbed during the cultivation period, g/m³, 2024

2024/56 days sample collection date	Non-fertilized	NPK fertilizer	Organic fertilizer
8.2.2024	0.34	1.30	-0.82
15.2.2024	1.20	1.49	0.34
22.2.2024	1.15	1.34	1.06
29.2.2024	1.70	1.70	1.68
7.3.2024	0.91	1.01	0.62
14.3.2024	2.09	2.06	1.77
21.3.2024	1.85	2.25	2.16
28.3.2024	0.84	1.06	0.70
3.4.2024	3.35	3.37	1.91
average value	1.49	1.73	1.05
Total amount of CO_2 absorbed during the cultivation period	83.54	96.97	58.56

The averaged values of carbon dioxide absorption in the chambers of the different experimental variants for the three experimental years repeat the trends observed in the individual years (Table 4). The averaged values somewhat eliminate the climatic factors influencing emissions. Mineral fertilizers increase absorption by 3.63% compared to unfertilized soil and by 17.92% compared to organic fertilization. Although NPK increases absorption, its production has a high carbon footprint, especially from nitrogen fertilizers-in synthesis through the Haber-Bosch process (Smith, 2020).

Table 4. Average absorption values of the three years (2022-2024), g/m³

Average absorption values of the three years, g/m³		
non-fertilized	NPK fertilizer	organic fertilizer
87.07	90.23	76.57

Biological fertilization leads to reduced absorption (by 12.08% compared to unfertilized soil), which is due to the slower decomposition of organic matter. However, organic fertilizers have a lower carbon footprint because they come from natural sources and do not require energy-intensive production processes. Optimizing greenhouse conditions (humidity, temperature, aeration) can improve absorption and reduce the need for intensive fertilization. Mineral fertilization (NPK) increases absorption but has a high carbon footprint. Biological fertilization has lower absorption but better

environmental sustainability. A combined approach (mixing mineral and organic fertilizers) may be optimal for reducing the carbon footprint while maintaining good productivity.

CONCLUSIONS

From the experiment conducted to determine the influence of fertilization methods (biological and conventional) on greenhouse lettuce production during the autumn-winter period, and specifically the studied fertilizers, the following conclusions can be made:

The highest CO_2 emissions are observed in the unfertilized variant.

When growing lettuce with mineral fertilization in greenhouse conditions during the autumn-winter period, less CO_2 is released.

Soil moisture modulates the respiration of microorganisms, affecting both emissions and absorption.

The cultivated crop plays a key role in stabilizing CO_2 dynamics, mitigating extreme fluctuations.

NPK fertilization improves CO_2 capture but can lead to increased emissions under specific soil moisture conditions.

Temperature and solar activity stand out as dominant factors for CO_2 dynamics in the soil.

At low temperatures (< 10°C) and weak radiation (< 0.025 kWh/m²), increased emissions are recorded, while under favorable conditions (T > 10°C, high solar activity), plants and soil processes support CO_2 absorption.

Biological fertilization shows a longer-lasting positive effect on the carbon balance, while mineral fertilization has a short-term but more dynamic effect.

The results provide an empirical basis for optimizing soil conditions to improve the carbon balance in agroecosystems.

ACKNOWLEDGEMENTS

This study was conducted with funding provided by the Center for Research, Intellectual Property Protection and Technology Transfer at the Agricultural University - Plovdiv.

REFERENCES

Borisova, D., Kostadinova, G., Petkov, G., Dospatliev, L., Ivanova, I., Dermendzhieva, D., Beev, G. (2023). Assessment of CH4 and CO2 Emissions from a Gas Collection System of a Regional Non-hazardous Waste Landfill, Harmanli, Bulgaria, using the Interrupted Time Series ARMA Model. *Atmosphere*, 14, 1089 https://doi.org/10.3390/atmos14071089

Borisova, V., G. Kostadinova, G. Petkov, D. Dermendzhieva, and G. Beev (2022). An Assessment of Two Types of Industrially Produced Municipal Green Waste Compost by Quality Control Indices. *Applied Sciences, 12*(20): 10668. https://doi.org/10.3390/app122010668 .

Dermendzhieva, D., T. Dinev, G. Kostadinova, G. Petkov, G. Beev (2021). Agro-ecological characterization of vermicomposted sewage sludge from municipal and poultry enterprise wastewater treatment plants. *Sains Malaysiana, 50*(8), 2167-2178.

Gruda, N. (2005). Impact of environmental factors on product quality of greenhouse vegetables for fresh consumption. *Critical Reviews in Plant Sciences, 24*(3), 227-247. https://doi.org/10.1080/07352680591008628

Gunady, M. G. A., Biswas, W., Solah, V. A., & James, A. P. (2012). Evaluating the global warming potential of the fresh produce supply chain for strawberries, romaine/cos lettuces (*Lactuca sativa*), and button mushrooms (*Agaricus bisporus*) in Western Australia using life cycle assessment (LCA). *Journal of Cleaner Production*, 28, 81-87. https://doi.org/10.1016/j.jclepro.2011.12.031

Huang, R., Liu, J., He, X. H., Xie, D. T., Ni, J. P., Xu, C., Zhang, Y. R., Ci, E., Wang, Z. F., & Gao, M. (2020). Reduced mineral fertilization coupled with straw return in field mesocosm vegetable cultivation helps to coordinate greenhouse gas emissions and vegetable production. *Journal of Soils and Sediments, 20*(4), 1834-1845. https://doi.org/10.1007/s11368-019-02477-2

Kostadinova, G., D. Dermendzhieva, G. Petkov, G. Beev, K. Koev (2018). Evaluation of wastewater quality at the inlet-outlet of the most modern wastewater treatment plant in Bulgaria. *Fresenius Environmental Bulletin*, *27*(12 B): 9723-9738.

Kuncheva, G., Ginchev, G., Ivanova, I. (2024). Influence of Long-Term Mineral Fertilization on Soil Microbiota, Organic Matter Content and CO2 Emissions. In: Chenchouni, H., et al. Recent Advancements from Aquifers to Skies in Hydrogeology, Geoecology, and Atmospheric Sciences. MedGU 2022. Advances in Science, Technology & Innovation. Springer, Cham. https://doi.org/10.1007/978-3-031-47079-0_31

Lättemäe, P., Särekanno, M., & Järvenpää, S. (2020). Life cycle assessment of nitrogen fertilizers. Agricultural and Food Science, 29(4), 258-268. https://doi.org/10.23986/afsci.93250

Pereira, B. de J., Cecilio Filho, A. B., & La Scala Jr., N. (2021). Greenhouse gas emissions and carbon footprint of cucumber, tomato and lettuce production using two cropping systems. *Journal of Cleaner Production*, 282, Article 124517. https://doi.org/10.1016/j.jclepro.2020.124517

Pereira, E. I. P., Suddick, E. C., Mansour, I., Mukome, F. N. D., Parikh, S. J., Scow, K., & Six, J. (2015). Biochar alters nitrogen transformations but has minimal effects on nitrous oxide emissions in an organically managed lettuce mesocosm. *Biology and Fertility of Soils, 51*(5), 573–582. https://doi.org/10.1007/s00374-015-1004-5

Qi, L., Pokharel, P., Ni, C., Gong, X., Zhou, P., Niu, H., Wang, Z., & Gao, M. (2020). Biochar changes thermal activation of greenhouse gas emissions in a rice-lettuce rotation microcosm experiment. *Journal of Cleaner Production*, 247, Article 119148. https://doi.org/10.1016/j.jclepro.2019.119148

Rahman, M. M., Alam, M. A., & Hossain, M. S. (2018). Environmental and economic impacts of lettuce production: A case study on resource efficiency. *Journal of Cleaner Production*, 172, 3374-3382. https://doi.org/10.1016/j.jclepro.2017.11.136

Schmalenberger, A., & Fox, A. (2017). Biofertilizers: Harnessing the power of beneficial soil microbes. *Advances in Agronomy*, 145, 95-135. https://doi.org/10.1016/bs.agron.2017.02.002

Smith, J. (2020). Industrial Chemistry and the Haber-Bosch Process. Oxford University Press.

Thapa, R., Chatterjee, A., & Awale, R. (2016). Nitrous oxide emissions and mitigation strategies for agriculture: A review. *Sustainability*, *8*(10), 877. https://doi.org/10.3390/su8100877

Wang, J., Hamoud, Y. A., Zhu, Q., Shaghaleh, H., Chen, J., Zhong, F., & Hou, M. (2024). Soil gaseous carbon emissions from lettuce fields as influenced by different irrigation lower limits and methods. Agronomy, 14(3), Article 563. https://doi.org/10.3390/agronomy14030563

Windisch, L., Atzler, C., Heintz-Buschart, A., Huson, D. H., Soman, C., Hubschmann, T., & Schloter, M. (2021). Impact of long-term organic and mineral fertilization on rhizosphere microbiota, root exudates, and defense-related gene expression in lettuce. Frontiers in Microbiology, 12, 783854. https://doi.org/10.3389/fmicb.2021.783854

Zhang, J., Sun, H., Ma, J., Zhang, X., Wang, C., & Zhou, S. (2023). Effect of straw biochar application on soil carbon, greenhouse gas emissions, and nitrogen leaching: A vegetable crop rotation field experiment. *Soil Use and Management, 39*(2), 729–741. https://doi.org/10.1111/sum.12877

Zhang, Y., Liu, X., Wang, J., Li, H., & Chen, Q. (2024). Soil gaseous carbon emissions from lettuce fields as influenced by different irrigation lower limits and methods. *Agronomy, 14*(3), 563. https://doi.org/10.3390/agronomy14030563

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

THE INFLUENCE OF MICROORGANISMS ON THE QUANTITATIVE AND QUALITATIVE CHARACTERISTICS OF TOMATO FRUITS

Claudia-Loredana DRAGOMIR[1], Gheorghița HOZA[1], Alexandra BECHERESCU[3], Mihaela PARASCHIV[4], Claudia NICOLA[5], Mihai FRÎNCU[1, 2], Liliana BĂDULESCU[1, 2], Dorel HOZA[1]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăşti Blvd, 011464, District 1, Bucharest, Romania
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, Research Center for Studies of Food Quality and Agricultural Products, 59 Mărăști Blvd, 011464, District 1, Bucharest, Romania
[3]University of Life Sciences "King Mihai I" from Timişoara, 119 Calea Aradului Street, 300645, Timișoara, Romania
[4]Research and Development Institute for Vegetable and Flower Growing Vidra, 22 Calea Bucureşti, Vidra, Ilfov, Romania
[5]National Research and Development Institute for Biotechnology in Horticulture Ştefăneşti - Argeş, 37 Bucharest-Piteşti Road, Ştefăneşti, Argeş County, Romania

Corresponding author email: mihaela1mail@yahoo.ca

Abstract

Tomato (Solanum lycopersicum L.) is one of the vegetables with the highest global production value. In an effort to achieve an increasing both quantity and quality production, researchers have been and are still looking for strategies to improve crop technology using various plant protection and stimulation products.
Over the years, different technologies have been developed for cultivating and treating plants with Bacillus spp. in order to increase plant productivity but also to reduce residual elements in plants, thus helping to protect the consumer. The present study aims to highlight the effect of the microbial inoculants Rizobac and Bactilis on fruit quality indicators for the three tomato hybrids. Both microbial inoculations had a tendency to increase fruit size in the treated tomato hybrids, for total dry matter they ranged between 4.03% and 4.38% for Buffalosun F1, pH ranged from 4.07 to 4.12 for Kingset F1, from 4.05 to 4.18 for Bucanero F1 and from 4.01 to 4.21 for Buffalosun F1, when Bactilis was applied.

Key words*: beneficial bacteria, fertilization, microorganisms, production, environmental protection, tomatoes.*

INTRODUCTION

The tomato *(Solanum lycopersicum* L.) is one of the most valuable vegetables globally in terms of production (Nanu et al., 2024). It is consumed in a wide variety of forms and provides numerous health benefits due to its high content of lycopene, folic acid, ascorbic acid, flavonoids, α-tocopherol, potassium, and phenolic compounds (Erba et al., 2013).
Tomatoes are part of the human diet both in fresh form and in various processed products such as tomato juice, tomato paste, puree, and dried tomatoes (Wu et al., 2022). These products are consumed in greater quantities than fresh tomatoes (Reimers & Keast, 2016), while green or semi-ripe tomatoes are used for pickling, candying, and preservation. Due to their versatility, tomatoes are highly valued and widely consumed around the world, being incorporated into numerous culinary recipes (Javaria et al., 2012). Their high antioxidant content provides significant health benefits (Li et al., 2024), and their seeds can be used to extract a valuable edible oil (Rubatzky & Yamaguchi, 1997).
Praised for their distinct aroma, tomatoes owe their unique taste to the combination of sugars, organic acids, free amino acids, volatile organic compounds (VOC), and the complex interactions between these elements (Tieman et al., 2012).
High serum levels of lycopene, resulting from tomato consumption and derived products,

contribute to reducing the risk of stroke and ischemic stroke in men (Karppi et al., 2012). Additionally, carotenoids, predominantly from tomatoes, may protect against breast cancer (Aune et al., 2012; Sato et al., 2002) and reduce the incidence of prostate, lung, and stomach cancer (Giovannucci, 2002).

Due to their rich content of minerals and antioxidants, tomatoes are essential in human nutrition, helping to neutralize free radicals in cells and preventing various diseases that can affect health (Kalogeropoulos et al., 2012; Porto et al., 2016).

To achieve higher and better-quality agricultural yields, researchers have explored and continue to develop technological solutions that include the use of plant protection and stimulation products. A key step in this direction has been the adoption of products that do not harm the environment or the treated plants.

Over time, cultivation and treatment technologies based on *Bacillus* spp. have been developed, aiming to increase plant productivity while reducing the accumulation of chemical residues, thus protecting consumers. *Bacillus* spp. is a bacterium recognized for its benefits in various fields, and research continues to fully assess its usefulness. This study aims to highlight the beneficial effects of biological products based on *Bacillus* spp. through their application at the root level. The research targets both agricultural producers, by providing efficient alternatives for conventional and organic technologies, and consumers, by promoting less polluted products.

The use of *Bacillus* spp. in agriculture is a current and innovative practice due to its multiple roles: natural biocontrol against pathogens (inhibiting the development of fungi and harmful bacteria), plant growth stimulation (by colonizing roots and enhancing nutrient absorption), bioremediation and ecological agriculture (degrading toxic compounds and fixing atmospheric nitrogen, thereby reducing pollution and protecting biodiversity), as well as emerging technologies based on *Bacillus* spp. for optimizing agricultural production.

Recent research explores the integration of these bacteria into precision agriculture, using advanced technologies such as drones and automated systems for intelligent treatment application.

Biological control, through the use of microorganisms to combat diseases and pests in vegetable crops, represents a valuable alternative to chemical treatments applied to tomatoes. *Bacillus* and *Paenibacillus* species help reduce pathogens either by producing metabolites with antibiotic effects or by directly stimulating the plant's natural defense mechanisms. Additionally, some strains improve nutrient absorption by promoting symbioses or directly fixing atmospheric nitrogen (Gardener, 2004).

The increasing concentration of heavy metals in the soil, caused by unsustainable agricultural practices, excessive use of chemical fertilizers, and large-scale pesticide application, poses a serious threat to soil ecosystems and environmental health (Jaishankar et al., 2014). In this context, the use of organic fertilizers and manure, alongside sustainable management practices, can reduce dependence on chemical fertilizers. This method helps farmers lower costs by providing a slow-release source of nutrients, maintaining soil fertility and long-term agricultural productivity (Praharaj, 2007).

The degradation of agricultural land due to intensive chemical fertilizer use has led farmers to explore alternative cultivation methods. Thus, there is a growing interest in organic farming and the implementation of efficient water and nutrient management techniques (Verma et al., 2023).

Unicellular microorganisms, such as bacteria and protozoa, play an essential role in soil ecology, being present everywhere - in water, air, soil, and food (Kadner & Kara Rogers, 2024). These microorganisms contribute to carbon fixation in low-vegetation areas and transform atmospheric nitrogen into organic compounds (Peng et al., 2021). Soil fertility depends on its biological component, which includes bacteria, viruses, fungi, and other essential microorganisms that maintain soil health.

Although bacteria are often associated with pathogens, most are harmless and play a beneficial ecological role, supporting life and ecosystem balance (Singh et al., 2019). The rhizosphere provides a crucial habitat for soil microorganisms, facilitating plant-

microorganism interactions. Among these, species from the *Bacillus* spp. genus are plant growth-promoting rhizobacteria (PGPR), improving mineral nutrient absorption and crop yields (Kalam et al., 2020).

Tomatoes, as a high-yield crop, require a substantial nutrient supply to support growth and maximize production. They respond positively to various technological interventions, such as increasing the number of stems to boost yield or using biofertilizers containing substances like arginine and cysteine (Apahidean et al., 2021; Becherescu et al., 2021; Hoza et al., 2019).

Applying nitrogen (N) in large quantities is a widespread agricultural practice. However, rising fertilizer costs have led farmers to optimize their usage and manage financial resources more efficiently. An alternative method for reducing nitrogen consumption is soil inoculation with *Bacillus pumilus*, which biologically fixes nitrogen and enhances its absorption by tomatoes. Additionally, *Bacillus pumilus* induces leaf transpiration, facilitating nitrogen transport to the shoots (Masood et al., 2020).

Among the bacteria recently used in agriculture is *Bacillus velezensis* 83, which, after application, has shown a positive impact on the productivity of greenhouse-grown tomatoes (Balderas-Ruíz et al., 2021). Furthermore, bacterial use can begin at the seed stage, with *Bacillus cereus* demonstrating improved germination rates and a higher vigor index compared to untreated seeds (Guo et al., 2019).

In addition to nitrogen absorption, *Bacillus* spp. promotes plant growth by enhancing phosphorrus uptake and producing phytohormones such as auxins, enzymes like ACC deaminase, and volatile organic compounds (VOC) such as 2,3-butanediol and acetoin (Asari et al., 2016; Borriss, 2020; Fazle Rabbee & Baek, 2020).

Another major benefit of using microorganisms is stimulating the absorption and transport of nutrients, particularly the solubilization of insoluble zinc compounds and their assimilation by plants (Mumtaz et al., 2017).

Bacterial colony metabolism significantly enhances biological processes in the soil and nutrient assimilation efficiency. These effects can lead to a remarkable increase in tomato production, estimated at up to 36.82%, supporting both productivity and agricultural sustainability (Dragomir & Hoza, 2022; Wang et al., 2024).

In conclusion, bacteria play a crucial role throughout the entire technological process, from sowing and harvesting to fertilization and fruit storage. These microorganisms represent a valuable resource, continuously supporting every stage of plant cultivation.

MATERIALS AND METHODS

Location and Experimental Design

The experiment was conducted in Scărișoara, Olt County, located in the Oltenia region of Romania. The locality is situated in the southern part of the county, approximately 30 km from Caracal and 75 km from Slatina, with coordinates 43.9934 latitude and 24.5719 longitude (Romanian Population, 2024). The area's terrain is predominantly flat, and the climate is temperate-continental, characterized by very hot summers and relatively mild winters.

The experiment was organized using a completely randomized block design, including two fertilization treatments and one unfertilized control, applied to three tomato hybrids. Each treatment had three replications, with 15 plants per replication.

Experimental Factors

Factor A - Indeterminate-growth tomato hybrids intended for greenhouse and tunnel cultivation: Kingset F1, Bucanero F1, Buffalosun F1.

Factor B - Fertilization treatments: Control (untreated), Rizobac fertilizer (5 L/ha), Bactilis fertilizer (5 L/ha). Both fertilizers are certified for organic farming. By combining the two experimental factors, nine experimental variants were obtained.

Fertilizer Application

Fertilizers were applied via fertigation, starting immediately after crop establishment in the greenhouse, according to Table 1, which details the biofertilizer application schedule at regular intervals in each decade of the month, over three years (2022-2024).

The application frequency remained constant, with minor variations across the years, reflecting efficient fertilizer management. The structured application plan ensured optimal plant nutrition.

Table 1. The application schedule of treatments

Number of applications	Application period		
	Year 2022	Year 2023	Year 2024
1	Early April	Early April	Early April
2	Late April	Mid-April	Mid-April
3	Mid-May	Late April	Early May
4	Late May	Mid-May	Mid-May
5	Early June	Late May	Early June
6	Mid-June	Early June	Mid-June
7	Early July	Mid-June	Late June
8	Mid-July	Early July	Early July

Tomato Cultivation Technology

The tomato cultivation followed standard greenhouse production practices. The study was conducted over three years (2022-2024) in the greenhouses of a private producer in Scărișoara, Olt County.

Biological Material

Kingset F1 - Indeterminate tomato hybrid, ideal for early production in protected spaces.

Bucanero F1 - A fleshy tomato variety with dark brown, nearly black fruits. This vigorous hybrid has short internodes and produces black tomatoes of the Kumato type.

Buffalosun F1 - Indeterminate, vigorous, and compact tomato hybrid, known for its extra-early maturity. The fruits are large with a yellow-orange coloration.

Product Descriptions

Rizobac - A microbial inoculant rich in nutrients and beneficial microorganisms, designed to improve root establishment, facilitate rapid crop stabilization, and enhance root penetration and expansion. It contains a population of beneficial soil bacteria with a concentration of 1×10^{11} CFU/L, contributing to maintaining a healthy soil environment.

Bactilis - A microbial inoculant containing beneficial bacteria in the form of endospores. After application, the spores germinate rapidly, and the resulting bacteria multiply and colonize plant roots, promoting plant growth.

Measurements and Determinations

Number of fruits per plant - Obtained by summing all fruits from the inflorescences.

Fruit set percentage - Determined using the formula: % Fruit set = Total number of fruits × 100)/Total number of flowers

Yield per plant - Calculated using the formula: Yield per plant = Number of fruits per plant × Average fruit weight. The value was expressed in kg/plant.

Yield per hectare - Calculated using the formula: Yield per ha = Yield per plant × Number of plants per hectare (35 000). The value was expressed in t/ha.

Average fruit weight - Measured using the precision balance PS 6000.R2. The value was expressed in grams (g).

Fruit height and circumference - Measured using a digital caliper micrometer. The value was expressed in millimeters (mm).

Firmness N - Determined using a digital penetrometer (53205 TR Italia) with an 8 mm piston.

Total soluble solids - Measured with a Kruss DR301-95 digital refractometer.

Total dry matter - Determined by weighing fresh material, drying in a MEMERT UN 110 oven at 105˚C until constant weight, cooling in a desiccator, and reweighing. The value was expressed in percentage (%).

Total titratable acidity (citric acid %) - Measured using an automatic TitroLine Easy titrator.

Ascorbic acid content - Determined by extracting 1 g of raw material, ground with 2 mL of 2% (v/v) orthophosphoric acid for 1 minute at room temperature. The mixture was quantitatively transferred to a 15 mL centrifuge tube and brought to a final volume of 10 mL with 2% (v/v) orthophosphoric acid. After extraction, all samples were centrifuged, filtered, and stored for HPLC analysis (Stan et al., 2019). Quantification of ascorbic acid was performed using HPLC-DAD equipment (Agilent Technologies 1200 chromatograph), with results expressed in mg/100 g fresh matter of product.

Statistical Analysis

For data processing and interpretation, IBM SPSS 20 (SPSS Inc., Chicago, IL, USA) was used. Statistical analysis was conducted using a one-way ANOVA test (p=0.05), followed by Duncan's post-hoc test (p=0.05) for pairwise comparisons of sample means.

The results are presented as means and standard deviations, with means accompanied by letters; different letters indicate statistically significant differences (p=0.05). Similar to the multiple t-test, Duncan's test allows the comparison of multiple samples but employs a different calculation method and stricter significance levels.

RESULTS AND DISCUSSIONS

Number of Fruits per Plant

The obtained data indicate that the application of the biofertilizers Rizobac and Bactilis led to a significant increase in fruit production for the three hybrids compared to the unfertilized control.

Unfertilized plants recorded the lowest fruit counts (7.91-14.97 fruits/plant), indicating reduced physiological efficiency in the absence of biofertilizers. This may be attributed to limited absorption of essential nutrients, negatively affecting the generative development of plants.

The application of Rizobac resulted in a significant increase of the number of fruits, with values ranging from 17.14 to 9.31 fruits/plant. This effect is due to the presence of nitrogen-fixing bacteria and growth-promoting agents in its composition, which enhance nutrient uptake and plant metabolism, stimulating fruit formation.

Similarly, treatment with Bactilis led to significant increases of the number of fruits, with values between 8.89 and 16.69 fruits/plant. This biofertilizer contains beneficial sporulated bacteria capable of solubilizing phosphates in the soil and stimulating the synthesis of phytohormones, thereby promoting fruit set and reproductive development (Figure 1).

These results are consistent with findings from other scientific studies (Zhou et al., 2022), where a single inoculation with PGPR led to a 19% increase in the number of fruits - comparable to the 11-25% range observed in this study.

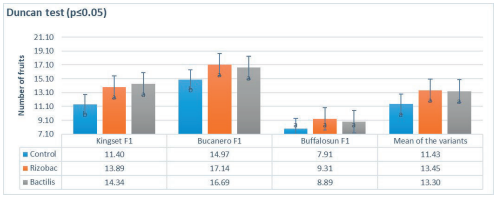


Figure 1. Influence of biofertilizer application on the average number of fruits per plant depending on the hybrids used

Fruit Set Percentage

Figure 2 illustrates the effects of Rizobac and Bactilis treatments on the fruit set percentage of Kingset F1, Bucanero F1, and Buffalosun F1, compared to the control variant.

For Kingset F1, the application of biofertilizers resulted in a significant increase in the fruit set percentage. The control variant recorded 65.47%, while treatments with Rizobac (72.36%) and Bactilis (71.35%) showed notable improvements, confirming their efficiency in optimizing reproductive processes.

In the case of Bucanero F1, biofertilizers contributed to an increase in the fruit set percentage compared to the control (60.10%), reaching 66.53% for Rizobac and 64.85% for Bactilis. The differences were significant, and the treated variants were classified in a higher-performing group than the control, indicating a beneficial effect on floral fertility.

For Buffalosun F1, the application of biofertilizers led to a moderate increase in the fruit set percentage, though without significant differences from the control. The recorded values were 49.84% for the control, 56.24% for Rizobac, and 55.87% for Bactilis, suggesting a limited influence on this hybrid.

The mean analysis of the variants indicates that biofertilizers had an overall positive effect on the fruit set process. The average fruit set percentage was 58.47% for the control, significantly increasing to 65.05% for Rizobac and 64.02% for Bactilis. These results confirm the contribution of biofertilizers to improving fruit formation, with a more pronounced impact on Kingset and Bucanero hybrids.

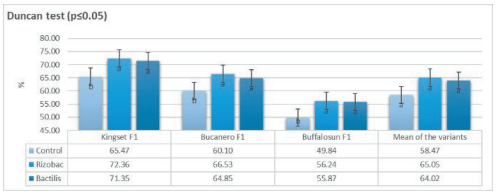


Figure 2. Influence of biofertilizer application on the set percentage depending on the hybrids used (%)

Tomato Yield per Plant

Figure 3 presents the effects of Rizobac and Bactilis treatments on tomato yield for Kingset F1, Bucanero F1, and Buffalosun F1, compared to the control variant.

For Kingset F1, the application of biofertilizers led to a significant increase in yield. The control variant recorded 2.35 kg/plant, while

treatments with Rizobac (3.08 kg/plant) and Bactilis (3.23 kg/plant) showed notable improvements, confirming their effectiveness in stimulating generative development.

In the case of Bucanero F1, biofertilizers increased yield compared to the control (3.02 kg/plant), reaching 3.27 kg/plant for Rizobac and 3.29 kg/plant for Bactilis.

For Buffalosun F1, the application of biofertilizers resulted in a moderate yield increase, but without significant differences from the control. The recorded values were 3.20 kg/plant for the control, 3.45 kg/plant for Rizobac, and 3.52 kg/plant for Bactilis, suggesting a limited influence of biofertilizers on this hybrid.

The mean analysis of the variants indicates that biofertilizers had an overall positive effect on tomato yield. The average yield was 2.86 kg/plant for the control, increasing significantly to 3.27 kg/plant for Rizobac and 3.34 kg/plant for Bactilis. These results confirm that biofertilizers contribute to improving productivity, with a more pronounced impact on Kingset and Bucanero hybrids.

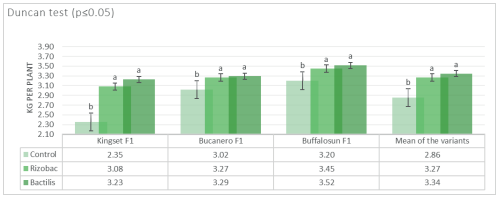


Figure 3. Influence of biofertilizer application on fruit production per plant kg)

Tomato Yield per Hectare

Tomato yield is influenced by essential physiological and morphological factors, such as the number of flowers per plant, fruit set percentage, number of fruits per plant, and fruit size. These parameters directly impact crop productivity, and their interaction determines the efficiency of metabolic processes and overall plant yield.

The results indicate a significant increase in yield in the biofertilizer-treated variants compared to the control.

For Kingset F1, the control yield was 69.7 t/ha, while Rizobac application led to 81.45 t/ha, and Bactilis resulted in the highest yield, 89.3 t/ha.

A similar effect was observed in Bucanero F1, where yield increased from 77.92 t/ha (control) to 93.34 t/ha (Rizobac) and 95.76 t/ha (Bactilis).

For Buffalosun F1, biofertilizer application also led to a significant yield increase, ranging from 71.24 t/ha (control) to 92.69 t/ha (Rizobac) and 88.14 t/ha (Bactilis) (Figure 4).

The mean yield analysis confirms the efficacy of biofertilizers, with Rizobac leading to an average yield of 89.16 t/ha and Bactilis reaching 91.07 t/ha, compared to 72.95 t/ha in the control variant.

The significant differences between treatments demonstrate that biofertilizers stimulate physiological processes and plant metabolism, leading to higher yields.

Species of Bacillus, recognized for their symbiotic efficiency, play a crucial role in promoting plant growth, improving yield, and enhancing stress resistance (Haile et al., 2024).

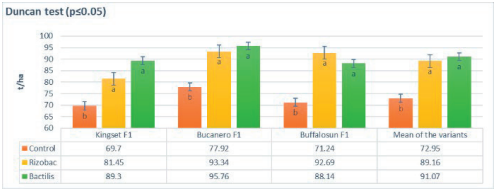


Figure 4. Influence of biofertilizer application on production per hectare depending on the hybrids used (t/ha)

Average Fruit Weight

Fruit weight is a key agronomic trait in many crops (Knaap et al., 2014).

The Duncan test analysis indicates a significant variation in fruit mass, primarily influenced by genetic characteristics.

Among the analyzed hybrids, Buffalosun F1 stands out for its larger fruit size compared to the others.

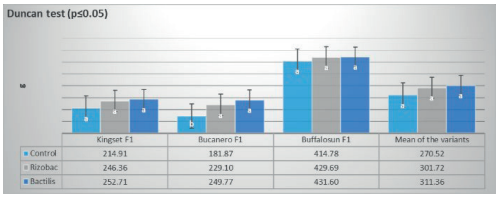


Figure 5. Influence of biofertilization variant on average fruit weight depending on hybrid (g)

Overall, all three hybrids responded positively to biofertilizers in terms of fruit mass (Figure 5).

For Kingset F1, the average fruit weight increased from 214.91 g (control) to 246.36 g (Rizobac) and 252.71 g (Bactilis).
For Bucanero F1, the control recorded the lowest value (181.87 g), while Rizobac (229.10 g) and Bactilis (249.77 g) led to a significant increase.
For Buffalosun F1, the average fruit weight was significantly higher than in the other hybrids, ranging from 414.78 g (control) to 429.69 g (Rizobac) and 431.60 g (Bactilis).
On average, Bactilis resulted in the highest average fruit weight (311.36 g), followed by Rizobac (301.72 g) and the control (270.52 g).
The differences between treatments are statistically significant, with a more pronounced effect in Bucanero F1 and Buffalosun F1.

Fruit Firmness

Microbial inoculations with Rizobac had a positive impact on fruit firmness across all three investigated hybrids (Figure 6). Although a consistent improvement in tomato firmness was observed after Rizobac application, the results were not statistically significant.
For the Bactilis biofertilizer, fruit firmness showed a slight decrease in Kingset F1 (-6.0%) and Bucanero F1 (-6.6%), but in Buffalosun F1, a significant increase (+18.57%) in fruit firmness was recorded. However, these differences were not statistically significant.
The results align with findings reported by Bilalis et al. (2018) and Wang et al. (2021).
The overall trend in fruit firmness remained constant, regardless of the applied fertilization. While the control variant did not show significant differences among hybrids, after biofertilizer application, Buffalosun F1 stood out with the highest firmness, exceeding 7.50 N.

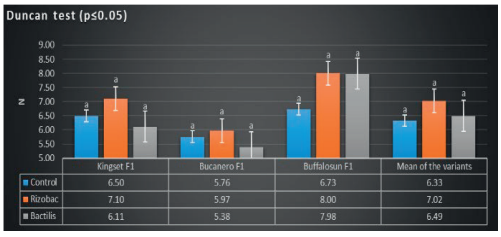


Figure 6. Influence of biofertilization variant on average fruit firmness depending on hybrid (N)

Soluble Solid Content

Figure 7 illustrates the effect of biofertilizers on the soluble solid content in the three analyzed tomato hybrids.
Rizobac treatments did not have a significant influence on the soluble solid content in any of the studied hybrids. On average, the total soluble solid content was very similar between the control variant (4.41°Brix) and the Rizobac-treated variant (4.40°Brix).
In contrast, the Bactilis biofertilizer had a significant impact on the total soluble solid content, forming two homogeneous value groups. However, in Buffalosun F1 and Kingset F1, the differences compared to the untreated variant were not statistically significant.
The results presented in Figure 7 indicate a consistent trend for this quality parameter across all hybrids. Buffalosun F1, followed by Bucanero F1, recorded the highest soluble solid content.

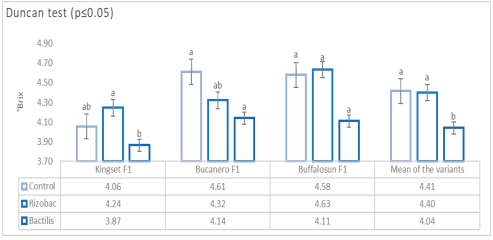


Figure 7. Influence of biofertilization variant on average total soluble substance of fruits depending on hybrid (°Brix)

Total Dry Matter

Various factors, including genotype, environmental conditions, cultivation technology, and water supply, influence the dry matter composition of tomatoes.
The highest total dry matter values were recorded in the untreated control for Kingset F1 (4.26%) and Bucanero F1 (4.31%), while for Buffalosun F1, the highest value was observed in the Bactilis-treated variant (4.38%). These values are lower than those reported by Davies and Hobson (1981).
On average, across the biofertilization treatments, the highest values were recorded in the untreated control, while the lowest values were found in the Rizobac-treated variant. However, the differences between variants were not statistically significant (Figure 8).

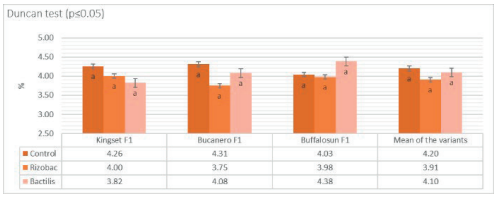


Figure 8. Influence of biofertilization variant on average total dry matter of fruits depending on hybrid (%)

The fluctuations in mean total dry matter values in this study ranged between 4.03% and 4.13%. The dry weight of Buffalosun F1 fruits was higher than that of Kingset F1 and Bucanero F1 over the three-year study period, despite being lower in the untreated control (Figure 8).

Fruit Acidity

Overall, the concentration of organic acids in tomatoes showed a slight decrease in Kingset F1 and Buffalosun F1, but a significant reduction in Bucanero F1 following the application of biofertilizers (Figure 9). However, when analyzing the mean values across variants, the reduction was not statistically significant, according to the Duncan test ($p \leq 0.05$).

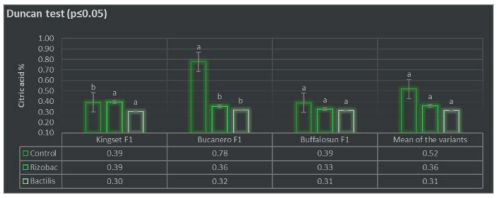


Figure 9. Influence of biofertilization variant on average fruit acidity depending on hybrid (citric acid %)

The ANOVA variance analysis indicates an insignificant variation in fertilization treatments and genetic factors on tomato acidity. However, scientific literature confirms that organic acid levels are influenced by environmental factors and cultivation conditions (Fandi et al., 2010; Hernández-Pérez et al., 2020; Rusu et al., 2023), as well as genotypic variations (Dufera et al., 2018).

In this study, biofertilizer application facilitated the availability of potassium and phosphorus in the soil, but the antagonistic effect between these nutrients may explain the non-significant variations in the treatments applied.

For the analyzed hybrids, the mean titratable acidity values varied as follows: Kingset F1: 0.30-0.39%, Bucanero F1: 0.32-0.78%, Buffalosun F1: 0.31-0.39%.

Titratable acidity was higher in Kingset F1 across all microbial inoculations. However, the differences in mean titratable acidity among the three hybrids were not statistically significant, although Bucanero F1 exhibited a slightly higher value (0.48%) compared to Kingset F1 (0.36%) and Buffalosun F1 (0.34%).

Ascorbic Acid Content

The ascorbic acid in tomatoes varies significantly, with reported values ranging from 6 to 50 mg/100 g of fresh matter, according to the literature (Gest et al., 2013; Sérino et al., 2019; Valšíková-Frey et al., 2017). In this study, the values obtained for the analyzed hybrids were below this range.

Statistical analysis indicates that fertilization variants significantly influenced ascorbic acid biosynthesis (Figure 10). In Kingset F1, vitamin C accumulation decreased after the application of biofertilizers. Conversely, in Bucanero F1, vitamin C content increased significantly by 55.06% following Rizobac application.

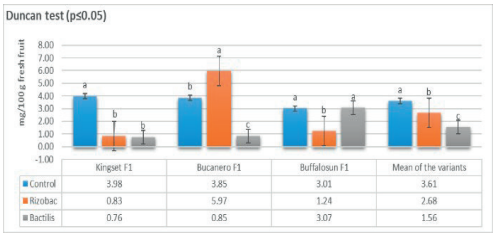


Figure 10. Influence of biofertilization variant on average ascorbic acid of fruits depending on hybrid (mg/100 g fw)

However, when analyzing the mean values across variants, Rizobac and Bactilis treatments led to a significant decrease in ascorbic acid levels, by 25.97% and 56.91%, respectively, over the three-year study period, compared to the control variant.

The response of hybrids to the applied treatments varied, showing significant differences depending on the type of biofertilizer used.

CONCLUSIONS

The application of biofertilizers can contribute to increased yield and improved crop productivity, depending on the floral development stage and the hybrid used.

In conclusion, Rizobac and Bactilis biofertilizers had a significant positive impact on fruit set, with more evident results in Kingset F1 and Bucanero F1. These treatments stimulated fertilization and optimized fruit formation, generating statistically significant differences compared to the control variant.

By activating physiological processes and enhancing nutrient absorption, biofertilizers contributed to higher tomato production, proving their efficiency as a solution for yield improvement. Their application represents a promising method for enhancing productivity and sustainability in tomato cultivation.

All three hybrids analyzed benefited from biofertilizer application, showing a positive impact on fruit weight. Microbial inoculations with Rizobac had a favorable effect on firmness, though without statistically significant differences, while Bactilis reduced firmness in some hybrids, except for Buffalosun F1, where an improvement was observed.

Rizobac treatments did not have a significant impact on the soluble solid content, with mean values nearly identical between the control and treated variants. In contrast, Bactilis significantly influenced total soluble solids, but the differences were not statistically significant for Buffalosun F1 and Kingset F1.

Fluctuations in total dry matter values were minimal, with the dry weight of Buffalosun F1 fruits surpassing that of the other hybrids. The application of biofertilizers led to a slight decrease in acidity in Kingset F1 and Buffalosun F1, while Bucanero F1 recorded a significant decrease, though without statistically significant differences when analyzing mean values across variants.

The vitamin C concentration varied significantly among hybrids, with Kingset F1 having the lowest value under Bactilis treatment, while Bucanero F1 recorded the highest value under Rizobac treatment.

The different responses of hybrids to treatments confirm the influence of genetic and environmental factors on quality and productivity parameters in tomatoes.

REFERENCES

Apahidean, A. I., Domocos, D., Carbunar, M., Bei, M., Hoza, G., Apahidean, A. S. (2019). Cultivar and fertilization influence on production and quality of tomatoes grown in polyethylene tunnels in ecological system. *Scientific Papers. Series B. Horticulture*, Vol. LXIII, Issue 1, Print ISSN 2285-5653, 307-314.

Asari, S., Matzén, S., Petersen, M., Bejai, S., & Meijer, J. (2016). Multiple effects of *Bacillus amyloliquefaciens* volatile compounds: Plant growth promotion and growth inhibition of phytopathogens. *FEMS Microbiology Ecology*, 92. https://doi.org/10.1093/femsec/fiw070.

Aune, D., Chan, D. S. M., Vieira, A. R., Navarro Rosenblatt, D. A., Vieira, R., Greenwood, D. C., & Norat, T. (2012). Dietary compared with blood concentrations of carotenoids and breast cancer risk: A systematic review and meta-analysis of prospective studies. *The American Journal of Clinical Nutrition*, 96(2), Article 2. https://doi.org/10.3945/ajcn.112.034165.

Balderas-Ruíz, K. A., Gómez-Guerrero, C. I., Trujillo-Roldán, M. A., Valdez-Cruz, N. A., Aranda-Ocampo, S., Juárez, A. M., Leyva, E., Galindo, E., & Serrano-Carreón, L. (2021). *Bacillus velezensis 83* increases productivity and quality of tomato (*Solanum lycopersicum* L.): Pre and postharvest assessment. *Current Research in Microbial Sciences*, 2, 100076. https://doi.org/10.1016/j.crmicr.2021.100076.

Basu, A., Prasad, P., Das, S., Kalam, S., Sayyed, R., Reddy, M., & El Enshasy, H. (2021). Plant Growth Promoting Rhizobacteria (PGPR) as Green Bioinoculants: Recent Developments, Constraints, and Prospects. *Sustainability*, 13, 1140. https://doi.org/10.3390/su13031140.

Becherescu, A., Hoza, G., Dinu, M., Iordănescu, O., Popa, D. (2021). The influence of biofertilizing and biostimulating products on the production of cornichon cucumber hybrids cultivated in heated solarium. S. *Scientific Papers. Series B. Horticulture*, Vol. LXV, No. 1, 2021. Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653.

Bilalis, D., Krokida, M., Roussis, I., Papastylianou, P., Travlos, I., Cheimona, N., & Dede, A. (2018). Effects of organic and inorganic fertilization on yield and quality of processing tomato (Lycopersicon esculentum Mill.). *Folia Horticulturae*, 30(2), 321–332.

Borriss, R. (2020). Phytostimulation and Biocontrol by the Plant-Associated Bacillus amyloliquefaciens FZB42: An Update. In: Kumar, M., Kumar, V., Prasad, R. (eds) Phyto-Microbiome in Stress Regulation. *Environmental and Microbial Biotechnology*. Springer, Singapore. https://doi.org/10.1007/ 978-981-15-2576-6_1.

Dragomir, C. L., Hoza, D. (2022). Review on improving tomato culture technology in protected system for

environmental protection and increasing productivity using PGPR. *Scientific Papers. Series B*, Horticulture, Vol. LXVI, Issue 1, Print ISSN 2285-5653, 444-448.

Dufera, J. T., Mohammed, W., Hussien, S., & Kumar, V. (2018). Genetic analysis for fruit yield and quality attributes in some processing and fresh market tomato genotypes. *International Journal of Vegetable Science*, 24(3), 227–235.

Erba, D., Casiraghi, M. C., Ribas-Agustí, A., Cáceres, R., Marfà, O., & Castellari, M. (2013). Nutritional value of tomatoes (*Solanum lycopersicum* L.) grown in greenhouse by different agronomic techniques. *Journal of Food Composition and Analysis*, 31(2), Article 2. https://doi.org/10.1016/j.jfca.2013.05.014

Fandi, M., Muhtaseb, J., & Hussein, M. (2010). Effect of N, P, K concentrations on yield and fruit quality of tomato (*Solanum lycopersicum* L.) in tuff culture. *Journal of Central European Agriculture*, 11(2), 179–184.

Fazle Rabbee, M., & Baek, K.-H. (2020). Antimicrobial Activities of Lipopeptides and Polyketides *of Bacillus velezensis* for Agricultural Applications. *Molecules*, 25(21), Article 21. https://doi.org/10.3390/molecules25214973

Gest, N., Gautier, H., & Stevens, R. (2013). Ascorbate as seen through plant evolution: The rise of a successful molecule?. J. *Exp. Bot*, 64, 33–53.

Giovannucci, E. (2002). A review of epidemiologic studies of tomatoes, lycopene, and prostate cancer. *Experimental Biology and Medicine (Maywood, N.J.)*, 227(10), Article 10. https://doi.org/10.1177/153537020222701003

Guo, Q., Li, Y., Lou, Y., Shi, M., Jiang, Y., Zhou, J., Sun, Y., Xue, Q., & Lai, H. (2019). *Bacillus amyloliquefaciens Ba13* induces plant systemic resistance and improves rhizosphere microecology against tomato yellow leaf curl virus disease. *Applied Soil Ecology*, 137, 154–166. https://doi.org/10.1016/j.apsoil.2019.01.015

Haile, D., Tesfaye, B., & Assefa, F. (2024). Plant growth promoting Rhizobacteria for sustainable tomato production. *South African Journal of Botany*, 174, 371–382. https://doi.org/10.1016/j.sajb.2024.08.059

Hernández-Pérez, O. I., Valdez-Aguilar, L. A., Alia-Tejacal, I., Cartmill, A. D., & Cartmill, D. L. (2020). Tomato fruit yield, quality, and nutrient status in response to potassium: Calcium balance and electrical conductivity in the nutrient solution. *Journal of Soil Science and Plant Nutrition*, 20, 484–492.

Hoza, G., Dinu, M., Soare, R., Becherescu, A. D., Apahidean, I. A., & Hoza, D. (2019). Influence of plant management systems on growth and fructification of tomato plants in protected culture. *Scientific Papers. Series B. Horticulture*, Vol. LXII, 2018, Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

Jaishankar, M., Tseten, T., Anbalagan, N., Mathew, B. B., & Beeregowda, K. N. (2014). Toxicity, mechanism and health effects of some heavy metals. *Interdisciplinary Toxicology*, 7(2), Article 2.

Javaria, S., Khan, M. Q., Rahman, H., & Bakhsh, I. (2012). Response of tomato yield and post harvest life to potash levels. Sarhad J. Agric., 28.

Kadner, R. J. & Kara Rogers. (2024). Bacteria-Prokaryotes, Microbes, Cells. *Britannica*. https://www.britannica.com/science/bacteria/Diversity-of-structure-of-bacteria

Kalam, S., Basu, A., & Podile, A. R. (2020). Functional and molecular characterization of plant growth promoting Bacillus isolates from tomato rhizosphere. *Heliyon*, 6(8), Article 8. https://doi.org/10.1016/j.heliyon.2020.e04734

Kalogeropoulos, N., Chiou, A., Pyriochou, V., Peristeraki, A., & Karathanos, V. T. (2012). Bioactive phytochemicals in industrial tomatoes and their processing byproducts. *LWT - Food Science and Technology*, 49(2), Article 2. https://doi.org/10.1016/j.lwt.2011.12.036

Karppi, J., Laukkanen, J. A., Sivenius, J., Ronkainen, K., & Kurl, S. (2012). Serum lycopene decreases the risk of stroke in men: A population-based follow-up study. *Neurology*, 79(15), Article 15. https://doi.org/10.1212/WNL.0b013e31826e26a6

Knaap, E., Chakrabarti, M., Chu, Y. H., Clevenger, J. P., Illa-Berenguer, E., Huang, Z., Keyhaninejad, N., Mu, O., L., S., & WU, S. (2014). What lies beyond the eye: The molecular mechanisms regulating tomato fruit weight and shape. *Frontiers in Plant Science*, 5, 227.

Li, J., Liu, F., Wu, Y., Tang, Z., Zhang, D., Lyu, J., Khan, K. S., Xiao, X., & Yu, J. (2024). Evaluation of nutritional composition, biochemical, and quality attributes of different varieties of tomato (*Solanum lycopersicum* L.). *Journal of Food Composition and Analysis*, 106384. https://doi.org/10.1016/j.jfca.2024.106384

Masood, S., Zhao, X. Q., & Shen, R. F. (2020). Bacillus pumilus promotes the growth and nitrogen uptake of tomato plants under nitrogen fertilization. *Scientia Horticulturae*, 272, 109581. https://doi.org/10.1016/j.scienta.2020.109581

McSpadden Gardener, B. B. (2004). Ecology of *Bacillus* and *Paenibacillus* spp. in Agricultural Systems. *Phytopathology*, 94(11), Article 11. https://doi.org/10.1094/PHYTO.2004.94.11.1252

Mumtaz, M. Z., Ahmad, M., Jamil, M., & Hussain, T. (2017). Zinc solubilizing *Bacillus* spp. Potential candidates for biofortification in maize. *Microbiological Research*, 202, 51–60. https://doi.org/10.1016/j.micres.2017.06.001

Nanu, Ş., Paraschiv, A. N., Iamandei, M., Sfîrloagă, L. M., Bîrsoghe, C., Ilina, M. D., Frătuțu, F., & Băjenaru, M. F. (2024). Evaluation of the effectiveness of some protection products against the Tuta absoluta pest in tomato crops from southern Romania. *Acta Horticulturae*, 1391, 373–380. https://doi.org/10.17660/ActaHortic.2024.1391.51

Peng, Y., Duan, Y., Huo, W., Xu, M., Yang, X.-Y., Wang, X., Wang, B., Blackwell, M., & Feng, G. (2021). Soil microbial biomass phosphorus can serve as an index to reflect soil phosphorus fertility. *Biology and Fertility of Soils*, 57, 1–13. https://doi.org/10.1007/s00374-021-01559-z

Porto, J. S., Rebouças, T. N. H., Moraes, M. O. B., Bomfim, M. P., Lemos, O. L., & Luz, J. M. Q. (2016). Quality and antioxidant activiy of tomato cultivated under different sources and doses of nitrogen. *Revista Caatinga*, 29(4), 780–788. https://doi.org/10.1590/1983-21252016v29n401rc

Praharaj, C. (2007). Long term quantitative and qualitative changes in cotton and soil parameters under cultivars, cropping systems and nutrient management options. *Indian Journal of Agricultural Sciences*, 77, 280–285.

Reimers, K., & Keast, D. (2016). Tomato Consumption in the United States and Its Relationship to the US Department of Agriculture Food Pattern: Results From What We Eat in America 2005–2010. *Nutrition Today*, 51, 1. https://doi.org/10.1097/NT.0000000000000152

Rubatzky, V. E., & Yamaguchi, M. (1997). *World Vegetables: Principles, Production, and Nutritive Values*. Chapman & Hall.

Rusu, O. R., Mangalagiu, I., Amăriucăi-Mantu, D., TELIban, G. C., Cojocaru, A., Burducea, M., G., M., M., R., G., C., A., S., & Stoleru, V. (2023). Interaction effects of cultivars and nutrition on quality and yield of tomato. *Horticulturae,* 9(5), 541.

Sato, R., Helzlsouer, K. J., Alberg, A. J., Hoffman, S. C., Norkus, E. P., & Comstock, G. W. (2002). Prospective study of carotenoids, tocopherols, and retinoid concentrations and the risk of breast cancer. Cancer Epidemiology, Biomarkers & Prevention: A Publication of the American Association for Cancer Research, *Cosponsored by the American Society of Preventive Oncology*, 11(5), Article 5.

Sérino, S., Costagliola, G., & Gomez, L. (2019). Lyophilized tomato plant material: Validation of a reliable extraction method for the analysis of vitamin C. *Journal of Food Composition and Analysis*, 81, 37–45.

Singh, M., Srivastava, M., Kumar, A., Singh, A., & Pandey, K. (2019). Endophytic bacteria in plant disease management. Microbial Endophytes Prospects for *Sustainable Agriculture Woodhead Publishing Series in Food Science*, Technology and Nutrition 2020, Pages 61-89 https://doi.org/10.1016/B978-0-12-818734-0.00004-8.

Stan, A., Bujor, O. C., Dobrin, A., Haida, G., Bădulescu, L., & Asănică, A. (2019). Monitoring the quality parameters for organic raspberries in order to determine the optimal storage method by packaging. *XII I8nternational Rubus and Ribes Symposium: Innovative Rubus and Ribes Production for High Quality Berries in Changing 1277*, 461–468. https://www.actahort.org/books/1277/1277_66.htm

Tieman, D., Bliss, P., McIntyre, L. M., Blandon-Ubeda, A., Bies, D., Odabasi, A. Z., Rodríguez, G. R., van der Knaap, E., Taylor, M. G., Goulet, C., Mageroy, M. H., Snyder, D. J., Colquhoun, T., Moskowitz, H., Clark, D. G., Sims, C., Bartoshuk, L., & Klee, H. J. (2012). The Chemical Interactions Underlying Tomato Flavor Preferences. *Current Biology*, 22(11), Article 11. https://doi.org/10.1016/j.cub.2012.04.016

Valšíková-Frey, M., Komár, P., & Rehuš, M. (2017). The effect of varieties and degree of ripeness to vitamin C content in tomato fruits. *Acta Horticulturae et Regiotecturae*, 20(2), 44–48.

Verma, S. B., Kumar, C., & Narayan, R. P. (2023). Sustaining soil health and tomato production through organic inputs and bacterial inoculations in alluvial soils detoxifying plant contaminants. *South African Journal of Botany*, 161, 404–417. https://doi.org/10.1016/j.sajb.2023.08.017

Wang, J., Cui, Y., Wu, K., Wu, S., Wu, K., Li, Y., & Niu, W. (2024). Micro/nanobubble-aerated drip irrigation affects saline soil microenvironments and tomato growth by altering bacterial communities. *Soil and Tillage Research*, 239, 106034. https://doi.org/10.1016/j.still.2024.106034.

Wang, J., Liu, L., Gao, X., Hao, J., & Wang, M. (2021). Elucidating the effect of biofertilizers on bacterial diversity in maize rhizosphere soil. *PLoS One*, 16(4), 0249834. https://doi.org/10.1371/journal.pone.0249834.

Wu, X., Yu, L., & Pehrsson, P. R. (2022). Are Processed Tomato Products as Nutritious as Fresh Tomatoes? Scoping Review on the Effects of Industrial Processing on Nutrients and Bioactive Compounds in Tomatoes. *Advances in Nutrition*, 13(1), Article 1. https://doi.org/10.1093/advances/nmab109.

Zhou, W., Zhang, M., Tao, K., & Zhu, X. (2022). Effects of arbuscular mycorrhizal fungi and plant growth-promoting rhizobacteria on growth and reactive oxygen metabolism of tomato fruits under low saline conditions. *BIOCELL*, 46(12), 2575–2582. https://doi.org/10.32604/biocell.2022.021910

https://populatia.ro/

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

PHYSICO-CHEMICAL CHARACTERISTICS OF WATERMELON FRUITS (*CITRULLUS LANATUS*) OF GENOTYPES CULTIVATED ON SANDY SOILS IN SOUTHWESTERN ROMANIA

Felicia-Constantina FRĂTUȚU, Ștefan NANU, Cristina BÎRSOGHE, Alina-Nicoleta PARASCHIV, Loredana-Mirela SFÎRLOAGĂ, Maria-Diana ILINA

Research - Development Station for Plant Culture on Sands Dăbuleni, Călărași, Dolj County, Romania

Corresponding author email: nanu.st@scdcpndabuleni.ro

Abstract

The study was carried out during 2022-2024 within the experimental field of the Research - Development Station for Plant Culture on Sands Dăbuleni. The method used to set up the experiment was the randomized block. The material used to set up the experiment consisted of six watermelon genotypes:'Dulce de Dăbuleni', 'Oltenia', 'De Dăbuleni', own creations of the Research - Development Station for Plant Culture on Sands Dăbuleni, and Burebista F1, Kratos F1, Fechete F1 of other origin. Physical characteristics such as length, diameter, and shape index of the fruits, quantitative characteristics such as fruit weight, fruit peel thickness and weight, core percentage, and also biochemical ones such as soluble dry matter content, total dry matter content, titratable acidity, vitamin C, and carbohydrates were evaluated. The weight of the fruits analyzed during the study period recorded average values that varied between 4.63 kg for Burebista F1(2024) and 9.32 kg for Fechete F1(2023). The soluble dry matter content varied between 10.00 and 11.80%, the highest average value being recorded for the Burebista F1 cultivar, respectively 11.00%.

Key words*: watermelon, physicochemical characteristics, sandy soils.*

INTRODUCTION

Watermelon (*Citrullus lanatus*) is a highly valued species due to its large, round, oval, or elongated fruits, rich in vitamins (Salk et al., 2008). Watermelon fruits are also an important source of nutrients, including lycopene, a carotenoid pigment with a special antioxidant role in the metabolism of the human body, protecting it against serious diseases such as cancer (Perkins-Veazie & Collins, 2004). Watermelons were first cultivated 5000 years ago in Egypt, often found in hieroglyphs and frescoes in ancient Egyptian tombs. In China, it was introduced in the 10th century, later making its way west to North America (Anonymus, 2014). Knowledge of the physicochemical characteristics of the fruits plays an important role in the sorting, classification, and handling process. The shape of the fruit plays an important role in determining the method of their valorization, either for retail sale or in the processing process in the form of different products (Bahansawy et al., 2004; Khater & Bahansawy, 2016; Dou et al., 2018). The accumulation of knowledge regarding the physicochemical characteristics facilitates the use and evaluation of local watermelon cultivars studied in breeding programs (McGregor, 2012). Therefore, the present study aimed to analyze the physicochemical characteristics of the fruits of watermelon genotypes grown in the sandy soil area.

MATERIALS AND METHODS

The experiment was established in the period 2022-2024 on sandy soils in the research field of the Research - Development Station for Plant Culture on Sands Dăbuleni. The randomized block method was used with four repetitions.

The study material was represented by 6 watermelon genotypes: ′Dulce de Dăbuleni′, ′Oltenia′, ′De Dăbuleni′, Burebista F1, Kratos F1, and Fechete F1, whose fruits were harvested at physiological maturity.

The determined physical characteristics were the length (cm), diameter (cm) and shape index of the fruits, as well as their quantitative characteristics, namely the weight of the fruits (kg), the thickness of the peel (cm) and the

percentage of the fruit core (%), according to the methodology described by Ionică M. E. (2014). Based on the results obtained in the three years of study, a classification of fruits by shape and size was performed.

The classification of fruits by size was performed according to the method described by Khater & Bhansawi in 2016 (< 5kg = small fruits; 5-8 kg = medium fruits; 8-11 kg = large fruits and > 11 kg = very large fruits), and the classification according to the shape index was performed according to the method described by Dou et al. in 2018 (< 1.8 = elongated shape; 1.4-1.6 = oval shape and between 1.0-1.10 = spherical shape).

The determination of the chemical properties of the fruits such as soluble dry matter content (%), total dry matter (%), carbohydrates (%), titratable acidity (g malic acid/ 100g fresh matter), and vitamin C (mg), was performed according to the methods described by Croitoru (2021).

The soluble dry matter (SUS) was determined by the refractometric method, the results being expressed in percentages (%). The determination of the total dry matter (SUT) content was carried out using the gravimetric method based on the removal of water by evapotranspiration from the average analytical sample used, keeping it in an oven at a temperature between 85-105°C, the results being expressed in percentages of total dry matter (%).

The determination of the titratable acidity (TA) was carried out according to the method described by Ionică (2014), the results being expressed in grams of malic acid/100 g of fresh substance. To determine the vitamin C content, the iodometric method described by Croitoru (2021) was used, which is based on the oxidation of ascorbic acid with excess iodine, the results being expressed in mg of ascorbic acid.

Carbohydrates were determined according to the Fehling Soxhelt method described by Croitoru (2021), with the results expressed in percentages.

Regarding the determination of plant productivity, at the end of the vegetation period, the total production for each experimental variant was calculated, and the production was reported in tons/ha.

The data obtained were statistically processed using the statistical analysis program (Stat Point Technologies, Warrenton, VA, USA). The relationships between the physical characteristics of the fruits were quantified using correlations.

RESULTS AND DISCUSSIONS

The literature review states that the evaluation of physical characteristics, plant growth form, and leaf and fruit shape are useful indicators in determining genetic variation and in the selection process. From the analysis of the data in Tables 1 and 2, it appears that during the analyzed period, there was a high variability between the analyzed genotypes, depending on the climatic year.

Regarding the physical characteristics of the fruits during the analyzed period, the highest coefficient of variability (15.99%) was calculated for the length of the fruits (′Oltenia′ in 2023).

The variation limits for fruit length were between 39.99 cm for ′De Dăbuleni′ in 2022 and 20.90 cm for Burebista F1 in 2024. The lowest value of fruit diameter for the study period was recorded in 2024 for Burebista F1 (20.18 cm), and the highest value of 24.40 cm was also recorded in 2024 for the ′Oltenia′ cultivar.

The results obtained are higher than those reported by Hakimi & El Madidi in 2015 for fruit length (31.70 cm) and diameter (23.20 cm).

From the point of view of the shape index, it presented the lowest value of 1.04 in 2024 for Burebista F1 and the highest of 1.97 in 2022 for ′De Dăbuleni′. In the literature, Tanaka & Mizutani (1995) reported lower shape index values for the analyzed cultivars than those in the present study, reporting an oval (if = 0.6) and spherical (if = 1.0) fruit shape.

Table 2 presents the results obtained regarding the main quantitative characteristics of watermelon fruits, such as weight, peel thickness, and fruit core percentage.

Table 1. Physical characteristics of the fruits of watermelon genotypes studied during 2022-2024

Genotype	Descriptive statistics	Morphological characteristics								
		Fruit length (cm)			Fruit diameter (cm)			FSI		
		2022	2023	2024	2022	2023	2024	2022	2023	2024
'Dulce de Dăbuleni'	Mean±SD	29.66±0.91	25.78±2.22	22.40±0.89	22,50±0.77	22.56±1.39	21.10±1.14	1.19±0.03	1.14±0.06	1.06±0.05
	Variation limits	25.50/27.80	23.70/29.20	21.00/23.00	21,50/23.50	21.00/24.00	20.00/22.50	1.14/1.23	1.08/1.22	1.02/1.15
	CV%	3.41	8.63	3.99	3.43	6.18	5.40	2.60	5.08	4.69
'Oltenia'	Mean±SD	29.04±2.22	26.64±4.26	27.00 ±1.73	22.94 ±0.94	21.78±2.58	24.40 ±1.52	1,27±0.09	1.23±0.17	1.11±0.05
	Variation limits	26.5/32.5	20.50/31.50	24.00/28.00	22.00/24.5	17.80/24.50	22.00/26.00	1.17/1.41	1.07/1.51	1.04/1.17
	CV%	7.63	15.99	6.42	4.12	11.87	6.22	7.43	13.77	4.25
'De Dăbuleni'	Mean±SD	39.98 ±3.79	36.06 ±4.14	37.88 ±1.94	20.24 ±0.81	20.70 ±1.33	21.32 ±2.02	1.97±0.15	1.75±0.25	1.79±0.24
	Variation limits	34.50/44.50	30.00/41.00	36.20/40.50	19.00/21.20	19.40/22.80	19.30/24.50	1.73/2.10	1.46/2.11	1.49/2.10
	CV%	9.47	11.74	5.12	4.02	6.41	9.46	7.40	14.03	13.43
Burebista F1	Mean±SD	25.84 ±1.82	23.28±1.42	20.90 ±1.56	22.23 ±0.73	21.44 ±1.53	20.18 ±1.28	1.16±0.06	1.09±0.03	1.04±0.02
	Variation limits	23.00/27.2	21.40/25.40	18.50/22.50	21.60/23.50	19.20/23.40	18.50/21.50	1.06/1.23	1.04/1.11	1.00/1.05
	CV%	7.06	6.12	7.45	3.28	7.15	6.34	5.42	2.6	2.22
Kratos F1	Mean±SD	31.56±2.16	31.84 ±1.05	30.18±2.49	21.52 ±1.24	23.62 ±2.89	22.84±1.30	1.47±0.16	1.36±0.15	1.33±0.14
	Variation limits	28.50/33.60	30.50/33.00	26.00/32.50	19.50/22.80	20.10/28.10	21.00/24.20	1.28/1.72	1.12/1.52	1.16/1.55
	CV %	6.85	3.28	8.24	5.78	12.22	5.71	10.93	11.05	10.94
Fechete F1	Mean±SD	25.04 ±1.68	26.58 ±1.52	22.36 ±2.09	21.86 ±1.02	23.86 ±1.77	21.70 ±1.52	1.15±0.06	1.13±0.12	1.03±0.07
	Variation limits	23.50/27.40	24.50/28.60	20.00/25.00	21.00/23.50	21.20/25.10	20.00/24.00	1.08/1.24	1.05/1.35	0.95/1.13
	CV%	6.71	5.73	9.33	4.68	7.49	7.02	5.27	11.03	6.81

Table 2. Quantitative characteristics of fruits in watermelon genotypes studied during 2022-2024

Genotype	Descriptive statistics	Morphological characteristics								
		Fruit weight (kg)			Peel thickness (cm)			Core percentage (%)		
		2022	2023	2024	2022	2023	2024	2022	2023	2024
'Dulce de Dăbuleni'	Mean ± SD	7.18±0.49	6.91±1.37	5.24±0.73	1.2±0.19	1.10±0.26	1.60±0.22	42.72±1.36	53.66±5.11	55.85±3.96
	Variation limits	6.53/7.66	5.39/8.70	4.50/6.36	1.00/1.50	0.80/1.50	1.50/2.00	41.11/44.14	44.67/57.06	49.17/59.31
	CV %	6.79	19.83	13.88	15.03	24.05	13.98	3.17	9.52	7.09
'Oltenia'	Mean ± SD	8.68±1.28	7.09±1.76	8.88±1.34	1.48±0.15	1.42±0.23	1.40±0.42	58.24±3.97	59.57±4.19	59.39±6.94
	Variation limits	7.32/10.20	4.34/9.12	6.94/9.93	1.30/1.70	1.20/1.70	1.00/2.00	53.92/63.51	54.27/65.16	53.25/70.31
	CV %	14.71	24.88	15.12	10.02	16.05	29.88	6.82	7.04	11.68
'De Dăbuleni'	Mean ± SD	8.89±1.40	8.57±1.13	9.26±0.98	1.38±0.16	1.68±0.26	1.32±0.20	58.30±5.74	54.77±6.82	59.24±3.28
	Variation limits	7.17/10.39	7.08/10.18	8.51/10.99	1.20/1.60	1.40/2.00	1.00/1.50	51.38/66.56	42.77/59.24	54.67/62.63
	CV %	15.72	13.18	10.63	11.91	15.41	15.53	9.84	12.46	5.53
Burebista F1	Mean ± SD	6.80±0.63	6.00±0.98	4.63±0.89	1.50±0.07	1.14±0.24	1.44±0.58	58.73±5.06	61.23±10.56	59.29±4.99
	Variation limits	6.11/7.57	4.65/7.27	3.40/5.77	1.40/1.60	0.80/1.40	0.80/2.00	52.84/66.26	44.43/69.60	53.30/66.40
	CV %	9.2	16.33	19.26	4.71	21.13	40.07	8.61	17.25	8.42
Kratos F1	Mean ± SD	7.58±0.63	8.96±0.34	8.04±0.95	1.46±0.27	1.48±0.44	1.22±0.22	54.38±9.31	55.85±5.78	62.91±4.08
	Variation limits	7.07/8.67	8.56/9.40	6.72/9.17	1.00/1.70	0.90/2.00	1.00/1.50	40.99/66.28	49.55/64.23	55.75/65.76
	CV %	8.28	3.74	11.78	18.51	29.99	17.77	17.13	10.35	6.49
Fechete F1	Mean ± SD	6.62±0.91	9.32±1.24	5.82±1.00	1.42±0.32	1.10±0.14	1.30±0.27	63.73±3.73	65.25±3.66	62.00±6.88
	Variation limits	5.62/8.09	7.62/11.05	5.25/7.60	1.00/1.80	1.00/1.30	1.00/1.50	60.27/67.96	59.87/70.15	53.14/70.85
	CV %	13.78	13.26	17.22	22.49	12.86	21.1	5.86	5.61	11.1

Fruit weight recorded values during the study period ranging from 4.63 kg in 2024 for Burebista F1 and 9.32 kg for Fechete F1 in 2023, followed by ′De Dăbuleni′ with 9.26 kg in 2024.

The data obtained regarding fruit weight are consistent with those reported in the literature by Hakimi & El Madidi, respectively 8.3 kg, but lower than those reported by Frătuțu et al. (2024) of 12.55 kg. Regarding the thickness of the peel of the watermelon fruits analyzed, it presented values ranging between 1.10 cm for ′Dulce de Dăbuleni′ and Fechete F1 in 2023, and the highest value of 1.68 cm was recorded for ′De Dăbuleni′ also in 2023. The highest percentage of core (65.27%) was recorded in 2023 for Fechete F1, and the lowest (42.72%) for ′Dulce de Dăbuleni′ in 2022. In the literature, Bahnasawy et al. (2004) mention that the thickness of the fruit peel is positively correlated with the increase in fruit weight. The data obtained by them regarding peel thickness and core percentage are higher (12.11-20.28 mm for peel thickness and 42.12-65.70% for core percentage) than those recorded in the present study.

The highest coefficient of variability for quantitative characteristics was calculated in 2024 at Burebista F1 for peel thickness, respectively 40.07%, and the lowest of 3.17% for core percentage at ′Dulce de Dăbuleni′ in 2022.

To classify the fruits according to the shape index and fruit weight, the scale proposed by Khater & Bhansawi (2016) was used (Table 3). Analyzing the data in Table 3, we can see that depending on the average weight obtained by each cultivar during the study period, 3 of the 6 cultivars presented large fruits with a weight ranging between 8.15 kg (Kratos F1) and 8.91 kg (′De Dăbuleni′), and the other 3 presented medium-sized fruits, with values ranging between 5.81 kg (Burebista F1) and 7.25 kg (Fechete F1).

Regarding their shape, the ′De Dăbuleni′ genotype presented elongated fruits with a shape index greater than 1.80. The cultivars Burebista F1 and Fechete F1 presented a spherical fruit shape (1.10) and the other 3 a spherical-oval shape with an index ranging between 1.13 (′Dulce de Dăbuleni′) and 1.39 (Kratos F1). Dou et al. (2018) mention in their study that the shape of the watermelon fruits analyzed ranged between elongated (if > 1.8) and spherical (if = 1.0-1.1).

Table 3. Classification of fruits in the watermelon genotypes studied, depending on their shape index and weight

Genotype	Fruit weight*(kg)	Size	FSI*	Shape
′Dulce de Dăbuleni′	6.44	medium	1.13	spherical-oval
′Oltenia′	8.22	large	1.20	spherical-oval
′De Dăbuleni′	8.91	large	1.84	elongate
Burebista F1	5.81	medium	1.10	spherical
Kratos F1	8.15	large	1.39	spherical-oval
Fechete F1	7.25	medium	1.10	spherical

*The data represents the average of the 2022-2024

The chemical characteristics of the fruits (Table 4) showed that during the study period, the total dry matter (%) recorded the highest value of 12.11% in the ′De Dăbuleni′ genotype and the lowest of 9.65% in ′Dulce de Dăbuleni′. The soluble dry matter (%) presented values ranging between 10.00% in ′Dulce de Dăbuleni′ and 11.00% in Burebista F1. The titratable acidity content determined in the fruits analyzed during the study period ranged between 0.25 g malic acid/100 g fresh matter in ′Oltenia′, and 0.37 g malic acid/100 g fresh matter in Burebista F1. The highest vitamin C content for the study period was recorded in Burebista F1 (17.11 mg) and the lowest in ′Dulce de Dăbuleni′ (10.79 mg). In terms of carbohydrate content, Burebista F1 stood out with 9.86% and Kratos F1 with 8.59%. In the specialized literature, Victoire et al. (2023) reported lower values for the soluble dry matter content (7.06-7.89%) and total dry matter content (1.53-5.37%) for the analyzed cultivars than those obtained in the present study. The data obtained in the present study are higher than those reported by Bîrsoghe et al., 2024 in terms of vitamin C content (8.80-14.96 mg/ 100 g fresh matter), and relatively similar in terms of total dry matter content (8.5-14.38%) and soluble dry matter (9.00-12.20%).

Table 4. Chemical properties of fruits of watermelon genotypes studied during 2022-2024

Genotype	Descriptive statistics	SUS (%)	SUT (%)	TA g (malic ac/100 g FW)	Vit C (mg)	Carbohydrates (%)
'Dulce de Dăbuleni'	Mean ± SD	10.00 ± 0.40	9.65 ± 0.71	0.26 ± 0.01	10.79 ± 3.62	9.74 ± 4.65
	Variation limits	9.60 / 10.40	8.90 / 10.32	0.25 / 0.26	8.60 / 14.96	6.02 / 14.96
	CV (%)	4.00	7.40	2.25	33.52	47.76
'Oltenia'	Mean ± SD	10.10 ± 0.10	9.59 ± 0.07	0.25 ± 0.03	13.76 ± 0.49	8.81 ± 0.19
	Variation limits	10.00 / 10.20	9.52 / 9.65	0.23 / 0.28	13.20 / 14.08	8.60 / 8.94
	CV (%)	0.99	0.68	9.93	3.54	2.11
'De Dăbuleni'	Mean ± SD	10.80 ± 1.25	12.11 ±2.00	0.30 ± 0.05	10.90 ± 2.15	9.27 ± 1.08
	Variation limits	9.80 / 12.20	10.61 / 14.38	0.24 / 0.33	8.94 / 13.20	8.43 / 10.49
	CV (%)	11.56	16.53	16.63	19.73	11.64
Burebista F1	Mean ± SD	11.00 ± 0.92	11.65 ±0.65	0.37 ± 0.12	17.01 ± 3.97	9.86 ± 1.39
	Variation limits	10.00 / 11.80	11.09 / 12.36	0.28 / 0.51	13.20 / 21.12	8.60 / 11.35
	CV (%)	8.33	5.58	32.40	23.32	14.09
Kratos F1	Mean ± SD	10.47 ±0.92	11.79 ± 0.75	0.27 ± 0.01	12.32 ± 3.84	8.59 ± 1.54
	Variation limits	9.40 /11.00	11.34 /12.66	0.26 / 0.28	7.92 / 14.96	6.81 / 9.49
	CV (%)	8.83	6.37	4.33	31.13	17.92
Fechete F1	Mean ± SD	10.63 ±0.32	9.96±2.23	0.30 ±0.04	11.96 ±1.98	9.51 ± 1.63
	Variation limits	10.40 / 11.00	7.40 / 11.49	0.26 / 0.33	9.68 / 13.20	8.25 / 11.35
	CV (%)	3.02	22.42	11.84	16.53	17.11

*The data represents the average of the 2022-2024

Table 5 presents the statistical calculation of the average production obtained by each cultivar during the study period of 2022-2024.

Table 5. Average watermelon production recorded during the period 2022-2024

Genotype	Average production (t/ha)	Relative productio n (%)	Difference (t/ha)	LSD*
'Dulce de Dăbuleni'	23.18	100	0.00	Control
'Oltenia'	23.52	101.46	0.34	NS
'De Dabuleni'	32.65	138.82	9.47	NS
Burebista	43.97	134.69	20.79	***
Kratos	44.48	101.16	21.30	***
Fechete	47.86	107.59	24.68	***
			LSD 5% = 10.55 LSD 1% = 14.46 LSD 0.1% = 19.69	

LSD* - Least Significant Difference; NS – Not significant

Thus, according to the data, the highest production of 47.86 t/ha was recorded for Fechete F1 with a difference from the control of 24.68 t/ha, statistically ensured as very significant.

The lowest production of 23.18 t/ha was recorded for the control cultivar 'Dulce de Dăbuleni'. The results obtained are higher than those reported by Shrefler et al. (2015), respective productions ranging between 12 and 37 t/ha.

CONCLUSIONS

The observations and determinations obtained from the study carried out at the Research - Development Station for Plant Culture on Sands Dăbuleni, during the period 2022-2024 allowed the evaluation of watermelon genotypes so that they could be used in breeding programs as future progenitors;

The recorded production and quality data will be used as criteria in choosing the watermelon genotypes cultivated on sandy soils in the southern part of the country.

REFERENCES

Anonymous, (2014). Watermelon characteristics. http://www.ehow.com/info

Bahanasawy, A.H., El-Haddad, Z.A., El-Ansary, M.Y., Sorour H.M., (2004). Physical and mechanical properties of some Egyptian onion cultivars. *J. of Food* Eng., 62: 255-261.

Croitoru, M. (2021). *Chimie și biochimie vegetală. Lucrări practice de laborator*, Editura SITECH, Craiova.

Dou, J., Zhao, S., Lu, X., He, N., Zhang, L., Ali, A., ... & Liu, W. (2018). Genetic mapping reveals a candidate gene (ClFS1) for fruit shape in watermelon (*Citrullus lanatus* L.). *Theoretical and applied genetics*, *131*, 947-958.

Frătuțu, F., Nanu.Șt., Sfîrloagă, L., Bîrsoghe, C., & Ilina, D. (2024). The behavior of some watermelons hybrids in pedoclimatic conditions from SCDCPN Dăbuleni." *Annals of the University of Craiova-Agriculture Montanology Cadastre Series"*, *54*(1), 131-137.

Hakimi, F., & El Madidi, S. (2015). Variability of agro-morphological traits in some Moroccan watermelon landraces (*Citrullus lanatus* Thunb. Matsum. and Nakai). *International Journal of Current Sciences*, *17*, 90-96.

Ionică, M.E. (2014). *Methods of analysis and quality control of fresh and diverse processed fruits and vegetables* (in Romanian). Ed Universitaria, Craiova.

Khater, E. S. G., & Bahansawi, A. (2016). Watermelon fruits properties as affected by storage conditions. *Misr Journal of Agricultural Engineering*, *33*(1), 101-122.

McGregor, C. (2012). *Citrullus lanatus* germplasm of southern Africa. *Israel Journal of Plant Sciences, 60*(4): p. 403-413https://doi.org/10.1560/IJPS. 60.1.403.

Perkins-Veazie, P., Collins, J.K. (2004)**.** Flesh quality and lycopene stability of fresh-cut watermelon. *Postharvest Biol. Technol*., 31: 159-166.

Shrefler, J., Brandenberger, L., Rebek, E., Damicone, J., & Taylor, M. (2015). Watermelon production. *Oklahoma Cooperative Extension, Oklahoma State University, Oklahoma, United States of America, Fact Sheets, HLA-6236. Available online at (http://osufacts. okstate. edu)*.

Tanaka, T., Wimol, S., & Mizutani, T. (1995). Inheritance of fruit shape and seed size of watermelon. *Journal of the Japanese Society for Horticultural Science*, *64*(3), 543-548.

Victoire, G. D., Désiré, A. Y., Marc, A., Thierry, A. P. M., & Achille, T. F. (2023). Comparative Study of the Physico-Chemical and Biochemical Parameters of the Pulp of Two Varieties of Watermelon (*Citrullus lanatus*) Grown in Côte D'Ivoire. *American Journal of Food Science and Technology*, *11*(5), 175-182.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

CONVENTIONAL AND BIOLOGICAL CONTROL OF PESTS IN PEPPERS AND EGGPLANTS CROPS IN THE GREENHOUSE. A REVIEW

Simona HOGEA, Gabriela ȘOVĂREL

Romania Research and Development Institute for Vegetable and Flower Growing Vidra, 22 Calea București, Vidra, Ilfov, Romania

Corresponding author email: gabriela_sovarel@yahoo.com

Abstract

The paper presents the main pests that affect peppers and eggplants crops and chemical and biological methods to control. In the 2010-2021 period, in Romania, the average area of eggplants was 9233,75 hectares and in peppers case was 18745.5 hectares. The most common pests in peppers and eggplants crops are trips, whiteflies, aphids, mites and caterpillars. Even though the use of pesticides can reduce the percentage of damage caused by pests, they are less and less used worldwide due to several disadvantages. To reduce the amount of pesticides used, more and more studies are being conducted on the application of biological products based on plant extracts (such as citrus, cinnamon, mimosa tenuiflora and seaweed extract) or microorganisms (Bacillus thuringiensis) and on the release of parasites and predators (Macrolophus pygmaeus, Amblyseius swirskii, Chrysoperla carnea etc.) to reduce risks to human health and the environment.

Key words*: control, eggplant, pepper, pest.*

INTRODUCTION

In recent years, agriculture has been subjected to excessive use of chemicals, which has caused significant damage to the soil, climate, water and global ecosystem of our planet. (Barcanu et al., 2023). Pesticides are often used to help increase pepper crop production by reducing damage caused by pests. This could improve farmers profitability in pepper production (Oerke et al., 2004; Shim et al., 2023). However, it is important to note that the use of pesticides can also have negative effects, including risks to human health and the environment (Radwan et al., 2005; Shim et al., 2023). The World Health Organization (WHO) reported that approximately three million cases of pesticide poisoning occur annually worldwide, of which 220,000 cases are fatal (Shim et al., 2023).

In response to these challenges, there is now a concerted effort to develop and adopt organic farming practices to remedy the damage and prevent further damage. Organic farming has become a vital objective of the European Commission, aiming for at least 25% of agricultural land to be dedicated to organic crops by 2030 (Barcanu et al., 2023). Practicing organic farming systems can be solutions to these problems, and their role will contribute to improving food security through survival (Sterie et al., 2023).

The global harvested area of eggplant was estimated at 1.85 million hectares in 2019, with a production of nearly 33 million tons, where China, India, Indonesia, Egypt, Turkey, the Philippines and Iran are major producers with 90% of the total global area (FAO, 2019; Nayak et al., 2021). The total vegetable area in Romania recorded 262.7 thousand acres in 2010, reaching 197.7 thousand hectares in 2021, showing a decrease of 24%. The average area under eggplants in Romania in the period 2010-2021 was 9233.75 hectares, varying between 8576 hectares (2020) and 10315 hectares (2010), presenting a negative growth rate (-1.13%). In the case of areas under peppers, they varied between 17188 hectares (2020) and 21005 hectares (2010), with a period average of 18745.5 hectares (Sterie et al., 2023). The harvested area of eggplants in 2022 was estimated at 4140 ha, with a production of 15594.2 kg/ha, and in 2021 it was 4940 ha and a production of 19404.9 kg/ha (FAOSTAT).

There are many advantages to growing plants in high tunnels, but there are also disadvantages such as reduced ventilation which can lead to an

increase in temperature and relative humidity, leading to a favorable environment for the emergence and development of pests (Weintraub, 2006).
Both eggplant and pepper crops are attacked by pests from the seedling stage to the mature plant stages (El Arnaouty et al., 2020). Pests that can reduce production through qualitative and quantitative depreciation are thrips, whiteflies, aphids, mites and caterpillars. Also, some of the insects listed can transmit viruses and bacteria (Shim et al., 2023).

PESTS IN PEPPERS AND EGGPLANTS CROPS

Thrips

Thrips is one of the main pests of crops in protected areas, causing damage and production losses in many vegetable crops. They can cause direct damage due to feeding and oviposition, as well as indirect damage by transmitting plant viruses such as Tospoviruses (Rosenheim et al., 1990; Visschers et al., 2023). It is a polyphagous insect that has the ability to quickly develop resistance to insecticides. The most harmful species are *Frankliniella occidentalis*, *Trips tabaci*, *Thrips palmi* and western flower thrips (WFT) (Lewis, 1997; Weintraub, 2006).
Larvae and adults of thrips can be indetified on the leaves, the vegetative tops, flowers and fruit and suck the sap out of tissue. As a result of the attack, discoloring spots appear, which in time become silver or even brown (Hoza et al., 2016). Thrips like many other pests are becoming resistant to many insecticides, but attempts to control the virus they vector are unsuccessful because thrips can transmit the pathogen faster than they acquire a lethal dose of insecticide (Todd et al., 1996; Weintraub, 2006).

Whiteflies

Among the whiteflies the two species, the sweetpotato whitefly, *Bemisia tabaci* and the greenhouse whitefly, *Trialeurodes vaporariorum* cause most of the damage (Nauen et al., 2014; Perring et al., 2018). These whiteflies, using mouthparts modified for sucking, damage plants by removing sap, excreting honeydew, vectoring plant-limiting viruses (Perring et al., 2018) and can be found both in greenhouses and open field. Severe infestation of whitefly adults and progeny can cause seedling death or a reduction in the vigor and yield of plants (Kumar et al., 2019; Horowitz et al., 2020; Zayed et al., 2022).
These pests can be found in crops like pepper, eggplant, cucumber, tomato, bean etc. (Șovărel et al., 2022).

Mites

The principal spider mites in horticulture crops is *Tetranychus urticae* known as the two-spotted spider mite. This mite has needle-like chelicerae which pierce plant cells, often feeding on chloroplasts on the undersurface of the leaf (Zhang, 2003; Weintraub, 2006). In Romania, the pest attacks crops of eggplant, peppers, cucumbers, beans, tomatoes etc. (Călin et al., 2017). As a result of the attack, upper leaf surfaces develop whitish or yellowish stippling and produce silk webbing which covers the leaves (Weintraub, 2006).
The broad mite, *Polyphagotarsonemus latus*, is an important pest in tropical and subtropical regions which attacks young, growing plant parts (Weintraub et al., 2003). This mite is 0.1-0.3 mm long, also known as the white mite, and is found in the apical portion of plants, especially in shoot structures (Venzon et al., 2008). Beeing very small and difficult to detect, usually feeds on the lower leaf surface and causes leaf edges to become rigid and roll under, and causes distortion and discoloration of flowers and blistering of fruits (Weintraub, 2006). Usually the damage begins in small clumps, but infestations can spread rapidly (Venzon et al., 2008).

Aphids

Among the aphids, the green peach aphid, *Myzus persicae* is widely distributed in temperate regions, beeing a polyphagous insect pest of protected vegetables (Wang et al., 2021), with more than 800 host plant species (Raeyat et al., 2021). Another problematic aphids are *Macrosiphum euphorbiae, Aphis gossypii* and *Aulacorthum solani* (Sanchez et al., 2011).
The aphids form crowded colonies on the underside and upper side of the leaves, later dispersed on the flowers (Costache et al., 2023). The main problems caused by aphids are that they are vectors of viruses and produce a large

amount of honeydew that they leave on the plants (Sanchez et al., 2011).
Aphid control is very important and can be achieved through preventive and curative methods: IPM, barrier crops integrating companion cropping and nitrogen application, agronomic, biological and botanical practices (Călin et al., 2020).

Caterpillars
The main insect species that cause damage on plants foliage and fruits are *Helicoverpa armigera* and *Spodoptera exigua.*
H. armigera damages 68 plant families, more than 350 species of plants (Kuznetsov, 1999; Cunningham & Zalucki, 2014; Terlemezyan et al., 2024). The larvae cause the main problems, because its feeding with vegetative parts of the plants and with buds, inflorescences, fruits and pods (Suzana-Milan et al., 2022; Terlemezyan et al., 2024).
S. exigua attacks the leaves and fruits, causing damages to over 185 species of plants belonging to 50 families (Terlemezyan et al., 2024). The caterpillars are usually found on the back of the leaves, where they feed, destroying the leaf tissues, leaving only the veins that maintain the leaf's shape (Terlemezyan et al., 2024).

AGROPHYTOTECHNICAL METHODS

The efficiency of control actions and the reduction of production losses caused by harmful insects in vegetable crops are conditioned by the application of a complex of agrophytotechnical measures (Costache et al., 2023). The main agrophytotechnical measures are crop rotation, cultivation of resistant/tolerant hybrids and varieties, planting on soil mulched with foil (inhibits weed growth, maintains soil temperature and moisture, protects the soil from erosion caused by wind or water, leaching of fertilizers, especially on sandy soils, as well as protecting against infection and the development of soil-borne diseases) (Moursy et al., 2015), plant nutrition: seaweed-based biostimulators can be used successfully in the eggplant fertilization program (Constatin et al., 2023) and peppers need a significant amount of nitrogen, which helps the plant grow (Constantin et al., 2024), crops irrigation, mechanized and manual plows, phytosanitary hygiene measures (periodic removal of affected organs and plants from the crop) etc.

CHEMICAL CONTROL

Pesticides are the main method for disease and pest management and the productivity of crops depends on their effective control (Jeyanthi & Kombairaju, 2005). Most chemical insecticides are very effective in controlling pests, however, few pesticides are safe for use and storage (Iosob et al., 2021).
Over time, pests have developed resistance to pesticides due to their excessive use (some example of conventional insecticides: organophosphates, carbamates and synthetic pyretrhroids; Kodandaram et al., 2010).
Pesticides with active substance thiamethoxam and acetamaprid were used to control whitefly (Kalaf & Dawood, 2020) in the past, this substances are banned now. The product Avaunt (indoxacarb) was used against whitefly nymphs, which reduced the pest population density from 7.51 to 1.01 after 14 days of treatment (Patel et al., 2014).
The active substance abamectine was used to control thrips, mites and leafminer flies and imidacloprid to control whitefly, thrips and aphids. Jodeh et al. (2016) noticed that the amount of imidacloprid and abamectine residues were very high even after the first day of treatment. Was demonstrate that photodegradation caused a decrease of the pesticide residues in the plant parts (Jodeh et al., 2016). The products with these substances are also banned.
In Romania, in this moment, the following substances are approved for pest control in pepper crops: hexythiazox, deltamethrin, acetamiprid, flonicamid, emamectin benzoate, and for eggplant crops: hexythiazox, chlorantraniliprole.
Hexythiazox is an acaricide used for the control of many phytophagous mites. Glăvan et al. (1993) claim that the product based on hexythiazox in a concentration of 0.1% caused a mortality, in the mobile stages of the pest *T. urticae*, of 100% (12 days after treatments) in the Iṣalniṭa area and of 93.44-97.07% in the Vidra area (Romania). They also found that the product's effectiveness on eggs is lower (67.70% - 12 days after treatment).

BIOLOGICAL CONTROL

Excessive use of pesticides increases the cost of production, causes human health problems and environmental pollution (Jeyanthi and Kombairaju, 2005). Controlling pests in crops under high plastic tunnels by chemical pesticides has conducted to several problems such as development of resistance and raising environmental and health concerns (El Arnaouty et al., 2020).

In order to reduce the amount of pesticides used, more and more studies are being carried out on the application of biological products based on plant extracts (such as citrus, cinnamon, mimosa tenuiflora and seaweed extract) or microorganisms (*Bacillus thuringiensis*) and on the release of parasitoids (*Aphidius colemani*) and predators (*Macrolophus pygmaeus, Amblyseius swirskii, Chrysoperla carnea, Coccinella septempunctata* etc.) to minimize risks to human health and the environment.

Biological products will not completely eliminate pest problems, but they can reduce pest populations and damage to a reasonable level (below the economic threshold). Applying biological control generally requires more time than chemical control to bring a pest population to an acceptable level of control (El Arnaouty et al., 2020). Despite their beneficial effect on environmental quality, biopesticides generally have several limiting characteristics (rapid degradation in sunlight, air and humidity) that make them less accepted by large farming communities, being recognized and used mainly in organic agriculture in developed countries (Cenușă et al., 2016).

Classes of insecticides have been marketed that are not persistent or bioaccumulative and have few effect on mammals, but can be very toxic to aquatic invertebrates. These are based on citrus, neem azadirachtin, garlic, chili pepper derivatives, and limonene from citrus peel (Sarwar & Salman, 2015).

Neem oil is extracted from the neem seeds of Azadirachta indica, a tree native to India that grows in poor, degraded soils and semi-arid climates. The oil extracted from these seeds has repellent, antifendant and insecticidal properties (Siegwart et al., 2015). Neem extracts work against chewing and sucking insects such as lepidopterous caterpillars and beetle larvae (Xu et al., 2008). The active ingredient accumulates in the growing tips of the treated plants, usually reaching functional levels within 24 hours of foliar spraying (Xu et al., 2008).

Bacillus thuringiensis is an aerobic bacterium, Gram-positive, of the bacilli family and is widely present in soil, water, air and plants in its vegetative form, beeing the most common and described bioinsecticide in the world (Raymond et al., 2010; Siegwart et al., 2015).

Aphidius colemani is a parasitic wasp of aphids which laid eggs directly in the aphid body, hatch and consume the aphid. After that the body of aphids bloats and develops a hard shell. When the wasp reachs maturity cuts a circulat hole and escapes (Weintraub, 2006). The advantage of parasitoids is that they can develop in large numbers before aphid pests are observed and a continuous supply of parasitoids is ensured over a period of several weeks (Weintraub, 2006).

Macrolophus pygmaeus is native to the Mediterranean region and often occurs in greenhouses ot tunnels, where pesticides have not been applied for a long time (Ramzi et al., 2022). It feeds on with plant tissue and attacks pests like whiteflies, thrips, aphids, spider mites, leaf miners and *Lepidoptera* species including *Tuta absoluta* (Urbaneja et al., 2009; Zhang et al., 2022).

Amblyseius swirskii is a mite used to control thrips in pepper, eggplant and cucumber crops grown in greenhouse. Some research showed that predator provided better control on the foliage of pepper plants, compared with the flowers (Călin et al., 2020). Another research made in Turkey showed that releasing 50 adults/sqm of *A. swirskii* can control the thrips attack, even on the long term in pepper crops in heated and not heated tunnels (Kutuk et al., 2011; Hoza et al., 2016).

Chrysoperla carnea is a generalist predator that spontaneously appears in crops (Weintraub, 2006). Have been recorded as an effective predator of aphids (Liu & Chen, 2001), coccids, mites and mealy bugs (Zaki & Gesraha, 2001; Sattar et al., 2011).

Coccinellid predators are usually utilized for aphid control, since many species can reduce aphis populations in greenhouses (Skouras et al., 2023). *Coccinella septempunctata* known as the seven-spotted lady beetle, is an important biological contron agent which can be found in

a wide rage of agricultural and/or natural habitats or crops all over the world (Singh et al. 2004) and can prey more than 20 aphid species, as well as species of *Psylloidea* and *Tetranychidae* (Skouras et al., 2023). Bratu (1998) states that the *T. urticae* attack superimposed on that of the *M. persicae* species, negatively influences the efficiency of the *C. septempunctata* predator, reducing it by up to 14.9 - 52.0%.

Another method to control is baculoviruses as insecticides, being ideal tools in IPM programs. About 60 baculovirus-based pesticides have been utilized to control diverse insect pest all over the world (Beas-Catena et al., 2014). Insect viruses are unable to infect mammals and humans, beeing very safe to handle. Because the most insect viruses are relatively specific the risk of non-target effects on beneficial insects is very low (Gupta & Dikshit, 2010) with no or negligible effects on non-target organisms (beneficial insects, vertebrates and plants) (Yaqoob et al., 2016; Gelaye & Negash, 2023). Most baculoviruses infect caterpillars, which are the immature form of moths and butterflies (Rodriguez et al., 2012; Gelaye & Negash, 2023).

CONCLUSIONS

To minimize pesticide residues, which directly and indirectly affect the human population, fauna and the environment, farmers globally are adopting biological control, less harmful. Biological control of insect pests can be achieved by applying biological pesticides and releasing parasites and predators (*Macrolophus pygmaeus, Amblyseius swirskii, Chrysoperla carnea, Coccinella septempunctata* etc.) into crops.

It is also important to apply a complex of agrophytotechnical measures to increase the efficiency of control measures and means (chemical or biological).

ACKNOWLEDGEMENTS

This research work was carried out with the support of Ministry of Agriculture and Rural Development, financed from Project ADER 6.3.15/2023 – Integrated management for the control of pest agent in the main vegetable species grown in greenhouses in conventional and organic system.

REFERENCES

Barcanu, E., Agapie, O., Gherase, I., Tanase, B., & Dobre, G. (2023). Evaluating cultivars for organic farming: tomatoes, peppers and aubergine in south Romania. *Agriculture & Food, Vol. 11*, ISSN 1314-8591.

Beas-Catena, A., Sanchez-Miron, A., Garcia-Camacho, F., Contreras-Gomez, A., & Molina-Grima, E. (2014). Baculovirus pesticides: an overview. The Journal of *Animal & Plant Sciences, 24*(2), page 362-373, ISSN: 1018-7081.

Bratu, E. (1998). Effiency of the predator *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) released in egg stage to reduce populations of the pest *Myzus persicae* Sulz. in pepper crops. *Anale I.C.D.L.F., Vol. XV*, pp.215-227, ISSN 0250-2917.

Călin, M., Cristea, T.I., Ambăruș, S., Brezeanu, C., Brezeanu, P. M., Muscalu, S. P., Costache, M., Șovărel, G., & Bratu E. (2017). Biological control of two-spotted spider mite in pepper and melon crops cultivated in tunnels. *Scientific papers. Seria B Horticulture*, Volume LVI, pag. 347–352.

Călin, M., Cristea, T.I., Ambăruș, S., Brezeanu, C., Brezeanu, P. M., Iosob, G.A., Muscalu, P.S., Calara, M., Bute, A., & Prisecaru, M. (2020). Behavior of vegetable plant seeds at aphids atack and control of these pests. *Biologie, 29*/2, pp 70-74, Universitatea ”Vasile Alecsandri” din Bacău.

Călin, M., Cristea, T.O., Brezeanu, P.M., Ambarus, S., Brezeanu, C., Muscalu, S.P., Sova, F., Antal, C.A., Prisecaru, M., Costache, M., Sovarel, G., Bratu, L., Burnichi, F., & Sbirciog, G. (2020). Biological control of pepper pests in organic agriculture. *Acta Hortic. 1269*. ISHS, DOI 10.17660/ActaHortic.2020.1269.22.

Cenuşă, A.E., Șovărel, G., Costache, M., & Bratu, E. (2016). Effect of some ”bio-insecticides” used against two spotted spider mites (*Tetranychus urticae* KOCH.) in the cucumbers crop under plastic tunnels conditions. *Scientific Papers. Series B, Horticulture*. Vol. LX, Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653.

Constantin, D. C., Gheorghe, M.C., Buzatu, M.A., & Scurtu, I. (2023). The role of biostimulants in the fertilization program in eggplant. *Romanian Journal of Horticulture, Vol. IV*, pp 59-64, 10.51258/RJH.2023.06.

Constantin, D. C., Paraschiv, M., & Sbirciog, G. (2024). The impact of foliar treatments on yield and quality of round pepper *(Capsicum annuum* L.) at *Asteroid 204 cv. Romanian Journal of Horticulture, Vol. V*, pp 35-42, 10.51258/RJH.2024.04.

Costache, M., Sovarel, G., Hogea, S., & Cenusa, E. (2023). *Bolile și dăunătorii culturilor de legume din spații protejate și câmp. Recunoaștere și combatere*. Editura PIM Iasi, pp 131, ISBN 978-606-13-8010-7.

Cunningham, J.P., & Zalucki, M.P. (2014). Understanding heliothine (Lepidoptera: Heliothinae)

pests: what is a host plant?. *J.Economic Entomology. 107*: 881-965. doi: 10.1603EC14036.

El Arnaouty, S. A., El-Heneidy, A. H., Afifi, A., Heikal, I. H., & Kortam, M. N. (2020). Comparative study between biological and chemical control programs of certain sweet pepper pest in greenhouses. *Egyptian Journal of Biological Pest Control 30*:28, https://doi.org/10.1186/s41938-020-00226-z

Gelaye, Y., & Negash, B. (2023). The role of baculoviruses in controlling insect pests: A review. *Cogent Food & Agriculture, 9*: 2254139. https://doi.org/10.1080/23311932.2023.2254139.

Glăvan, L., Negru, G., Popescu, A., Szabo, Al., Bratu, E., & Tudose, M. (1993). Effectiveness of some new biological products in the control of certain pests in the vegetable crops*. Anale I.C.D.L.F, vol. XII*, pp. 163–172, ISSN 0250-2917.

Gupta, S., & Dikshit, A. (2010). Biopesticides: An ecofriendly approach for pest control. *Journal of Biopesticides, 3*(1), 186–188.

Horowitz, A.R., Ghanim, M., Roditakis, E., & Nauen, R.; Ishaaya, I. (2020). Insecticide resistance and its management in *Bemisia tabaci* species. *J. Pest Sci., 93,* 893–910.

Hoza, G., Dobrin, I., Dinu, M., Becherescu, A., Ilie, V., & Catuneanu Bezdadea, I. (2016). Research regarding the use of natural predators for the control of pests for pepper in protected culture. *Agriculture and Agricultural Science Procedia 10,* 192–197.

Iosob, G.A., Cristea, T.O., Bute, A., & Avasiloaiei, D-I. (2021). Biological control of pests in cucurbitaceous vegetables: an overview. *Biologie, 30*/2, pp. 68-76, Universitatea ”Vasile Alecsandri” din Bacău.

J.A. Sanchez , M. La-Spina , J.M. Michelena , A. Lacasa & A. Hermoso de Mendoza (2011). Ecology of the aphid pests of protected pepper crops and their parasitoids. *Biocontrol Science and Technology, 21*:2, 171-188.

Jeyanthi, H., & Kombairaju, S. (2005). Pesticide use in vegetable crops: frequency, intensity and determinant factors. *Agricultural Economics Research Review,* vol. 18, pp 209-221.

Jodeh, S., Al Masri, S., Haddad, M., Hamed, O., Jodeh, D., Salghi, R., Radi, S., Amarah, J., El-Hajjaji, F., & Warad, I. (2016). Evaluation of potential Residue of Imidacloprid and Abamectin in cucumber and pepper plants after spraying using high performance liquid chromatography (HPLC). *J. Mater. Environ. Sci. 7*(3), pp. 1037 – 1047, ISSN: 2028-2508.

Kalaf J., M., & Dawood, S., H. (2020). Test of the chemical pesticides effect and some Biological agents in the control of white fly nymphs, *Bemisia tabaci* (Genn) on Eggplant. *International Journal of Agricultural and Statistical Sciences., Vol. 16*, Supplement 1, pp. 1289-1294. DocID: https://connectjournals.com/03899.2020.16.1289.

Kodandaram, M.H., Rai, A.B., & Halder, J. (2010). Novel insecticides for managament of insect pests in vegetable crops: A review. *Veg. Sci. 37*(2): 109-123.

Kumar, P., Naqvi, A.R., Meena, R.S., & Mahendra, M. (2019). Seasonal incidence of whitefly, *Bemisia tabaci* (Gennadius) in tomato (*Solanum lycopersicum* Mill). *Int. J. Chem. Stud., 7,* 185–188.

Kutuk, H., Yigit, A., Canhilal, R., & Karacaoglu, M. (2011). Control of western flower thrips (*Frankliniella occidentalis*) with *Amblyseius swirskii* on greenhouse pepper in heated and unheated plastic tunnels in the Mediterranean region of Turkey. *African Journal of Agriculture Research vol. 6*(24), pg. 5428-5433.

Kuznetsov, V.I. (1999). *Insects and mites are pests of agricultural crops. Volume III*. *Lepidoptera. Part 2*. St. Petersburg: Publishing house “Nauka”. 410 p.

Lewis T (1997). *Thrips as Crop Pests.* CAB International, Wallingford.

Liu, T-X., & Chen, T-Y. (2001). Effects of three aphid species (Homoptera: Aphididae) on development, survival and predation of *Chrysoperla carnea* (Neuroptera: Chrysopidae). *Appl. Entomol. Zool. 36*(3): 361-366.

Moursy, F. S., Mostafa, F. A., & Solieman, N. Y. (2015). Polyethylene and rice straw as soil mulching: reflection of soil mulch type on soil temperature, soilborne diseases, plant growth and yield od tomato. *Global Journal of Advanced Research, vol. 2,* Issue 10, pp. 1497-1519, ISSN: 2394-5788.

Nauen, R., Ghanim, M., & Ishaaya, I. (2014). Whitefly special issue organized in two parts. *Pest Management Science, 70*, 1438–1439.

Nayak, S.B., Rao, K.S., & Mekala, S. (2021). *Management of important insect-pest o eggplant (Solanum melongena* L)*. Solanum melongena: Production*. *Cultivation and Nutrition*, ISBN: 978-1-68507-311-4.

Oerke, E.C., & Dehne, H.W. (2004). Safeguarding production - Losses in major crops and the role of crop protection. *Crop Prot., 23*,275–285.

Patel, R., T.M. Bharpoda, N.B. Patel, & P.K. Borad (2014). Bio-efficacy of cyantraniliprole 10% od-ananthranilic diamide insecticide against sucking pests of cotton*. International Quarterly Journal of life sciences Bioscan, 9*(1), 89-92.

Perring, T.M., Stansly, P.A., Liu, T.X., Smith, H.A., & Andreason, S.A. (2018). Whiteflies: Biology, Ecology, and Management. In: Wakil, W., Brust, G.E., Perring, T.M. (Eds.), *Sustainable Management of Arthropod Pests of Tomato*. *Academic Press, Elsevier*, pp. 73–110.ISBN: 9780128024416.

Radwan, M.A., Abu-Elamayem, M.M., Shiboob, M.H., & Abdel-Aal, A. (2005). Residual behavior of profenofos on some field-grown vegetables and its removal using various washing solutions and household processing. *Food Chem. Toxicol., 43*, 553–557.

Raeyat, Z., Razmjou, J., Naseri, B., Ebadollahi, A., & Krutmuang, P. (2021). Evaluation of the Susceptibility of Some Eggplant Cultivars to Green Peach Aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae). *Agriculture, 11*, 31. https://doi.org/10.3390/agriculture11010031.

Ramzi, S., Madahi, K., Lotfollahi, P. & Azimi, S. (2022) Can host plants affect egg predation of two-spotted spider mite by *Macrolophus pygmaeus* (Hemiptera: Miridae)?, *Persian Journal of Acarology, 11*(2): 309–322.

Raymond, B., Johnston, P., Nielsen-Leroux, C., Lereclus, D., & Crickmore, N. (2010). *Bacillus thuringiensis*: an

impotent pathogen? *Trends Microbiol. 18*, 189–194. doi: 10.1016/j.tim.2010.02.006.

Rodriguez, V. A., Belaich, M. N., & Ghiringhelli, P. D. (2012). Baculoviruses: Members of Integrated Pest Management Strategies. *In Integrated Pest Management and Pest Control-Current and Future Tactics*, pp. 463–480. https://doi.org/10.5772/32779.

Rosenheim, J.A., Welter, S.C., Johnson, M.W., Mau, R.F.L., & Gusukuma-Minuto, L.R. (1990). Direct Feeding Damage on Cucumber by Mixed-Species Infestations of *Thrips palmi* and *Frankliniella occidentalis* (Thysanoptera: Thripidae). *J. Econ. Entomol., 83*, 1519–1525.

Sarwar, M., & Salman, M. (2015). Success stories of eco-friendly organically acceptable insecticides as natural products discovery. *International Journal of Materials Chemistry and Physics Vol. 1*, No. 3, pp. 388-394 http://www.aiscience.org/journal/ijmcp.

Sattar, M., Abro, G.H., & Syed, T.S. (2011). Effect of different hosts on biology of Chrysoperla carnea (Stephens) (Neuroptera: Chrysopidae) in Laboratory Conditions. *Pakistan J. Zool., vol. 43* (6), pp. 1049-1054.

Shim, J.-H., Eun, J.-B., Zaky, A.A., Hussein, A.S., Hacimüftüoglu, A., & Abd El-Aty, A.M. (2023). A Comprehensive Review of Pesticide *Residues in Peppers. Foods, 12*, 970. https://doi.org/10.3390/foods12050970.

Siegwart, M., Graillot, B., Blachere Lopez, C., Besse, S., Bardin, M., Nicot, P.C. & Lopez-Ferber, M. (2015). Resistance to bio-insecticides or how to enhance their sustainability: a review. *Front. Plant Sci. 6:*381. doi: 10.3389/fpls.2015.00381.

Singh, S.R., Walters, K.F.A., Port, G.R., & Northing, P. (2004). Consumption rates and predatory activity of adult and fourth instar larvae of the seven spot ladybird, *Coccinella septempunctata* (L.), following contact with dimethoate residue and contam-inated prey in laboratory arenas. *Biol. Control, 30,* 127–133.

Skouras, P.J., Karanastasi, E., Lycoskoufis, I., Demopoulos, V., Darras, A.I., Tsafouros, A., Tsalgatidou, P.C., Margaritopoulos, J.T., & Stathas, G.J. (2023). Toxicity and Lethal Effect of Greenhouse Insecticides on *Coccinella septempunctata* (Coleoptera: Coccinellidae) as Biological Control Agent of Myzus persicae (Hemiptera: Aphididae). *Toxics, 11,* 584. https://doi.org/10.3390/ toxics11070584

Șovărel, G., Costache, M., & Hogea, S. (2022). *Managementul integrat pentru controlul agenților de dăunare la culturile de legume din familia Solanaceae*, Editura Pim, Iași, p. 47, ISBN 978-606-13-7222-5.

Sterie, M.C., Stoica, G.D., Giucă, A.D., & Bogos, I.B. (2023). An overview of the vegetable sector in Romania. Conference: Competitiveness of Agro-Food and Environmental Economy DOI: 10.24818/CAFEE/2022/11/01

Suzana-Milan, C.S., Bruschi, L., Schaeffer, A.H., Fiorentin, F.J.R., & Salvadori, J.R. (2022). Feeding preference of *Helicoverpa armigera* larvae (Lepidoptera: Noctuidae) on vegetative and reproductive organs of soybean plants. *Research, Society and Development. 11*(4): e5311426 789. doi: 10.33448/rsd-v11i4.26789.

Terlemezyan, H.L., Manvelyan, A.A., Harutyunyan, H.R., Mkrtchyan, H.N., & Ghazaryan, M.H. (2024). Pests of *Helicoverpa armigera* (Hübner) and *Spodoptera exigua* (Hübner) in Pepper Fields in Armenia. *Indian Journal of Agricultural Research*. 1-6. doi: 10.18805/IJARe.AF-898.

Todd, J.W., Culbreath, A.C., & Brown, S.L. (1996). Dynamics of vector populations and progress of tomato spotted wilt disease relative to insecticide use in peanuts. *Acta Hortic., 431*:491–198.

Urbaneja, A., Montón, H., & Mollá, O. (2009). Suitability of the tomato borer *Tuta absoluta* as prey for *Macrolophus pygmaeus* and *Nesidiocoris tenuis. J Appl Entomol, 133*: 292–296. https://doi.org/10.1111/j. 1439-0418.2008.01319.x

Venzon, M., Rosado, M.C., Molina-Rugama, Duarte, V.S., Dias, R., & Pallini, A. (2008). Acaricidal efficacy of neem against *Polyphagotarsonemus latus* (Banks) (Acari: Tarsonemidae). *Crop Protection, 27,* 869-872. doi:10.1016/j.cropro.2007.10.001.

Visschers, I.G.S., Macel, M., Peters, J.L., Sergeeva, L., Bruin, J., & van Dam, N.M. (2023). Exploring Thrips Preference and Resistance in Flowers, Leaves, and Whole Plants of Ten Capsicum Accessions. *Plants, 12*, 825. https://doi.org/10.3390/ plants12040825.

Wang, J., Li, S., Fang, Y., Zhang, F., Jin, Z.Y., Desneux, N., & Wang, S. (2021). Enhanced and sustainable control of *Myzus persicae* by repellent plants in organic pepper and eggplant greenhouses. *Pest Manag Sci*, DOI 10.1002/ps.6681.

Weintraub, P.G. (2006). Review. Integrated control of pests in tropical and subtropical sweet pepper production. *Pest Management Science*, DOI: 10.1002/ps

Weintraub, P.G., Kleitman, S., Mori, R., Shapira, N., & Palevsky, E. (2003). Control of the broad mite (*Polyphagotarsonemus latus* Banks) on organic greenhouse sweet peppers (*Capsicum annuum* L.) with the predatory mite, *Neoseiulus cucumeris* (Oudemans). *Biological Control, 27*, 300-309. doi:10.1016/S1049-9644(03)00069-0.

Xu, H-L., Xu, R., Qin, F., Ma, G., Yu, Y., & Kumar Shah, S. (2008). Biological pest and disease control in greenhouse vegetable production. *Acta Hort., 767*, ISHS.

Yaqoob, A., Shahid, A. A., Samiullah, T. R., Rao, A. Q., Khan, M. A. U., Tahir, S., Mirza, S. A., & Husnain, T. (2016). Risk assessment of Bt crops on the non-target plant-associated insects and soil organisms. *Journal of the Science of Food and Agriculture, 96*(8), 2613–2619. https://doi.org/10.1002/jsfa.7661.

Zaki, F.N., & Gesraha, M.A. (2001). Production of the green lacewing, *Chrysoperla carnea* (Steph.) (Neuroptera: Chrysopidae) reared on semi-artificial diet based on algae, Chlorella vulgaris. *J. appl. Ent., 125:* 97-98.

Zayed, M.S., Taha, E.-K.A., Hassan, M.M., & Elnabawy, E.-S.M. (2022). Enhance Systemic Resistance Significantly Reduces the Silverleaf Whitefly Population and Increases the Yield of Sweet Pepper,

Capsicum annuum L. var. *annuum*. *Sustainability, 14*, 6583. https://doi.org/10.3390/su14116583.

Zhang, N. X., Andringa, J., Brouwer, J., Alba, J. M., Kortbeek, R. W. J., Messelink, G. J., & Janssen, A. (2022). The omnivorous predator *Macrolophus pygmaeus* induces production of plant volatiles that attract a specialist predator. *Journal of Pest Science, 95*(3), 1343-1355. https://doi.org/10.1007/s10340-021-01463-3.

Zhang, Z-Q. (2003). Mites of Greenhouses, Identification, Biology and Control. *CAB International*, Wallingford, UK.

WHO. Pesticide Poisoning and Public Health. 2017. Available online: https://www.who.int/whr/1997/media_centre/executive_summary1/en (https://www.who.int/home)

https://www.fao.org/faostat/en/#data/QCL

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

PARAMETRIC EVALUATION OF POD YIELD STABILITY IN BUSHING SNAP BEAN (*PHASEOLUS VULGARIS* L.)

Corneliu IACOB, Sorin CIULCA

Faculty of Engineering and Applied Technologies,
University of Life Sciences "King Mihai I" from Timişoara,
119 Calea Aradului Street, 300645, Timişoara, Romania

Corresponding author email: c_i_sorin@yahoo.com

Abstract

In the context of climate change, the achievement of economically efficient yields largely depends on the cultivar's stability. A snap bean variety is considered more stable if it achieves a high green pod yield with a low variation of mean yield over diverse environments. The study was carried out to identify snap bean varieties that have high and stable yield across different years, to be used in breeding and sustainable production of this species. The plant material was composed of 20 varieties of bushing snap bean, genetically and ecologically differentiated. The varieties were evaluated for their green pod yield over three years using a randomized block design with three replications. To assess the performance of the varieties, a combined analysis of variance and yield stability statistics were determined. 'Sondella', 'Plador', 'Doge' and 'Maxidor' varieties, with relatively high pod yield expressed a good stability and can be used to develop new diverse cultivars with stable yields. 'Super Nano Yellow' variety expressed the highest sensitivity to the change of climatic conditions, being specific adapted to favorable conditions.

Key words*: genotype x environment interaction, pod yield, snap bean.*

INTRODUCTION

Consumers' preferences and palatability of snap bean are influenced by pod quality traits, while the pod yield related traits influence farmer's decision to cultivate new varieties (García-Fernández et al., 2024). The size of the pods holds significance not only for yield but also for commercial viability, impacting consumer satisfaction and influencing pricing in wholesale and retail markets (Li et al., 2023). As well as other yield traits like pod weight per plant and pods per plant, pod size traits such as length, thickness and width, exhibit quantitative inheritance (García-Fernández et al., 2021; Hagerty et al., 2016; Singh and Singh, 2015; Wu et al., 2020), being strongly influenced by environmental factors (Campa et al., 2018). Temperature is one of the most aggressive factors that affects snap bean yield, given that high temperatures cause a decrease of both marketable yield and pod quality (Richmond and Maness, 2024).

Yield stability expresses how stable the yield of a crop is over time, e.g., from year to year in case of temporal yield stability and over space, e.g., across different environments in case of spatial yield stability (Weih et al., 2021). The temporal yield stability is relevant for farmers, because it determines economic predictability and reduces risk (Rieckling et al., 2021). Analyses of genotypes yield stability are very important in recent years given that the increased variability of climate is associated with a decreased stability of crop yields (Müller et al., 2018; Tigchelaar et al., 2018). The stability of yield for different crops has a global dimension in the context of food security (Kalkuhl et al., 2016).

Under the current climate changes, the effectiveness of plant breeding programs depends on their ability to deliver varieties that consistently exhibit superior yield and quality performance across diverse environmental conditions (Wondaferew et al., 2024). To reduce the magnitude of the genotype x environment interaction in snap bean, the pre-breeding manipulations resulted in gaining pod yield stability by using the isolation environment for planting and the yield performance per se for evaluating genotypes (Traka-Mavrona et al., 2002).

The performance of any traits is a combined result of the genotype, the environment and the

interaction between genotype and environment (Dinsa et al., 2022). The adaptability as related to crop plants applies not only to the plants' ability to survive but also to maintain yield stability under varying environments. Genotype x environment interaction can make it difficult to identify the best genotypes, since in instances of complex interactions like in the case of yield; some genotypes may be superior only in a certain environment (Marinho et al, 2013).

Two main contrasting concepts of stability, static and dynamic, are commonly used in plant breeding (Becker and Léon, 1988). According to the static concept, a stable genotype maintains a constant yield across environments, while in the dynamic concept a stable genotype implies a yield response correlated with the mean response of all tested genotypes in each environment (Annicchiarico, 2002). There are several statistical methods which allow assessing stability by means of identifying the varieties with the highest stability and the most predictable response to different environmental variations (Viella et al., 2011).

Yield stability is especially important for grain legume crops including snap bean, given that these crops are considered to be less stable than others (Reckling et al., 2020). Under the current climate changes, the study was carried out to identify snap bean varieties that have high and stable yield across different years, to be used in breeding and sustainable production of this crop.

MATERIALS AND METHODS

The biological material was composed by 20 varieties of bushing snap bean genetically and ecologically differentiated. The experiment was conducted at University of Life Sciences "King Mihai I" from Timisoara, on a on a black chernozem during 2022-2024. The plots were composed of four rows with 4 m length and 0.9 m width that makes a plot area of 3.6 m^2. The spacing of 40 x 10 cm between rows and plants was used. All standard technological practices for snap beans were uniformly applied. During the experiment the rainfall deficit was supplemented by drop irrigation. Based on the values of mean temperatures and rainfall presented in Table 1, it is observed that 2022 was the most favorable year, and 2024 characterized by higher temperatures in July-August associated with low levels of precipitation, was considered the most unfavorable

Table 1. Mean temperatures and precipitation from April to August during 2022-2024

Year	2022		2023		2024	
Month	T (°C)	P (mm)	T (°C)	P (mm)	T (°C)	P (mm)
April	15.1	51.3	17.1	67.7	21.9	16.2
May	22.8	56.2	25.2	22.2	24.6	25.1
June	26.8	37.6	30.6	15.9	30.5	48.5
July	31.9	28.2	32.5	10.4	34.9	14.3
August	30.9	28.4	31.7	39.7	33.2	3.2

The experimental design was a randomized block with three replications. From each plot five randomly selected plants were evaluated for pod yield. The data were analyzed using ANOVA as per method for randomized block design, while the varieties mean were compared using Least Significant Difference Test as described by Ciulca (2006).

In order to evaluate pod yield stability of the snap bean varieties, the following methods were used; ANOVA according to Eberhart and Russel (1966) model, associated with estimates of regression coefficient (*bi*) and deviations from regression (S_{di}^2); CV_i , coefficient of variation (Francis and Kannenberg, 1978); σ_i^2, stability variance (Shukla, 1972); D_i, desirability index (Hernandez et al., 1993); R_1i, R_2i, ranks parameters (Langer et al., 1979); W_i^2, ecovalence (Wrike, 1962); *SFi,* stability factor (Lewis, 1954); *Pi,* superiority index (Linn and Binn, 1988); D_i^2, genotypic stability (Hanson, 1970).

RESULTS AND DISCUSSIONS

The pooled analysis of variance for pod yield displayed significant differences among varieties, years and variety x year interactions (Table 2). The highest difference was observed

for the main effect of variety (61.63%), while the differences related to year were about 21.66%, and variety x year interactions totally reach 16.71%. As such, the varieties did not differ only genetically but also some of these exhibited differential response to the climatic conditions during the three years. These results revealed that there is a genetic variability for pod yield associated with the presence of genotype x environment interaction. The magnitude of the linear components for environment and environment x year were higher than the pooled deviation, indicating that the prediction of stability for the studied varieties could be reliable, as reported by Hosamani et al. (2010). Similar results regarding the significance of different linear components in snap bean were also reported by Pan et al. (2007) and Satish et al. (2017). The significance of the pooled deviation indicates that non-linear component of variety x year interactions was predominant. The high percentage of environmental variation indicates that the environment is a major factor influencing the yield performance of snap bean varieties, in accordance with the findings of Dinsa et al. (2022).

Table 2. Analysis of variance for stability (Eberhert and Russel Model) of pod yield in snap bean varieties during 2022-2024

Source of variation	SS	DF	MS	F
Variety (Var)	109484	19	5762	7.38**
Year (Env)	38483	2	19241	24.63**
Variety x Year	29685	38	781	1.71*
Env + (Var x Env)	68168	40	1704	
Env (Linear)	38483	1	38483	55.16**
Env x Var (Linear)	15732	19	828	1.19
Pooled Deviation	13953	20	698	4.59**
Pooled Error	51965	114	456	
Total	177651	59		

Considering the data from Table 3 it is noted that generally the climatic conditions of 2022 have favored the achievement of significantly higher yield, while in 2024 the achieved levels were lower than other years. Under the conditions from 2022 the pod yield recorded values between 70.83 g at 'Roquencourt' and 273.66 g at 'Plador' variety, amid a high variability among varieties performances. In this year the varieties 'Plador', 'Doge', 'Super Nano Yellow', 'Minidor', 'Berggold', 'Voletta' and 'Ileana' were highlighted, these achieved a pod yield of over 200 g.

Given the conditions of 2023 the snap bean varieties showed smaller amplitude of pod yield, with the limits from 70.12 g at 'Processor' to 213.28 g at 'Nassau' variety. Against the background of significantly lower yields than the previous year, only the 'Nassau' variety exceeded 200 g, but also the varieties 'Volleta', 'Dodge' and 'Plador', properly capitalized on this year's conditions, achieving yields of approximately 190 g.

The variability of yield in 2024 was higher than that recorded in previous years, amid lower values ranging from 66.18 g in 'Roquencourt' to 231.35 g for 'Plador' followed by 'Doge' variety with 212.55 g, given that nine of the other varieties achieved yields over 100 g. Regarding the annual values of the achieved yields, it was found that the varieties 'Sondella', 'Processor' and 'Velondrom' did not show significant variations during the study, while for 'Berggold', 'Meraviglia di Venezia' and 'Super Nano Yellow' varieties the pod yields differ significantly from one year to another.

Static stability is analogous with the biological concept of homeostasis, considering that a stable genotype tends to maintain a constant yield across environments. The term environmental sensitivity has also been used, considering that greater sensitivity corresponds to lower stability.

Table 3. Pod yield of snap bean varieties during 2022-2024

No.	Variety	Year			Variety mean
		2022	2023	2024	
1	Aurie de Turda	109.90 x	94.26 xy	73.76 y	92.64 jk
2	Berggold	213.45 x	146.95 y	107.24 z	155.88 de
3	Ileana	200.99 x	110.31 y	137.3 y	149.54 e
4	Minidor	223.08 x	131.45 y	125.45 y	159.99 cd
5	Maxidor	187.43 x	129.98 y	136.93 y	151.45 e
6	Roquencourt	70.83 x	80.62 y	66.18 x	72.55 m
7	Sondella	181.92 x	159.41 x	171.42 x	177.59 bc
8	Voletta	205.08 x	190.39 x	96.68 y	164.05 c
9	Doge	270.43 x	194.99 y	212.55 y	233.42 a
10	Meraviglia di Venezia	183.07 x	135.32 y	92.21 z	136.87 f
11	Plador	273.66 x	189.57 z	231.35 y	246.53 a
12	Super Nano Yellow	245.45 x	147.13 y	74.21 z	155.60 de
13	Tytania	137.41 x	71.83 y	83.82 y	97.69 ij
14	Contender	148.29 x	98.98 y	113.42 y	120.23 g
15	Domino	162.58 x	97.31 y	98.76 y	119.55 gh
16	Marconi	104.33 x	81.39 xy	67.18 y	84.30 l
17	Nassau	195.89 x	213.28 x	119.64 y	176.27 b
18	Processor	93.97 x	70.12 x	102.49 x	92.19 kl
19	Velodrom	120.53 x	97.11 x	118.43 x	115.36 hi
20	Wotter	147.62 x	118.82 x	64.49 y	110.31 i
	Year mean	173.80 A	127.96 B	114.68 B	

Different letters indicate significance at p< 0.05: x, y, z -for genotype x years comparisons.
a, b, c - for varieties comparisons; A, B, C - for years comparisons.

The highest type I stability was observed in the varieties 'Roquencourt', 'Sondella', 'Processor', 'Velodrom', which achieved constant values of yield according to the static concept, regardless of the environmental conditions in which they were tested (Becker and Leon, 1988; Annicchiarico P., 2002). The lowest type I stability in terms of production was expressed at 'Super Nano Yellow', 'Minidor', 'Berggold', who achieved very different levels of yield during the study (Table 4).

Table 4. Stability statistics b_i, S_{di}^2, CV_i, W_i^2, σ_i^2, Di, for pod yield of snap bean varieties

No	Variety	b_i	S_{di}^2	CV_i	W_i^2	σ_i^2	D_i
1	Aurie de Turda	0.558	18.81	19.56	460.10	202.44	95.67
2	Berggold	1.795	47.29	34.43	1396.36	722.59	165.61
3	Ileana	1.458	108.09	31.14	1109.50	563.22	157.43
4	Minidor	1.879	5.74	34.20	1341.95	692.36	170.18
5	Maxidor	1.047	16.92	20.70	122.21	14.73	157.12
6	Roquencourt	-0.017	15.46	10.16	1849.33	974.24	72.45
7	Sondella	0.053	76.54	9.26	2046.59	1083.82	171.20
8	Voletta	1.433	493.68	35.85	3771.96	2042.36	171.82
9	Doge	1.000	166.17	16.17	1163.18	593.05	231.41
10	Meraviglia di Venezia	1.467	72.17	33.21	872.90	431.78	144.82
11	Plador	0.598	609.62	20.02	4539.12	2468.57	234.77
12	Super Nano Yellow	2.828	185.79	55.23	6927.41	3795.39	170.92
13	Tytania	1.146	32.31	35.75	262.11	92.45	103.90
14	Contender	0.796	31.31	21.09	289.58	107.71	124.54
15	Domino	1.269	9.62	31.18	188.94	51.80	126.43
16	Marconi	0.626	6.23	22.24	279.83	102.29	87.69
17	Nassau	0.800	554.72	28.26	3950.14	2141.35	180.61
18	Processor	-0.047	128.37	23.04	2745.41	1472.06	88.60
19	Velodrom	0.079	74.29	14.12	1947.88	1028.99	112.45
20	Wotter	1.231	144.66	38.27	1102.30	559.22	116.98

Type II stability according to the dynamic concept involves changing the performance of a genotype in an anticipated direction from one year to another depending on climatic

conditions. The measure of dynamic stability depends on the specific set of tested genotypes (Linn et al., 1988). According to this concept, the highest dynamic stability was shown by the varieties 'Doge' şi 'Maxidor', where the pod yield in the climatic conditions of the three years was parallel to the average yields of the other studied varieties.

According to Lin et al. (1988), a genotype is considered stable if the residual mean square from the regression model on the environmental index is small. The highest type III stability, respectively the minimum values of deviations from the regression line were observed at varieties: 'Minidor' ranked first, 'Marconi' ranked second, 'Domino' ranked third, 'Roquencourt' ranked fourth and 'Maxidor' ranked fifth, for which this regression model is appropriate to describe yield stability. The most unstable varieties with the highest S_{di}^2 values were 'Plador' ranked last, 'Nassau' ranked second last and 'Voletta' ranked third from last.

Low values of Shukla's stability variance indicate high stability. The most stable varieties as indicated by this stability parameter were 'Maxidor' with a yield above the overall mean, while 'Domino' and 'Tytania' express high stability with yield below the overall mean. The hybrids with poor stability according to this procedure were: 'Super Nano Yellow', 'Plador', 'Nassau' and 'Voletta'," which achieved yields above the overall mean.

Genotypes with low ecovalence (W_i^2) have smaller fluctuations across environments and therefore are stable. The most stable varieties according to Wricke's ecovalence were 'Maxidor', 'Domino', Tytania', Marconi' and 'Contender', who were ranked 9th, 13th, 16th, 19th and 12th for mean yield, respectively. The most unstable varieties according the ecovalence were 'Super Nano Yellow', 'Plador', 'Nassau' and 'Voletta'.

Desirability index (D_i) is based on both the level of production an the regression coefficient, representing "the area under the linear regression function divided by the difference between the extreme values of the environmental indices" (Hernandez et al., 1993). According to this parameter, the varieties 'Plador', 'Doge', 'Nassau', 'Volleta', exhibit the highest levels of yield associated with good dynamic stability that allows them to effectively capitalize the favorable conditions for the cultivation of this crop. In 'Roquencourt', 'Marconi' and 'Processor' varieties, low pod yield values are associated with low type II stability.

Table 5. Stability statistics $R1i$, R_2i, SFi, Pi, D_i^2, for pod yield of snap bean varieties

No	Variety	R_1i	R_2i	SFi	Pi	D_i^2
1	Aurie de Turda	36.14	36.14	1.49	9866	578
2	Berggold	106.21	106.21	1.99	3123	4092
3	Ileana	90.68	63.69	1.46	3514	1360
4	Minidor	97.63	97.63	1.78	2754	2806
5	Maxidor	57.45	50.50	1.37	3257	738
6	Roquencourt	14.44	4.65	1.07	13143	89
7	Sondella	22.51	10.50	1.06	2071	288
8	Voletta	108.40	108.40	2.12	3037	7285
9	Doge	75.43	57.87	1.27	56	1633
10	Meraviglia di Venezia	90.86	90.86	1.99	4698	3307
11	Plador	84.10	42.32	1.18	154	2485
12	Super Nano Yellow	171.24	171.24	3.31	4023	11181
13	Tytania	65.58	53.59	1.64	9045	852
14	Contender	49.31	34.87	1.31	6301	405
15	Domino	65.27	63.82	1.65	6338	1176
16	Marconi	37.15	37.15	1.55	11019	505
17	Nassau	93.64	76.26	1.64	2364	5400
18	Processor	32.37	-8.52	0.92	10624	471
19	Velodrom	23.42	2.10	1.02	7470	230
20	Wotter	83.13	83.13	2.29	7654	3365

Based on rank parameters (R_1i, R_2i), it can be observed that the two values for most varieties are very close (Table 5), which indicates that in general the varieties achieved extreme

productions against the background of the most favorable and unfavorable conditions during the study period (Das et al., 2010). As such, in this sense the highest yield stability was observed in the varieties 'Roquencourt', 'Velodrom', 'Sondella', while at the 'Super Nano Yellow', 'Voletta' and, 'Berggold' varieties, the climatic conditions had a higher influence, causing large yield variations.

According to the values of the stability factor (*SFi*) 'Processor', 'Velodrom', 'Roquencourt', 'Sondella', varieties showed constant yield values against the background of different climatic conditions in 2022 (the most favorable) and 2024 (less favorable). In the case of 'Super Nano Yellow', 'Voletta', 'Wotter' varieties, the higher values of the stability factor confirm that their yield was considerably influenced by the climatic conditions during the study period. According to the performance measure (*Pi*) of Lin & Binns (1988), the genotypes with the lowest (*Pi*) values are considered the most stable. As such, the most stable variety ranked first for *Pi* and second for mean yield was 'Doge', followed by 'Plador' ranked second for *Pi* and first for mean yield. The most unstable varieties according to this statistic were 'Roquencourt', 'Marconi' and 'Nassau'.

Genotypic stability (D_i^2) is based on the biological concept of stability and evaluates genotypes according to the deviation of their yield from the average yield of a genotype considered stable (Hanson, 1970). As such, according to this parameter the highest stability was observed in 'Roquencourt', 'Velodrom' and 'Sondella' varieties while 'Super Nano Yellow', 'Voletta', 'Nassau' and 'Berggold' varieties, showed a high yield variation over the three years.

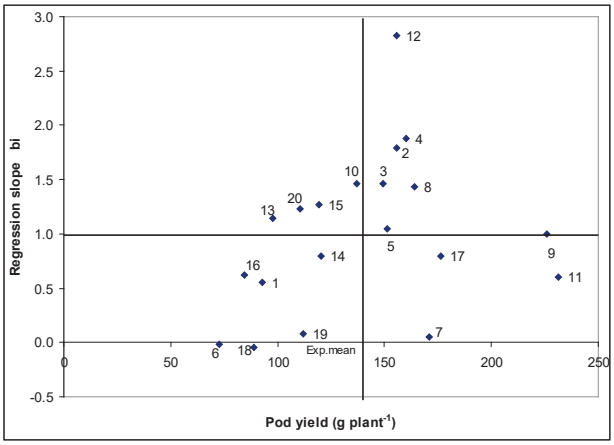


Figure 1. Mean values and regression coefficients for pod yield of snap bean varieties

Depending on the position of the varieties in Figure 1, it is observed that 'Sondella' variety presents a high static stability associated with a production above the average of the experience, while in 'Roquencourt', 'Processor', 'Vellodrom' the high stability is associated with values below the average. Varieties 'Maxidor' and 'Doge' show good dynamic stability, achieving values above the general average of experience and correlated with the favorability of environmental conditions. The varieties: 'Super Nano Yellow', 'Minidor', 'Berggold', 'Ileana' and 'Voletta', express high yield instability being specifically adapted to favourable environmental conditions.

According to Figure 2 'Plador', 'Doge', 'Sondella' and 'Maxidor' varieties, located in the lower right part of the graph, with relatively high pod yield and low coefficient of variation are considered stable. The varieties 'Berggold', 'Ileana', 'Minidor', 'Voletta', 'Super Nano Yellow' and 'Nassau', located in the upper right part of the graph, with higher mean yield than overall average and high variability, showed a specific stability to favourable conditions.

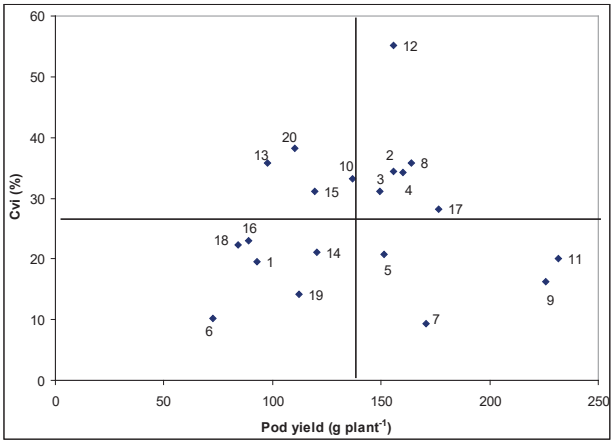


Figure 2. Mean values and variation coefficients for pod yield of snap bean varieties

Varieties with relatively low mean yield and low coefficient of variation like 'Roquencourt', 'Velodrom', 'Contender', 'Aurie de Turda', 'Marconi' and 'Processor', located in the lower left part of the graph, expressed a good stability but a lower yield potential. In the case of 'Tytania', 'Domino' and 'Wotter', varieties located in the upper left part of the graph, the low mean yield and high coefficient of variation indicate that they are highly influenced by the variety x year interaction.

CONCLUSIONS

Based on the variety's performances over the study period, it was seen that there is a considerable amount of variation available for the genetic improvement of snap bean cultivars and the enlargement of their genetic basis.

The obtained results support the importance of evaluating the yield performances of different snap bean varieties across multiple climatic conditions.

Understanding the varieties specific responses to climatic variations and their effect on yield are useful information for farmers to select the most suitable varieties for growing.

'Sondella' variety was found to have the best stability over the different climatic conditions associated with a yield above the experience mean and could be used in the snap bean breeding programs for the development of high yielding stable genotypes over environments for future use. Also, 'Plador', 'Doge' and 'Maxidor' varieties, with relatively high pod yield expressed a good stability and can be used to develop new diverse cultivars with stable yields. The above-mentioned varieties could be used for cultivation under unfavorable conditions.

REFERENCES

Annicchiarico, P. (2002). *Genotype x environment interactions - challenges and opportunities for plant breeding and cultivar recommendations*. FAO, Rome, Italy.

Becker, H.C., & Léon J. (1988). Stability analysis in plant breeding. *Plant Breed. 101, 1–23.*

Campa, A., Murube, E., Ferreira, J. (2018). Genetic diversity, population structure, and linkage disequilibrium in a Spanish common bean diversity panel revealed through genotyping-by-sequencing. *Genes. 9*, 518.

Ciulca, S. (2006). *Metodologii de experimentare în agricultura si biologie (Experimental methodologies in agriculture and biology).* Agroprint, Timisoara, Romania.

Das, S, Mishra, R.C., & Das S.R. (2010), G×E interaction, adaptability and yield stability of mid early rice genotypes. *Indian J. Agric. Res., 44,* 104-111.

Dinsa, T., Balcha, U., & Tadesse, F. (2022). AMMI Analysis for green pod yield stability of snap bean (*Phaseolus vulgaris* L.) genotypes evaluated in East Shewa Zone, Oromia, Ethiopia. *Journal of Scientific Agriculture, 6*, 46-50.

Eberhart, S.A., & Russell, W.A. (1966). Stability parameters for comparing varieties, *Crop. Sci*., *6*, 36-40.

Francis, T.R., & Kannenburg, L.W. (1978). Yield stability studies in short season maize. I. A descriptive method for grouping genotypes. *Canadian Journal of Plant Sciences, 58:* 1029-1034.

García-Fernández, C., Jurado, M., Campa, A., Brezeanu, C., Geffroy, V., Bitocchi, E., et al. (2022). A core set

of snap bean genotypes established by phenotyping a large panel collected in Europe. *Plants, 11*, 577.

García-Fernández, C., Jurado, M., Campa, A., Bitocchi, E., Papa, R., & Ferreira, J.J. (2024). Genetic control of pod morphological traits and pod edibility in a common bean RIL population. *Theor. Appl. Genet. 137*, 6.

Hagerty, C.H., Cuesta-Marcos, A., Cregan, P., Song, Q., Mcclean, P., & Myers, J.R. (2016). Mapping snap bean pod and color traits, in a dry bean × snap bean recombinant inbred population. *J. Am. Soc. Hortic. Sci., 141*, 131–138.

Hanson, W.D. (1970). Genotypic stability. *Theor. Appl. Genet. 40,* 226-231.

Hernández, C.M., Crossa, J., & Castillo, A. (1993). The area under the function: an index for selecting desirable genotypes. *Theoretical Applied Genetics. 87,* 409–415.

Hosamani, R., Patil, B.C., Ajjappapalavara, P.S., Naik, B.H., Smitha, R.P., Ukkund, K.C., & Mahammadali, A. (2010). Comparing stability of snap bean genotypes. *Indian Journal of Horticulture*. *67*, *Special Issue (Nov. 2010)*, 174–178.

Kalkuhl, M., von Braun, J., & Torero, M. (2016). Volatile and extreme food prices, food security, and policy: an overview. Food price volatility and its implications for food security and policy. In: Kalkuhl, M., von Braun, J., Torero, M. (eds), *Food Price Volatility and Its Implications for Food Security and Policy*. Springer Open, New York City.

Langer, I., Frey, K.J., & Bailey, T. (1979). Associations among productivity, production response and stability index in oat varieties. *Euphytica, 28,* 17-24.

Lewis, D. (1954). Gene-environment interaction. A relationship between dominance, heterosis, phenotypic stability and variability. *Heredity, 8*, 333-356.

Li, M., Wu, X., Wang, B., Wu, X., Wang, Y., Wang, J., et al. (2023). Genome-wide association analysis reveals the optimal genomic regions for pod size in bean. *Front. Plant Sci.,* 14.

Lin, C.S., & Binns, M.R. (1988). A superiority measure of cultivar performance for cultivar x location data. *Can. J. Plant Sci., 68*, 193-198.

Marinho, C.D., Gravina, G.A., Araújo, L.C., Almeida, S.N.C., Amaral Júnior, A.T., Daher, R.F., & Gonçalves L.S.A (2013). Snap bean recommendation based on different methods of phenotypic stability. *Genetics and Molecular Research. 12*(1), 248-255.

Müller, C., Elliott, J., Pugh, TAM., Ruane, A.C., Ciais, P., Balkovic, J., Deryng, D., Folberth, C., Cesar Izaurralde, R., Jones, C.D., Khabarov, N., Lawrence, P., Liu, W., Reddy, A.D., Schmid, E., & Wang, X. (2018). Global patterns of crop yield stability under additional nutrient and water inputs. *PLoS One, 13*(6), e0198748.

Pan, R.S., Singh, A.K., Rai, M., Kumar, S. (2007). Stability analysis of yield and its components in bush type French bean. *Veg. Sci., 33*(2), 145-148.

Reckling, M., Bergkvist, G., Watson, C.A., Stoddard, F.L., & Bachinger. J (2020). Re-designing organic grain legume cropping systems using systems agronomy. *Eur J Agron., 112*, 125951.

Reckling, M., Ahrends, H., Chen, T-W., Eugster, W., Hadasch, S., et al. (2021). Methods of yield stability analysis in long-term field experiments. A review. *Agronomy for Sustainable Development, 41(2)*.

Richmond, J., & Maness, N. (2024). Yield and quality of snap bean (*Phaseolus vulgaris* L.) cultivars from successive harvests in spring and fall production seasons in Oklahoma. *HortScience, 59*(12), 1815-1822.

Satish, D., Jagadeesha, R.C., Patil, R.K., & Masuthi. D. (2017). Genotype x environment interaction and stability analysis in Recombinant inbred lines of French bean for growth and yield components. *J Pharmacogn Phytochem. 6*(5), 216-219.

Singh, B.K., & Singh, B. (2015). Breeding perspectives of snap bean (*Phaseolus vulgaris* L.). *Vegetable. Sci.* 42, 1–17.

Shukla, G.K. (1972). Some aspects of partitioning genotype-environmental components of variability. *Heredity. 28,* 237-245.

Tigchelaar, M., Battisti, D.S., Naylor, R.L., Ray, D.K. (2018). Future warming increases probability of globally synchronized maize production shocks. *Proc Natl Acad Sci U S A. 115*(26), 6644–6649.

Traka-Mavrona, E., Georgakis, D., Spanomitsios, G., & Koutsika-Sotiriou, M. (2002). Pre-breeding manipulations for pod yield stability in a snap bean cultivar. *The Journal of Horticultural Science and Biotechnology*. *77*(6), 641–648.

Vilela, F.O., Amaral Junior A.T., Goncalves, L.S.A., Barbe, T.C., Gravina, G.A. (2011). Stability of F7:8 snap bean progenies in the Northern and Northwestern regions of Rio de Janeiro State. *Horticultura Brasileira. 29,* 84-90.

Weih, M., Karley, A.J., Newton, A.C., Kiær, L.P., Scherber, C., Rubiales, D., Adam, E., Ajal, J., Brandmeier, J., Pappagallo, S., et al. (2021). Grain yield stability of cereal-legume intercrops is greater than sole crops in more productive conditions. *Agriculture, 11,* 255.

Wricke G. (1962). Über eine methode zur erfassung der ökologischen streubreite in feldversuchen, *Z. Pflanzenzüchtung, 47,* 92-96.

Wu, J., Wang, L., Fu, J., Chen, J., Wei, S., Zhang, S., et al. (2020). Resequencing of 683 common bean genotypes identifies yield component trait associations across a north-south cline. *Nat. Genet., 52*, 118-125.

Wondaferew, D., Mullualem, D., Bitewlgn, W., Kassa Z., Abebaw Y., Ali H., Kebede K., Astatkie, T. (2024). Cultivating sustainable futures: multi-environment evaluation and seed yield stability of faba bean (*Vicia faba* L.) genotypes by using different stability parameters in Ethiopia. *BMC Plant Biol., 24*, 1108.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

CORRELATION BETWEEN CLIMATIC FACTORS AND DYNAMICS OF PATHOGENS AND PESTS: A CASE STUDY IN CABBAGE CROPS

Gabriel-Alin IOSOB, Tina Oana CRISTEA, Petre Marian BREZEANU, Claudia BĂLĂIȚĂ, Alexandru BUTE, Dan Ioan AVASILOAIEI

Vegetable Research and Development Station Bacău,
220 Calea Bârladului Street, Bacău, Romania

Corresponding author email: tinaoana@yahoo.com

Abstract

The study analyzes the impact of climatic conditions on the dynamics of diseases and pests in cabbage crop at the Vegetable Research and Development Station Bacău. High temperatures, with a maximum of 35.5°C in mid-summer, favored the development of pests such as Phyllotreta spp. and Plutella xylostella, while the August drought (rainfall of 0.3 mm) increased infestation. In contrast, high humidity in May-June supported the emergence of Brevicoryne brassicae, while heavy rainfall in September (170.4 mm) intensified the spread of fungal diseases, including Xanthomonas campestris. The analysis correlates variations in temperature, humidity and rainfall with the specific dynamics of the main pest species, highlighting the major challenges for phytosanitary control of cabbage crops in the context of climate change. The results provide a basis for improving integrated management strategies adapted to extreme and diversified weather conditions. The study underlines the importance of agro-meteorological monitoring in optimizing crop protection.

Key words*: agro-meteorological monitoring, cabbage pests, climate variability, integrated pest management, NE Region.*

INTRODUCTION

The term 'climate change' refers to long-term changes in temperature, precipitation patterns and other atmospheric conditions on Earth. These changes are driven primarily by human activities such as fossil fuel burning, deforestation and industrial processes, which increase the concentration of greenhouse gases in the atmosphere (Filonchyk et al., 2024). Climatic factors play a very important role in influencing the prevalence and behaviour of pathogens and pests that affect cabbage crops. The relationship between environmental conditions and the health of these crops is complex, and understanding this relationship is essential for the development of effective agricultural practices, especially in the context of climate change. Pathogens and pests are very sensitive to climatic variables such as temperature, humidity and precipitation (Skendžić et al., 2021). These factors can directly affect their life cycles, reproduction rates and survival. For example, temperature fluctuations can influence diapause of insects. Similarly, cold tolerance of insects is significantly affected by environmental temperatures, affecting their ability to infest crops (Muscalu et al., 2020; Subedi et al., 2023). For example, the development and spread of crop diseases are closely linked to climatic conditions (Zhou et al., 2024). A simulation model that integrates crop growth and pathogen development can quantify the direct and indirect effects of climate change on crop diseases. This model helps to understand how changes in temperature and humidity can influence plant disease dynamics (Dumitru et al., 2023). Host-pathogen dynamics are often independent of density, which means that the impact of climate change on temperature- and moisture-related factors is also crucial for predicting the behaviour of pests such as gypsy moth in different regions and climates over time (St. Leger, 2021). Changes in abiotic conditions, such as temperature and moisture, can disrupt the microclimate around plants, affecting soil microbial communities and canopy pathosystems. These changes can lead to significant shifts in disease manifestation, geographical distribution and economic implications, necessitating adaptations in

disease management strategies and cropping systems (Lahlali et al., 2024). The spread of invasive alien species is another critical issue influenced by climate change as it poses a substantial threat to global biodiversity and can cause significant economic losses in agriculture, forestry and aquatic ecosystems (Shrestha, 2019; Subedi et al., 2023). In Nepal, for example, the introduction of *Tuta absoluta* in 2016 had serious implications for local agriculture (Shrestha, 2019). Pathogen populations naturally adapt to climate change in both natural and agricultural systems. However, host populations, particularly in agricultural systems, may not adapt as quickly. This can lead to decreased genetic diversity and reduced resistance to pest and disease outbreaks. In agriculture, where genetically uniform crops are grown over large areas, new varieties need to be developed to improve their adaptation to changing climatic conditions (Pangga et al., 2011).

This study was conducted to examine the correlation between variations in temperature, humidity, and rainfall and the dynamic of key pathogens and pests population, highlighting key challenges in phytosanitary control of cabbage crops amid climate change. The findings serve as a foundation for enhancing integrated management strategies tailored to extreme and variable weather conditions. The study, conducted by researchers from the Vegetable Research and Development Station Bacău, emphasizes the critical role of agro-meteorological monitoring in optimizing crop protection.

MATERIALS AND METHODS

Experimental Site and Design

The study was conducted in 2024 from May to September, on a total of 1.85 ha conventional agricultural field at the Vegetable Research and Development Station of Bacău. The research aimed to analyze the correlation between climatic factors and the dynamics of pathogens and pests affecting cabbage crops.

Observations and Data Collection

Observations were made to determine the primary pathogens and pests affecting the cabbage crops. Data collection involved monitoring attack frequency (F %) and attack intensity (I %) every 10 days under natural infestation conditions. Using these parameters, the degree of attack (GA %) was calculated to identify key species and analyze their dynamics in relation to climatic factors.

Climatic Data Monitoring

To evaluate the influence of environmental conditions on pest and pathogen dynamics, climatic parameters were recorded using the iMetos 3.3 weather station installed at the research station. The monitored parameters included temperature, humidity, rainfall, and wind speed. These factors were analyzed in correlation with the specific dynamics of pathogens and pests.

Data Analysis

The collected data were statistically analyzed to determine the relationships between climatic variations and the occurrence and severity of pathogen and pest attacks in cabbage crops. The results provide insights into how weather conditions influence the prevalence and spread of key pest and pathogen species.

This study contributes to a better understanding of the correlation between climatic factors and pest and pathogen dynamics, supporting improved monitoring strategies for sustainable cabbage cultivation.

RESULTS AND DISCUSSIONS

Local climate conditions greatly influence cabbage production, primarily affecting plant growth and the occurrence and development of disease and harmful insects (Červenski et al., 2022).

Meteorological data for the year 2024 recorded at VRDS Bacău, with the iMmetos 3.3 weather station, shows how climate factors evolved over five months, from May to September.

1. Temperature (°C)

Average temperature

The average temperature follows a typical summer trend, rising steadily from May (15.5°C) to July (about 23.4°C), peaking in the second decade of July (27.0°C). After this, the temperature decreases towards September (16.9-17.1°C). The temperature differences reflect seasonal warming, which peaks in

midsummer before cooling in early autumn. August in particular is relatively warm (22.5-25°C).

Maximum temperature

In the summer months, maximum temperatures rise sharply, with notable peaks in July (35.5°C), while August also sees high highs (up to 33.5°C). September's maximum temperatures are lower (28°C), indicating the seasonal transition. The warmest period is the second decade of July, with a high of 35.5°C, typical of mid-summer heat waves.

Minimum temperature

Minimum temperatures rise gradually from May to July (7.9°C to 19.1°C), then fall towards September (to 10.3°C). Hot summer nights (July-August) are favorable for vegetative growth, but can also favor the emergence of certain pests and diseases.

2. Relative humidity (%)

Average humidity

The months of May and June had moderate moisture values (approximately 57-72%). July had relatively lower values (approximately 57-64%), which could be due to warmer and drier conditions. By September, moisture increased sharply, particularly in the second decade (83%), indicating higher moisture levels as the weather cools.

Maximum Humidity

Humidity often reached near saturation levels at night or in the early morning hours, as evidenced by consistent highs near or above 90%, particularly in June and September (up to 100%). This suggests periods of high atmospheric humidity, favorable for disease outbreaks.

Minimum humidity

Daytime minimum daytime humidity levels were quite low, especially in July and August (sometimes below 22%), characteristic of dry and scorching summer days. These dry periods can stress plants, especially drought-sensitive plants.

3. Precipitation (mm)

Precipitation showed significant variations over the months:

May: Moderate precipitation (from 9.6 mm to 21.6 mm), which supported vegetative crop growth in early summer.

June: Precipitation fluctuated (from 7.31 mm to 29.25 mm), with a decrease in the third decade, probably causing a short dry spell.

July: Significant rainfall, especially in the second dekad (40.65 mm), which provided the crop water requirements during the hot spell.

August: Dry period, especially in the second dekad, with only 0.3 mm of precipitation, a possible drought.

September: A sharp increase in rainfall in the second dekad (170.4 mm), suggesting heavy rainfall possibly related to seasonal changes, with potential for soil saturation or flooding.

4. Wind speed (m/s)

Wind speed remained generally low during the monitoring period (below 1.5 m/s), with occasional increases in May, July and September. Low wind speeds are typical for the summer months, although slightly higher values in May might suggest moderate winds, which could affect plant transpiration rates.

Analyzing the correlations between average temperature, relative humidity and precipitation in the period May-September 2024 at SCDL Bacău, we can see how these climatic factors influence plant development and the occurrence of pathogens and pests.

- The increase in temperatures and relatively moderate humidity in May, together with gradually increasing precipitation, are favourable for a good development of legume crops. These conditions stimulate vegetative growth (Chowdhury et al., 2021), but can also create a favourable environment for fungal diseases, such as mould or leaf spot (Juroszek et al., 2022), especially in the third decade of May, where temperatures and humidity have increased.
- In June, higher temperatures and more abundant rainfall in the first two decades favor vegetative growth and flowering. High humidity and rainfall in the first two decades can lead to disease outbreaks, especially in sensitive crops such as tomatoes (Panno et al., 2021). Reducing rainfall and humidity in the third decade decreases the risk of fungal diseases, but can increase water stress, influencing the development of non-irrigated plants.
- The month of July brought high temperatures and low relative humidity, which favors rapid

evaporation of soil water and can lead to soil drying without irrigation (Naorem et al., 2023). Rainfall in the second decade (40.65 mm) counterbalanced this effect. Relatively low moisture can reduce the incidence of fungal diseases (Zhou et al., 2024), but drought resistant pests such as mites and/or thrips can occur. High temperatures, coupled with low moisture levels, can accelerate the growth of pests that affect plant leaves and stems.

- August was a severe drought month in the second decade. High temperatures and low humidity create stressful conditions for plants, resulting in reduced yields (Naorem et al., 2023). This drought can be associated with an increase in pests that thrive in dry conditions, such as aphids and mites (Subedi et al., 2023). Rainfall in the third decade (42.8 mm) is welcome for crops, but may be insufficient to fully recover from the effects of drought. Also, these sudden rains may increase the risk of fruit cracking in tomatoes and favor fruit rot.
- September was a month of extreme rainfall in the second decade. Cooler temperatures and high moisture levels created favorable conditions for fungal diseases and root rot, mainly due to excess water in the soil (Lahlali et al., 2024). Heavy rains can also cause erosion or puddling, affecting crops (Naorem et al., 2023). Heavy rainfall, combined with high humidity, are ideal conditions for mold growth (Mieslerová et al., 2022).

High temperatures in July and August, coupled with low humidity, can create a favorable environment for drought-specific pests such as mites and lead to water stress. Variable rainfall and dry spells in August and June can affect plant development and favor certain diseases (Lahlali et al., 2024) or pests (Skendžić et al., 2021), depending on the type of crop. High humidity and heavy rainfall in September can lead to fungal diseases, especially during heavy rainfall.

Analyzing the data on climatic conditions and the presence of diseases and pests in white cabbage crop at SCDL Bacău, we can make the following clarifications:

1. *Aleyrodes proletella* (cabbage whitefly, Figure 1)



Figure 1. Cabbage whitefly (*Aleyrodes proletella*) on cabbage leaf - original photo

Very low occurrence, observed only in June, with a maximum infestation of 2.2% in the first decade, followed by a rapid decline.

Climatic correlation: Cabbage whitefly prefers warm and dry conditions (Holý & Kovaříková, 2024), but its occurrence in June coincides with a relatively humid period (69.31-72.88%) and moderate temperatures (21.2-22.9°C). The low infestation can be explained by the rainfall in this month (up to 29.25 l/mp). Higher humidity reduces whitefly activity, which prefers drier and warmer conditions.

2. *Brevicoryne brassicae* (cabbage aphid, Figure 2)



Figure 2. Cabbage aphid - colony on cabbage leaf - original photo

Maximum infestation (16.3%) occurs in late May and early June, but declines rapidly in July and August, with a slight reappearance in September.
Climatic correlation: Grey plant louse thrives in moderate temperatures, around 15-25°C, and relatively high humidity. May, with average temperatures of 13.9-18.3°C and humidity around 60%, provides ideal conditions for infestation. As temperatures rise in July and August (above 23°C) and humidity decreases, the louse population declines. In September, with falling temperatures and rising humidity (82.97%), it appears again, but at a reduced intensity (below 0.5%).

3. *Phyllotreta atra* and *P. nemorum* (Cabbage flea beetles, Figure 3)



Figure 3. Cabbage flea beetle (*Phyllotreta* sp.) infestation on cabbage leaves - original photo

Constant infestation (Stoleru et al., 2012), with higher values in June (up to 21.2%) and July (up to 32%).
Climatic correlation: Crucifer flea beetles prefer moderate to high temperatures and low humidity, which explains their intense activity in June and July, when mean temperatures ranged between 21.2°C and 27°C and relative humidity was decreasing (57.78-63.86%). Also, the moderate rainfall in these months (up to 40.65 l/mp) did not create sufficiently humid conditions to inhibit the activity of these pests. In August and September, with higher rainfall and high humidity, their population decreases significantly.

4. *Plutella xylostella* (Diamondback moth)

P. xylostella infestation is low, with sporadic occurrences in July and August (less than 0.2%).

Climatic correlation: The cabbage moth prefers warm and dry conditions (Georgescu et al., 2023) and is more active during periods of high temperatures and low precipitation. Minimum infestation in July and August may be associated with high temperatures (up to 27°C) and relatively low humidity. Increased rainfall in the second dekad of August (42.8 l/mp) may have contributed to limiting this infestation.

5. *Xanthomonas campestris* (Black rot, Figure 4)



Figure 4. Black rot on cabbage leaves caused by *Xanthomonas campestris* - original photo

Significant infestation in July (up to 22%) and August (up to 17.1%), decreasing thereafter in September.
Climatic correlation: Blackfly is favored by warm and humid conditions, especially during periods with frequent precipitation and high temperatures. In July and August, high average temperatures (up to 27°C) and moderate precipitation in July (up to 40.65 l/mp) create a favorable environment for the development of the bacterium. Also, lower relative humidity in July (below 65%) and uneven rainfall in August (0.3 l/mp in one decade and 42.8 l/mp in another) may favor the disease. In September, the heavy rainfall in the second decade (170.4 l/mp) may be too extreme for the bacterium to develop, leading to a decrease in infestation.

CONCLUSIONS

In conclusion moderate temperatures and relatively high humidity in May and early June favor the development of gray cabbage looper and whitefly, although whitefly was significantly less present in the crop. Crucifera

flea beetles (*Phyllotreta* spp.) are most active in June and July, periods with higher temperatures and lower humidity, suggesting that moderate drought is favorable for them. Their growth is limited by heavy rainfall in August and September. The cabbage moth has a low presence, with limited activity under drier and warmer conditions in July and August. This suggests vulnerability to sudden climatic changes such as sudden increases in rainfall. Black scurf is a significant problem in July and August, when moderate heat and rainfall create a favorable environment for the spread of this bacterial disease. Excessive rainfall in September appears to inhibit further bacterial growth.

ACKNOWLEDGEMENTS

This work was supported by a grant from the Romanian Ministry of Agriculture and Rural Development, ADER 6.3.18./2023-2026 and ADER 6.3.6, Building the Research Center Leg(O)Nest - cofounded by the European Union, Romanian Government, Regio Nord Est Programm 2021-2027, ADR NORD EST, call PR/NE/2024/P1/RSO1.1/1/2 - Infrastructuri CDI, contract nr. 651/26.03.2025 Smis code, 335392.

REFERENCES

Červenski, J., Vlajić, S., Ignjatov, M., Tamindžić, G., & Zec, S. (2022). Agroclimatic conditions for cabbage production. *Ratarstvo i Povrtarstvo*, *59*(2), 43–50. https://doi.org/10.5937/ratpov59-36772

Chowdhury, M., Kiraga, S., Islam, M. N., Ali, M., Reza, M. N., Lee, W.-H., & Chung, S.-O. (2021). Effects of Temperature, Relative Humidity, and Carbon Dioxide Concentration on Growth and Glucosinolate Content of Kale Grown in a Plant Factory. *Foods*, *10*(7), 1524. https://doi.org/10.3390/foods10071524

Dumitru, E. A., Berevoianu, R. L., Tudor, V. C., Teodorescu, F.-R., Stoica, D., Giucă, A., Ilie, D., & Sterie, C. M. (2023). Climate Change Impacts on Vegetable Crops: A Systematic Review. *Agriculture*, *13*(10), 1891. https://doi.org/10.3390/agriculture13101891

Filonchyk, M., Peterson, M. P., Zhang, L., Hurynovich, V., & He, Y. (2024). Greenhouse gases emissions and global climate change: Examining the influence of CO2, CH4, and N2O. *Science of The Total Environment*, 173359.

Georgescu, E., Toader, M., Brumă, I. S., Cană, L., Rîșnoveanu, L., Fătu, C., & Zaharia, R. (2023). Population Dynamics and Effect of Seed Treatment on Plutella xylostella Control in Romania. *Agronomy*, *13*(5), 1236. https://doi.org/10.3390/agronomy13051236

Holý, K., & Kovaříková, K. (2024). Spring Abundance, Migration Patterns and Damaging Period of Aleyrodes proletella in the Czech Republic. *Agronomy*, *14*(7), 1477. https://doi.org/10.3390/agronomy14071477

Juroszek, P., Laborde, M., Kleinhenz, B., Mellenthin, M., Racca, P., & Sierotzki, H. (2022). A review on the potential effects of temperature on fungicide effectiveness. *Plant Pathology*, *71*(4), 775–784. https://doi.org/10.1111/ppa.13531

Lahlali, R., Taoussi, M., Laasli, S.-E., Gachara, G., Ezzouggari, R., Belabess, Z., Aberkani, K., Assouguem, A., Meddich, A., El Jarroudi, M., & Barka, E. A. (2024). Effects of climate change on plant pathogens and host-pathogen interactions. *Crop and Environment*, *3*(3), 159–170. https://doi.org/10.1016/j.crope.2024.05.003

Mieslerová, B., Cook, R. T. A., Wheater, C. P., & Lebeda, A. (2022). Ecology of Powdery Mildews – Influence of Abiotic Factors on their Development and Epidemiology. *Critical Reviews in Plant Sciences*, *41*(6), 365–390. https://doi.org/10.1080/07352689.2022.2138044

Muscalu, A., Tudora, C., Coța, C., Gyorgy, Z., Burnichi, F., & Bârsan, M. (2020). Climate changes and methods to protect vegetable crops. *E3S Web of Conferences*, *180*, 03016. https://doi.org/10.1051/e3sconf/202018003016

Naorem, A., Jayaraman, S., Dang, Y. P., Dalal, R. C., Sinha, N. K., Rao, Ch. S., & Patra, A. K. (2023). Soil Constraints in an Arid Environment - Challenges, Prospects, and Implications. *Agronomy*, *13*(1), 220. https://doi.org/10.3390/agronomy13010220

Pangga, I. B., Hanan, J., & Chakraborty, S. (2011). Pathogen dynamics in a crop canopy and their evolution under changing climate. *Plant Pathology*, *60*(1), 70–81. https://doi.org/10.1111/j.1365-3059.2010.02408.x

Panno, S., Davino, S., Caruso, A. G., Bertacca, S., Crnogorac, A., Mandić, A., Noris, E., & Matić, S. (2021). A Review of the Most Common and Economically Important Diseases That Undermine the Cultivation of Tomato Crop in the Mediterranean Basin. *Agronomy*, *11*(11), 2188. https://doi.org/10.3390/agronomy11112188

Shrestha, S. (2019). Effects of Climate Change in Agricultural Insect Pest. *Acta Scientific Agriculture*, *3*(12), 74–80. https://doi.org/10.31080/ASAG.2019.03.0727

Skendžić, S., Zovko, M., Živković, I. P., Lešić, V., & Lemić, D. (2021). The Impact of Climate Change on Agricultural Insect Pests. *Insects*, *12*(5), 440. https://doi.org/10.3390/insects12050440

St. Leger, R. J. (2021). Insects and their pathogens in a changing climate. *Journal of Invertebrate Pathology*, *184*, 107644. https://doi.org/10.1016/j.jip.2021.107644

Stoleru, V. V., Munteanu, N. C., Stoleru, C. M. V., & Rotaru, L. G. (2012). Cultivar Selection and Pest Control Techniques on Organic White Cabbage

Yield. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, *40*(2), 190. https://doi.org/10.15835/nbha4027993

Subedi, B., Poudel, A., & Aryal, S. (2023). The impact of climate change on insect pest biology and ecology: Implications for pest management strategies, crop production, and food security. *Journal of Agriculture and Food Research*, *14*, 100733. https://doi.org/10.1016/j.jafr.2023.100733

Zhou, J., Zhang, X., Qu, Z., Zhang, C., Wang, F., Gao, T., Yao, Y., & Liang, J. (2024). Progress in research on prevention and control of crop fungal diseases in the context of climate change. *Agriculture*, *14*(7), 1108.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

RESPONSE OF TARRAGON (*ARTEMISIA DRACUNCULUS* L.) TO THE APPLICATION OF ZINC SULFATE AND GLYCINE BETAINE UNDER WATER STRESS CONDITIONS

Soheil KHANCHI[1, 2], Asad ROKHZADI[1, 2], Sirwan MOHAMMDIAZAR[3], Elena Maria DRĂGHICI[4]

[1]Department of Agronomy and Plant Breeding, Sa.C., Islamic Azad University, Sanandaj, Iran
[2]Research Center for Agriculture, Animal Husbandry, and Medicinal Plants, Sa.C., Islamic Azad University, Sanandaj, Iran
[3]Department of Chemistry, Sa.C., Islamic Azad University, Sanandaj, Iran
[4]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăşti Blvd, District 1, Bucharest, Romania

Corresponding author emails: asadrokh@iau.ac.ir, draghiciem@yahoo.com

Abstract

An experiment was conducted to investigate the effects of zinc sulfate and glycine betaine on tarragon (Artemisia dracunculus L.) under different irrigation regimes, using a central composite design (CCD) in greenhouse conditions. The experimental factors included irrigation at 30, 65, and 100% of field capacity (FC), zinc sulfate at 0, 3, and 6 g/L, and glycine betaine (GB) at 0, 75, and 150 mM. The imposition of drought stress led to a decrease in plant height and shoot dry weight (DW), while the highest rate of essential oil (EO) content was obtained under mild stress conditions. The highest plant height was obtained with moderate amounts of zinc sulfate, while the maximum DW was obtained with the application of 6 g/L of zinc sulfate. GB application had no significant effect on plant height and DW, but applying intermediate GB level maximized the EO content. In general increasing stress intensity reduced plant growth traits, but by imposing a moderate drought stress and applying 75 mM of GB, the EO content can be maximized.

Key words*: tarragon, drought stress, zinc sulfate, glycine betaine, essential oil.*

INTRODUCTION

Tarragon (*Artemisia dracunculus* L.) is a perennial herb species from Asteraceae family with numerous edible, spice, and medicinal uses (Kordali et al., 2005; Karimi et al., 2015; Koul and Taak, 2017). This plant is widespread in most temperate regions of Asia, Eastern and Central Europe, and Western North America (Obolskiy et al., 2011). It is a medicinal and culinary plant with antioxidant, anti-inflammatory, and hypoglycemic properties. Used traditionally in Asia for various ailments, it is now studied for its pharmacological benefits and is gaining importance in food, cosmetics, and biotechnology (Ekiert et al., 2021)

Hassanzadeh et al. (2016) stated that *Artemisia dracunculus* L., an aromatic plant from the *Asteraceae* family, is rich in essential oils containing methyl ethers, ocimene, and other compounds. Due to its pharmacological, antibacterial, and antifungal properties, it is extensively used in food preservation as a natural substitute for synthetic additives.

Mumivand et al. (2021) studied *Artemisia dracunculus* under drought, finding reduced water content and chlorophyll but increased antioxidant activity. HPLC analysis showed enhanced phenolic acids and flavonoids, with Hamadan, Varamin, and Estahbanat accessions proving drought-tolerant, aiding tarragon breeding and cultivation.

Zinc (Zn) is essential for plant and human growth, with dietary zinc deficiencies being a global issue. A study conducted by Majid et al. (2023) over two years evaluated the effects of foliar application of zinc sulfate (0.44 g Zn/L) at different phenological stages on wheat agronomic traits and grain zinc content. Foliar application during the branching and grain filling stages significantly improved grain yield and plant height, while early application at stem elongation had no effect on zinc

concentration. The most effective strategy for alleviating zinc deficiency was application during the branching and grain filling stages.

Salinity stress affects crop growth and yield, prompting research on mitigation strategies. This study examined the effects of NaCl salinity (0, 50, 100 mM) and foliar sprays (ZnO, nano-ZnO, Fe-chelate, magnetized-Fe, graphene-oxide) on tarragon. Graphene spray improved K^+/Na^+ balance, while Zn sprays enhanced essential oil content. Salinity increased antioxidant activity and phenolics, with GC/MS identifying estragole (81-91.8%) as the dominant oil component. Despite salinity's adverse effects, Fe and Zn foliar treatments helped mitigate stress (Hassanpouraghdam et al., 2021).

Kordali et al. (2005) studied the effects of NaCl salinity (0, 50, 100 mM) and foliar sprays (ZnO, nano-ZnO, Fe-chelate, magnetized-Fe, graphene-oxide) on tarragon. The results indicated that graphene improved the K^+/Na^+ ratio, while Zn sprays enhanced essential oil content. Salinity induced oxidative stress, but foliar applications of Fe and Zn partially mitigated these effects. GC/MS analysis revealed estragole as the dominant compound, with increased concentrations under ZnO treatment at 50 and 100 mM NaCl.

Zinc (Zn) is essential for plants as a micronutrient and is involved in crucial physiological processes like protein and carbohydrate metabolism, auxin hormone regulation, and enzyme formation (Hafeez et al., 2013). Due to its numerous physiological roles in plants, Zn can protect plants from damage under stress conditions (Umair Hassan et al., 2020).

Hassanpouraghdam et al. (2022) investigated the impact of NaCl salinity (0, 50, 100 mM) and foliar sprays (ZnO, Fe-chelate, graphene-oxide) on tarragon. The study found that graphene enhanced the K+/Na+ ratio, while salinity elevated oxidative stress markers. Zinc treatments increased catalase activity and essential oil content, with estragole being the predominant compound. Both Fe and Zn sprays helped reduce salinity-induced stress, improving the overall physiological response of tarragon.

Glycine betaine (GB) serves as a compatible osmolyte in plant cells, aiding in cell protection from environmental stressors like drought by facilitating osmotic regulation within the cell (Hernandez-Leon and Valenzuela-Soto, 2023). It has been shown that external application of GB to plants can improve the effects of drought stress in plants (Ashraf and Foolad, 2007; Obolskiy et al., 2011; Mohammad, 2023).

The study conducted by Oliveira et al. (2023) evaluated the response of tarragon to different concentrations of nutrient solution in hydroponic cultivation. The standard solution (100%) provided the best overall results. A 15% reduction maximizes fresh mass but significantly decreases dry mass, stem number, and height. For fresh consumption, a 25% reduction minimally affects production (1.3%).

Water deficit stress is one of the most important abiotic stresses damaging to plants. Today, due to global warming and climate change, this stress has shown more detrimental effects on plants than in the past (Liu et al., 2022).

This study aimed to model the response of tarragon plant to the application of zinc sulfate and glycine betaine under different levels of drought stress using the response surface methodology (RSM).

MATERIALS AND METHODS

The experiment was conducted in 2023 at the Yashil Inji Azarbayjan research extension greenhouse in Urmia, Iran (37° 44' N, 45° 10' E, 1338 m above sea level). The experimental layout was based on a central composite design (CCD) with three experimental factors The experimental factors included water deficit stress (30% FC, 65% FC, and 100% FC), zinc sulfate foliar application (0, 3, and 6 g/L), and glycine betaine (GB) foliar application (0, 75, and 150 mM).

The 4-leaf tarragon seedlings were transplanted into pots and subjected to drought stress treatments after reaching the 8-leaf stage. Foliar spraying with zinc sulfate and glycine betaine was carried out in three stages with 20-day intervals. At the flowering stage, plant height, shoot dry weight (DW), and essential oil (EO) content of tarragon plants were measured.

The collected data were subjected to analysis of variance and the effect of experimental factors on the measured responses of tarragon was

modeled using the response surface methodology (RSM). Statistical operations and graphing were performed using Design Expert 12 software.

RESULTS AND DISCUSSIONS

The fitted models for plant height, DW, and EO content were statistically significant (Tables 1-3). The effect of water stress on the measured parameters was significant. Zinc sulfate application had a significant quadratic effect on plant height and a significant linear effect on DW, but had no significant effect on EO content. Foliar application of GB had no significant effect on plant height and DW, but its quadratic effect on EO content was significant (Tables 1-3).

The response surface plot of plant height versus water stress and zinc sulfate indicates that plant height decreased linearly by reducing irrigation from 100 to 30% FC (Figure 1). The response of plant height to zinc sulfate was quadratic, so that under severe stress conditions (30% FC), the application of a moderate amount of zinc sulfate (3 g/L) resulted in the maximum plant height, and under normal irrigation conditions (100% FC), the plant height was maximized with the application of about 4 g/L of zinc sulfate (Figure 1). The plot of the interaction effect of water stress and zinc sulfate on DW shows that the highest amount of DW was obtained by applying the maximum rate of zinc sulfate at an irrigation level close to 100% FC (Figure 2).

Water deficit stress decreases plant growth by decreasing cell division and development, lowering stomatal conductance, and ultimately reducing the photosynthesis rate (Farooq et al., 2012). In accordance with our results, Thakur and Thakur (2018) declared that drought stress caused a significant decrease in plant height and biomass of various medicinal plants.

The positive impact of Zn on the height and DW of tarragon plants could be due to its influence on the meristematic regions of the plant, where it triggers the biosynthesis of the hormone indole-acetic acid (IAA) and promotes plant growth (Castillo-González et al., 2018).

The response plot of DW to water stress and GB indicates that although the application of GB did not have a statistically significant effect on DW, it did lead to an increasing trend in DW (Figure 3). It has been reported that GB can affect plant growth and development, especially under stress conditions, through interactions with nucleic acids and proteins (Hernandez-Leon and Valenzuela-Soto, 2023).

The EO content reacted as a parabolic graph versus GB and water stress (Figure 4). In other words, the highest amount of EO was obtained with the use of medium levels of GB (75 mM) and under medium stress conditions (about 80% FC).

In accordance with the present result, Feiz et al. (2019) found that using 100 mM of GB and moderate levels of water stress led to the highest amount of EC in marigold plants.

The response surface plot of water stress and zinc sulfate interaction shows that despite the non-significant effect of zinc sulfate on EO content, an increasing trend in EO content was observed with zinc sulfate application. (Figure 5). The improving effect of zinc sulfate on EO content could be attributed to the role of Zn in photosynthesis and carbohydrate metabolism, as carbohydrates are known to serve as an energy source for the production of terpenoids and EO constituents (Said-Al Ahl and Mahmoud, 2010).

Table 1. The ANOVA results and regression coefficients of the fitted models for plant height

Source	df	Plant height	
		Regression coefficient	*p*-value
Model	9		**0.0038**
Intercept		53.9	
Linear			
x_1 (Water stress)	1	4.625	**0.0040**
x_2 (Zinc sulfate)	1	1.525	0.2486
x_3 (GB)	1	-1.375	0.2952
Quadratic			
x_1^2	1	-3.625	0.1577
x_2^2	1	-5.925	**0.0317**
x_3^2	1	-0.375	0.8776
Interaction			
$x_1 x_2$	1	4.6875	**0.0071**
$x_1 x_3$	1	2.9375	0.0610
$x_2 x_3$	1	0.3125	0.8268
Lack of fit	5		0.5428
Pure error	5		
R^2		0.852	
CV (%)		8.04	

Table 2. The ANOVA results and regression coefficients of the fitted models for shoot dry weight (DW)

Source	df	Shoot DW	
		Regression coefficient	*p*-value
Model	9		**0.0040**
Intercept		1.15	
Linear			
x_1 (Water stress)	1	0.2450	**0.0003**
x_2 (Zinc sulfate)	1	0.1280	**0.0164**
x_3 (GB)	1	0.0370	0.4248
Quadratic			
x_1^2	1	-0.1991	**0.0408**
x_2^2	1	-0.0591	0.5017
x_3^2	1	0.0009	0.9917
Interaction			
$x_1 x_2$	1	0.0525	0.3158
$x_1 x_3$	1	0.0275	0.5923
$x_2 x_3$	1	0.0675	0.2044
Lack of fit	5		
Pure error	5		
R^2		0.851	
CV (%)		13.76	

Table 3. The ANOVA results and regression coefficients of the fitted models for essential oil (EO) content

Source	df	EO content	
		Regression coefficient	*p*-value
Model	9		**0.0098**
Intercept		1.51	
Linear			
x_1 (Water stress)	1	0.2743	**0.0042**
x_2 (Zinc sulfate)	1	0.0543	0.4822
x_3 (GB)	1	-0.0257	0.7367
Quadratic			
x_1^2	1	-0.3221	**0.0465**
x_2^2	1	0.0636	0.6632
x_3^2	1	-0.3649	**0.0277**
Interaction			
$x_1 x_2$	1	0.0679	0.4335
$x_1 x_3$	1	-0.0036	0.9666
$x_2 x_3$	1	-0.0036	0.9666
Lack of fit	5		
Pure error	5		
R^2		0.817	
CV (%)		19.69	

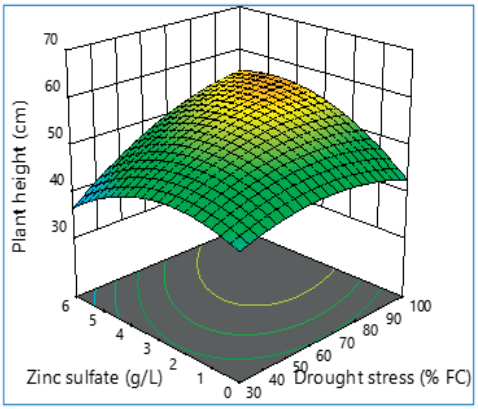


Figure 1. Response surface plot for the effect of drought stress and zinc sulfate on plant

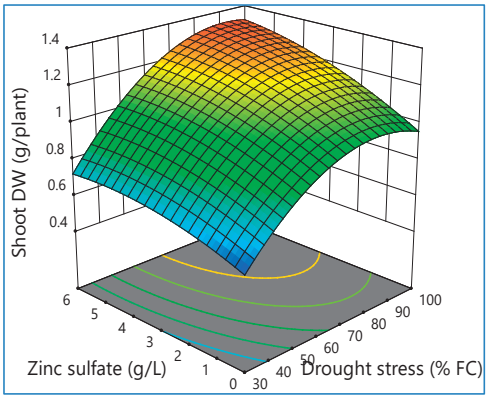


Figure 2. Response surface plot for the effect of drought stress and zinc sulfate on shoot dry weigh (DW)

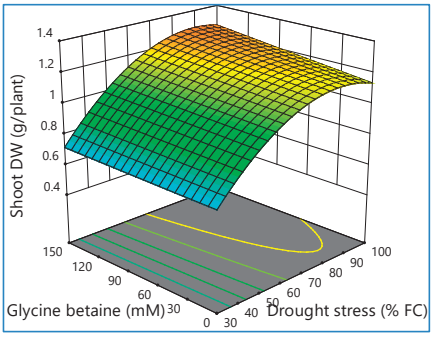


Figure 3. Response surface plot for the effect of drought stress and glycine betaine on shoot dry weigh (DW)

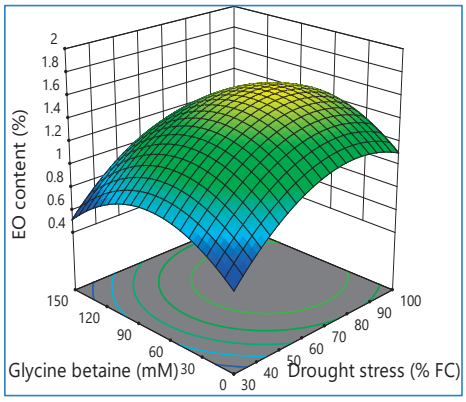


Figure 4. Response surface plot for the effect of drought stress and glycine betaine on EO content

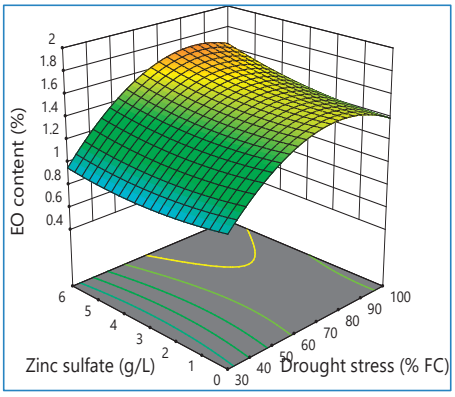


Figure 5. Response surface plot for the effect of drought stress and zinc sulfate on EO content

CONCLUSIONS

In this experiment, the effects of drought stress and the application of zinc sulfate and glycine betaine (GB) on tarragon growth and its essential oil (EO) content were modeled using the RSM technique. The fitted models for the studied traits were significant and had the necessary efficiency in predicting the responses. The effect of drought stress on the plant characteristics was significant. Increasing the intensity of drought stress caused a decrease in plant height and shoot dry weight (DW), while the EO content increased with the imposition of mild drought stress. The application of zinc sulfate increased the plant height and DW. The effect of GB on plant height and DW was insignificant, while its effect on the EO content was significant. In general, the results showed that despite the negative effects of severe drought stress on plant growth traits, the EO content of tarragon can be optimized by applying 75 mM GB and 6 g/L zinc sulfate, under a mild drought stress conditions.

REFERENCES

Ashraf, M. F. M. R., & Foolad, M. R., 2007. Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environmental and experimental botany*, *59*(2), 206-216.

Castillo-González, J., Ojeda-Barrios, D., Hernández-Rodríguez, A., González-Franco, A. C., Robles-Hernández, L., & López-Ochoa, G. R., 2018. Zinc metalloenzymes in plants. *Interciencia, 43*(4), 242-248.

de Oliveira R. C.; Ferraz-Almeida R.;. Luz J. M. Q, 2023. Performance on nutritive solution utilization by Tarragon (*Artemisia dracunculus* L.) cultivated in hydroponic, *Scientia Plena*, *vol 19*, no. 8, pp 1-7.

Ekiert Halina, Joanna Świątkowska, Ewa Knut, Paweł Klin, Agnieszka Rzepiela, Michał Tomczyk, Agnieszka Szopa, 2021. *Artemisia dracunculus* (Tarragon): A Review of Its Traditional Uses, Phytochemistry and Pharmacology, *Front Pharmaco,* Vol. 12, https://www.frontiersin.org/journals/pharmacology/articles/10.3389/

Farooq, M., Hussain, M., Wahid, A., & Siddique, K. H. M., 2012. Drought stress in plants: an overview. In: Aroca R (ed). *Plant responses to drought stress: From morphological to molecular features*, 1-33.

Feiz, F. S., Hakimi, L., Mousavi, A., & Ghanbari Jahromi, M., 2019. The effects of glycine betaine and L-arginine on biochemical properties of pot marigold (*Calendula officinalis* L.) under water stress. *Iranian Journal of Plant Physiology, 9*(3), 2795-2805.

Hafeez, B. M. K. Y., Khanif, Y. M., & Saleem, M., 2013. Role of zinc in plant nutrition-a review. *American Journal of Experimental Agriculture, 3*(2): 374-391.

Hassanpouraghdam M. B., 2021. Foliar application of graphene oxide, Fe, and Zn on *Artemisia dracunculus* L. under salinity, *Plant Physiology and Biochemistry, vol 80,* https://www.scielo.br/j/.

Hassanpouraghdam, M. B., Mehrabani, L. V., Kheirollahi, N., Soltanbeigi, A., & Khoshmaram, L., 2022. Foliar application of graphene oxide, Fe, and Zn on *Artemisia dracunculus* L. under salinity. *Scientia Agricola, vol. 80,* https://acikerisim.afsu.edu.tr/xmlui/handle/20.500.12933/1028

Hassanzadeh M. K., Zahra Tayarani Najaran, Maryam Nasery, Seyed Ahmad Emami, 2016. Chapter 92 - Tarragon (*Artemisia dracunculus* L.) Oils, *Essential Oils in Food Preservation, Flavor and Safety,* Pages 813-817,

Hernandez-Leon, S. G., & Valenzuela-Soto, E. M., 2023. Glycine betaine is a phytohormone-like plant growth

and development regulator under stress conditions. *Journal of Plant Growth Regulation*, *42*(8), 5029-5040.

Karimi, A., Hadian, J., Farzaneh, M., & Khadivi-Khub, A., 2015. Phenotypic diversity and volatile composition of Iranian *Artemisia dracunculus*. *Industrial Crops and Products*, *65*, 315-323.

Kordali, S.; Kotan, R.; Mavi, A.; Cakir, A.; Ala, A.; Yildrim, A., 2005. Determination of the chemical composition and antioxidant activity of the essential oil of *Artemisia dracunculus* and of the antifungal and antibacterial activities of Turkish *Artemisia absinthium, A. dracunculus, Artemisia santonicum,* and *Artemisia spicigera* essential oils. *Journal of Agricultural and Food Chemistry*, 53: 9452-9458.https://doi.org/10.1021/jf0516538.

Koul B., Taak P., 2017, The Artemisia genus: a review on traditional uses, phytochemical constituents, pharmacological properties and germplasm conservation*. Journal of Glycomics & Lipidomics, Vol 7*, issue 1, 1-7. 10.4172/2153-0637.1000142

Liu, X., Liu, W., Tang, Q., Liu, B., Wada, Y., & Yang, H., 2022. Global agricultural water scarcity assessment incorporating blue and green water availability under future climate change. *Earth's Future*, *10*(4), e2021EF002567.

Majid Abdoli, Ezatollah Esfandiari, Seyed Bahman Mousavi & Behzad Sadeghzadeh, 2014. Effects of foliar application of zinc sulfate at different phenological stages on yield formation and grain zinc content of bread wheat (cv. Kohdasht), *Azarian Journal of Agriculture,* vol 1, issue 1, pp 1-17, https://www.researchgate.net/publication/26990591

Mohammad, B.H.; Lamia, V.M. Data acquisition: Lamia, V.M.; Mohammad, B.H.; Nahideh, K.; Amir, S., 2023. Foliar application of graphene oxide, Fe, and Zn on *Artemisia dracunculus* L. under salinity, *Plant Physiology and Biochemistry, Sci. agric.* (Piracicaba, Braz.), vol. 80.

Mumivand H., Ebrahimi A., Shayganfar A., & Hamid Hassaneian Khoshro, 2021. Screening of tarragon accessions based on physiological and phytochemical responses under water deficit, *Scientific Report*, vol. 8, issue 11: pp. 17839.

Obolskiy, D., Pischel, I., Feistel, B., Glotov, N., & Heinrich, M., 2011. *Artemisia dracunculus* L. (tarragon): a critical review of its traditional use, chemical composition, pharmacology, and safety. *Journal of agricultural and food chemistry*, *59*(21), 11367-11384.

Said-Al Ahl, H. A. H., & Mahmoud, A. A., 2010. Effect of zinc and/or iron foliar application on growth and essential oil of sweet basil (*Ocimum basilicum* L.) under salt stress. *Ozean Journal of Applied Sciences*, *3*(1), 97-111.

Thakur, A., & Thakur, C. L., 2018. Evaluation of four medicinal herb species under conditions of water-deficit stress. *Indian Journal of Plant Physiology*, *23*(3), 459-466.

Umair Hassan, M., Aamer, M., Umer Chattha, M., Haiying, T., Shahzad, B., Barbanti, L. & Guoqin, H., 2020. The critical role of zinc in plants facing the drought stress. *Agriculture*, *10*(9), 396.

Yildrim, A., 2005. Determination of the chemical composition and antioxidant activity of the essential oil of *Artemisia dracunculus* and of the antifungal and antibacterial activities of Turkish *Artemisia absinthium, A. dracunculus, Artemisia santonicum,* and *Artemisia spicigera* essential oils, *Journal of Agricultural and Food Chemistry,* vol 53, 9452-9458. https://doi.org/10.1021/jf0516538

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

PRODUCTION OF POLYPHENOLIC COMPOUNDS WITH HIGH ANTIOXIDANT ACTIVITY IN *MELISSA OFFICINALIS* L. CALLUS LINE

Elena Monica MITOI, Alexandra Gabriela CIOCAN, Florentina ALDEA, Irina HOLOBIUC

Institute of Biology Bucharest of Romanian Academy,
296 Splaiul Independentei, District 6, Bucharest, Romania

Corresponding author email: alexandra.ciocan@ibiol.ro

Abstract

The importance of antioxidant components for disease prevention and health quality improvement has attracted much research attention over the last decades. Aromatic herbs have been recognized as important sources of antioxidants, Melissa officinalis L. being one of the species with many therapeutic actions. Plant extracts represent mixtures of numerous compounds and their biological actions are often attributed to polyphenol components, such as flavonoids and phenolic acids, which possess antioxidant activities. The aim of our research was to obtain and characterize a highly metabolite-producing callus line of lemon balm, with antioxidant activity. Our callus' performances were represented by a very high antioxidant capacity (18.74 mM Trolox equivalents/g DW), a high content of total polyphenols (2.57 mg gallic acid equivalents/g DW) and an especially rich content of flavonoids (19.21 mg rutin equivalents/g DW). This lemon balm callus line was compared to plant tissues grown in ex vitro conditions and proved to have clearly superior productive qualities. Also, our results showed values 4 to 18 times higher than those reported in other studies which investigated different types of lemon balm extracts.

Key words*: callus line, Melissa officinalis L., antioxidant activity, polyphenols.*

INTRODUCTION

Polyphenols are secondary metabolites specific to higher plants and, currently, over 8000 such compounds are known (Koleva et al., 2021). Half of them belong to the flavonoid group and are found as aglycones, glycosylated forms or methylated derivatives with strong antioxidant activity (Kumar & Pandey, 2013), most of them being of interest mainly for the food, medicine, cosmetics and pharmaceutical industries.

Due to the growing economic importance of polyphenols, it was vital to reconsider genetic resources, but also to direct research towards finding new methods of producing and extracting bioactive compounds from plants (Radomir et al., 2019).

In many medicinal plants which are part of the spontaneous flora, the risk of overexploitation by excessive and unsustainable harvesting causes the destruction of natural habitats and the decline of species (Coskun & Kapdan, 2024). Also, the changing climatic conditions significantly affect the biological potential of medicinal plants in terms of population density, but also have effects on harvesting periods. The spontaneous flora is subject to different types of stress which can lead to genetic changes that can compromise the biosynthetic value of the plant material. All this highlights the need for new approaches to improve the production of secondary metabolites.

The introduction of *in vitro* cultures as biological model has led to obtaining important scientific data on valorisation through unconventional methods. *In vitro* cultures have proven to be efficient and reproducible alternatives for the production of plant material in increased quantities, but also with improved qualities. The use of *in vitro* culture techniques for the production of aromatic plants has made it possible to regulate the production in accordance with the requirements, independent of season, phenomena of dormancy, with a high content of active principles, which can be supplied in fresh condition (Radomir et al., 2019).

Mellissa officinalis L., known as lemon balm, is one of the aromatic plants with many medicinal and economical values. It is a perennial herb belonging to the mint family, characteristic to

the Mediterranean region, distributed from Central and Southern Europe to Central Asia, Russia, Caucasus and Iran (Petrova et al., 2021). This Mediterranean species grows spontaneously in southern and western Romania (Hanganu et al., 2008). It is cultivated in Europe, Asia and the United States as a culinary plant with a particular taste, but also for medicinal purposes, due to its chemical composition rich in essential oils with health benefits (Doğan et al., 2021).

M. officinalis plants are rich sources of various secondary metabolites such as phenolic acids, flavonoids, terpenes, volatile oils or tannins (Petrova et al., 2021). It is used in traditional medicine for the multitude of proven pharmacological effects, such as: anti-inflammatory, antispasmodic, sedative, anxiolytic, cardioprotective, antipsychotic, antitumor, antiviral, antimicrobial or antioxidant (Shakeri et al., 2016; Ieri et al., 2017).

Cultivation of lemon balm by conventional methods is not difficult, however to obtain a homozygous population producing valuable compounds in constant quantity and quality is an endeavour (Meftahizade et al., 2010). Through *in vitro* culture it is possible to obtain a stable quantity, but also to stimulate the production of certain metabolites by using growth regulators or elicitors. However, the performances are dependent on the genotype, the endogenous hormones' concentrations and the initial explant response, i.e. the interaction between the exogenous hormone concentrations and the endogenous hormones receptors (Phillips & Garda, 2019).

Since lemon balm is one of the plant species intensively exploited for volatile oils with significant commercial value, in recent years a series of studies have been carried out specifically on the production of secondary metabolites in *in vitro* cultures (Barros et al., 2013; Rahman et al., 2015; Mokhtarzadeh et al., 2017; Mousavi & Shabani, 2019; Petrova et al., 2021; Kim et al., 2020; Kianersi et al., 2022; Coskun & Kapdan, 2024). In light of the above, this study aims to present a simple, reproducible and optimized protocol for obtaining callus in *M. officinalis* with a metabolic profile rich in polyphenols and flavonoids with increased antioxidant activity and monitoring, on long term, the production of secondary metabolites in this callus culture.

MATERIALS AND METHODS

Plant material

The plant material used for our experiment was represented by *M. officinalis* callus culture initiated from *in vitro* plantlets obtained by aseptically germination of seeds. Seeds were purchased from commercial site (www.semintelegumeflori.ro).

Seeds of lemon balm were sterilized in 70 % ethanol for 30 sec, washed with sterilized distilled water, transferred to 0.1% $HgCl_2$ for 15 min and finally washed three times with sterilized distilled water. Sterilized seeds were inoculated on MS basal medium (Murashige & Skoog, 1962) with 0.7% agar and incubated at 24°C, with 16-h photoperiod, in a growth chamber - Fitotron Gallenkamp SCG 120 (Weiss Technik, Loughborough, UK).

Production of callus cultures

One-month old plantlets were used as a source of explants. Leaf explants were excised and used for callus cultures establishment. Callus cultures were initiated on MS basal medium supplemented with B5 vitamins (Gamborg et al., 1968) and 0.3 mg/l 2,4-D, 1 mg/l NAA (1-naphthalen acetic acid), 0.5 mg/l Kinetin, 0.2 mg casein hydrolysate, 30 g/l sucrose and 7 g/l agar. Cultures were incubated in a growth room at 24±2°C under dark condition. Primary calli were initiated after incubation for one month and callus fragments were subcultured on fresh media every three weeks.

The optimized callus culture was obtained by successive passages for two years on adjusted formula of culture medium with increased levels of 2,4-D at 1mg/l and casein hydrolysate at 0.3 mg/l.

The callus stock from this culture was maintained for twelve years as *M. officinalis* callus long-term culture of in the *in vitro* culture collection of the Institute of Biology Bucharest, Romanian Academy.

The gravimetrical parameters of this callus culture were registered as:

- callus growth rate was calculated as W_f/W_i, where W_f and W_i are the fresh weights (FW, g) at the beginning and the end of the growth period (three weeks);
- dry weight (DW, g) was obtained by drying of 1g from each sample was at 80ºC, until

constant weight, in an Ecocell oven (MMM Group, Germany);

- callus moisture content was calculated as percentage using the formula: M.C (%) = (FW-GW)/FW × 100.

Extracts Preparation

For extract preparation, 1 gram of fresh weight callus was crushed using a mortar and pestle, then extracted with different types of solvents (water, ethanol, methanol) in different proportions (1:1; 1:3; 1:5 m/v). After vortexing vigorously for 1 minute, extracts were placed on an orbital shaker (Heidolph Instruments GmbH, Germany) for 2 days under continuous shaking, in the dark, at room temperature (RT). After this maceration, samples were centrifuged two times (15 min, 12000 rpm, 4°C) using an Eppendorf centrifuge (Germany). The supernatant was transferred into a clean tube and stored at 4 °C.

Evaluation of Total Phenolic Content

The classical Folin-Ciocalteu method was used for the total phenolic content estimation (Stankovic et al., 2012). A calibration curve with gallic acid between 10-100 µg/mL ($R^2 = 0.9968$) was made and the results were calculated as mg of gallic acid equivalents (GAE)/g of callus DW.

Evaluation of Flavonoid Content

A protocol with aluminium chloride adapted from Cai et al. (2010) was used for flavonoid concentrations' estimation. The results were calculated by extrapolation on a standard curve with rutin between 100-1000 µg/ml ($R^2 = 0.9996$) and expressed as mg of rutin equivalents (RE)/g of callus DW.

Evaluation of Antioxidant Activity Through DPPH Assays

The 2,2-Diphenyl-1-picrylhydrazyl (DPPH) method was applied according to Marxen et al. (2007). Results were calculated as mM of Trolox equivalents (TE)/g of callus DW, according to a standard curve between 50-150 µg Trolox/ml (R2 = 0.9873).

For all colorimetric determinations, a Spectronic Helios Gamma UV-Vis spectrophotometer (Thermo Fisher Scientific) was used and biochemical determinations were performed in triplicate.

Evaluation of flavonoids and phenolic acids production by HP-TLC

The methanolic extracts were separated using HP-TLC system (CAMAG, Muttenz, Switzerland) on glass TLC silica gel 60 F_{254} plates. Two mobile phases were used: one for phenolic acids (A) ethyl acetate: acetic acid: formic acid: water (100:10:10:18, v/v) and one for flavonoids (B) chloroform: ethyl acetate: acetone: formic acid (40:30:20:10, v/v), according to Jesionek et al. (2015). The plates were visualized under UV light (254 nm and 365 nm). The standards caffeic acid, gallic acid, chlorogenic acid, salicylic acids, cinnamic acid, maslinic acid, ursolic acid, catechin, proanthocyanin B1, resveratrol, rutin, quercetin, myricetin 3-O-glucoside and kaempferol 3-O-glucoside were purchased from Sigma and Extrasynthesis. The flavonoids' separation was highlighted with 1% NP reagent combined with 5% PEG 4000 solution. For the assessment of antioxidant activity, a 0.2% methanolic DPPH solution was sprayed onto the plate.

Statistical analysis

All the experiments consisted of 3 to 9 replicates for biochemical and gravimetrical parameters. The experimental data were subjected to normality and homogeneity of variance tests, after which a one-way analysis of variance (ANOVA) was performed, to assess the statistical significance. To determine the differences between means, we used the Tukey HSD test. All analyses were performed using MS Excel (Microsoft) and Origin, Version 2025 (OriginLab Corporation, Northampton, MA, USA). The results were reported as means ± standard deviation (SD) and *p*-values $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***) were considered statistically significant.

RESULTS AND DISCUSSIONS

In the last decades, plant cell and tissue culture techniques, specifically biotechnologies applied for the elicitation of biologically active compounds biosynthesis, have proven to be valuable tools for the study of secondary metabolite production in plants.

In our callus induction experiment from leaf fragments, primary calli were obtained and multiplied by successive subcultivations on the same type of medium. Subsequently, a callus line was selected (Figure 1), which showed high metabolic performances, especially flavonoids, in concentration of 19.21±2.75mg RE/g FW, a rich content of total polyphenols of 2.57±0.05

mg GAE/g FW and a high antioxidant activity of 18.47±1.96 mM TE/g FW.



Figure 1. Callus initiated from leaf explants of *M. officinalis*

Our callus tissues had a high content of water over 95% and by expressing the metabolite concentrations as g dry weight, our ethanolic extracts of lemon balm callus presented increased values. The flavonoid concentration was 626±89.84 mg RE/g DW, clearly superior to those presented in literature: 25.8±6.26 mg RE/g in extracts from plants from the Southeast region of Romania (Spiridon et al., 2011); 12.5± 2.11 mg RE/g DW in commercial plants from Iran (Moradi et al., 2016); 57.76 mg RE/g in *M. officinalis* leaves from Bulgaria (Koleva et al., 2021). Using other standards, other authors reported flavonoid concentrations of 72.28 mg quercetin equivalents (QE)/g DW for plants yield in farms from Egypt (Hassan et al., 2019); 12.65±0.66 mg QE/g DW for methanolic extracts of *M. officinalis* leaves from southern Bulgaria (Dogan et al., 2021) or 45.71 ± 0.40 mg catechin equivalents (CE)/g DW for plants harvested from the Macedonian area (Tusevski et al., 2014). Our callus ethanolic extracts displayed an antioxidant activity of 610.43±22.70 mM TE/g DW, which can be compared with the value of 406.03 ± 13.57 µmol TE/g DW obtained in methanolic plants extracts by Tusevski et al. (2014).

The increased concentration of flavonoids and enhanced antioxidant activity negatively impacted the growth of the callus culture, therefore optimization of culture conditions was necessary. The administered hormonal balance was modified by increasing the auxin concentration from 0.3 to 1 mg/l 2,4-D to promote cell proliferation, but also the casein hydrolysate concentration from 0.2 to 0.3 mg/l.

Subsequently, the methanol extracts, obtained from the optimized callus line, were compared with the leaf, stem or root extracts coming from potted plants grown in greenhouse conditions. The concentrations of polyphenols and flavonoids and the antioxidant activity of the callus extracts were significantly superior to potted plants' extracts (Figure 2).

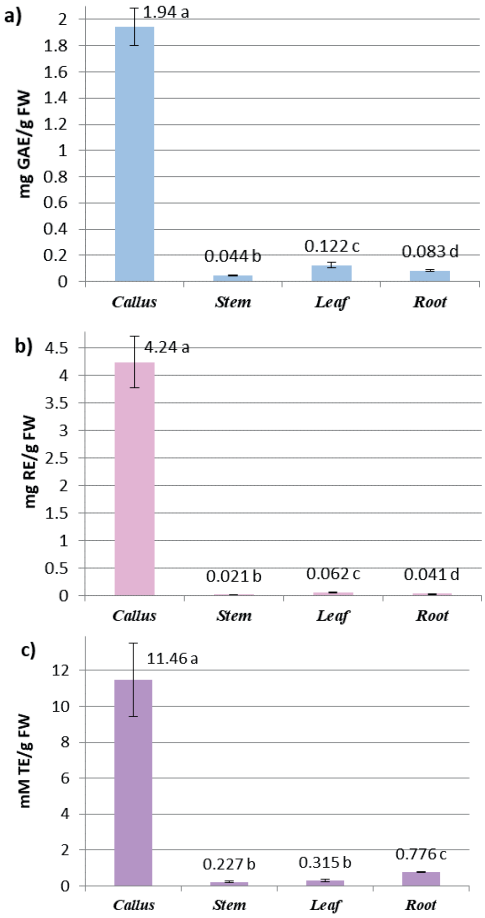


Figure 2. The total polyphenolic content (a), flavonoid concentration (b) and antioxidant activity (c) in methanolic extracts of *M. officinalis*. The data are presented as mean ± SD. Values with different letters are significantly different ($p < 0.001$).

Two years post-initiation, the optimized callus line (Figure 3) was subjected to a new extraction with different solvents (water, methanol and ethanol) to determine the levels of polyphenols and flavonoids and the antioxidant activity.



Figure 3. Optimized callus culture of *M. officinalis*

For the extraction of phenolic compounds, the best results were obtained in methanol, while for flavonoids, the ethanol extraction proved to be more advantageous (Figure 4).

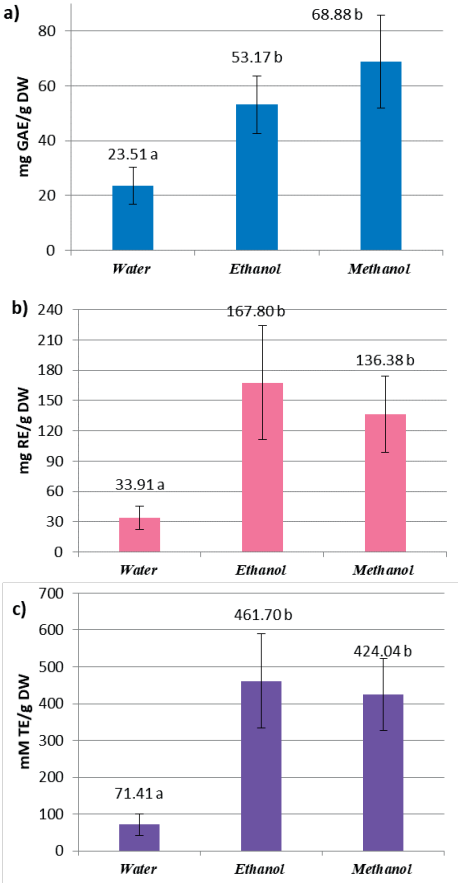


Figure 4. The total polyphenolic content (a), flavonoid concentration (b) and antioxidant activity (c) in different types of extracts from *M. officinalis* calli. The data were recorded at the end of the subcultivation period (3 weeks) and are presented as mean ± SD. Values with different letters are significantly different ($p < 0.01$)

The aqueous extract registered the weakest values. Moreover, the literature specifies that due to the high content of constituents with phenolic and flavonoid origin, extraction in ethanol, methanol and water would be recommended for *M. officinalis* extracts, as compared to acetone or hexane (Mabrouki et al., 2018; Stini et al., 2024). Hydroalcoholic solutions of methanol and ethanol proved to be much more efficient in the extraction of phenolic acids, in particular for rosmarinic acid, the main compound of *M. officinalis* extracts, compared to water (Angelov & Condoret, 2007; Wang et al., 2004; Duda et al., 2015).

The methanolic extracts presented a high concentration of polyphenols (68.88±16.98 mg GAE/ g DW), which is comparable to the values reported in other studies: 54.9±2.14 mg GAE/ g (Spiridon et al., 2011); 71. 02 mg GAE/ g DW (Hassan et al., 2019); 70.86 mg GAE/ g DW (Tusevski et al., 2014); 51±0.5 mg GAE/ g (Alizadeh Behbahani & Shahidi, 2019); 63 mg GAE/g dry ethanolic extract (Mambrouki et al., 2018) or with the results obtained by Koleva et al. (2021) in *M. officinalis* leaf extracts (65.17 mg GAE/g).

Depending on the extraction method used, other authors (Manolescu et al., 2022) reported, in a recent study, concentrations between 24.20-70.07 mg GAE/g, the highest values being obtained by using the microwave-assisted extraction technique, while by maceration, as in our study, a total polyphenol content of maximum 32.26±0.26 mg GAE/g was obtained. A polyphenol content ranging from 18.17±0.04 to 64.17±052 mg GAE/g was also reported by Petkova et al. (2017), using the infusion technique from lemon balm plants cultivated in Bulgaria.

Lower polyphenol concentrations were registered by other authors using water decoction and infusion: 43.51±1.50, respectively 27.17±0.51 mg GAE/g of DW (Popova et al., 2016) or between 32.86- 53.99 mg GAE/g DW by using ultrasound-assisted extraction in ethanol-water solvent (Manolescu et al., 2022).

However, higher concentrations were estimated in the literature for *M. officinalis* extracts, reaching to 184.33±0.5 mg GAE/g (Dogan et al., 2021) or over 200 mg GAE/g in the case of methanolic extracts reported by Moradi et al. (2016) or Papoti et al. (2019). Possible explanations for the large differences in the polyphenol

contents shown previously are: the techniques and extraction parameters;the harvesting period of the plant material; the drying protocol of the plants; the geographical area of cultivation, but also the characteristics of the plant material (Farhat et al., 2014; Sellami et al., 2012; Dent et al., 2017). Moreover, a recent study showed the difference in the production of polyphenols in two ecotypes of *M. officinalis* from Iran, Esfahan and Ilam, which had different biosynthetic capacities and the different responses to elicitation with methyl jasmonate (Kianeresi et al., 2022).
Although the metabolite concentrations of were lower in the optimized culture than in initial phases (Table 1), the advantage of producing plant biomass with a high concentration of metabolites supports a high production yield through *in vitro* callus culture of *M. officinalis* and stimulation with different elicitors would be inadvisable for this, already performing culture.

Table 1. Comparison of metabolites' accumulation in *M. officinalis* callus culture over time. The data were recorded at the end of subcultivation period (3 weeks) and are presented as mean ± SD. Values from the same column are significantly different ($p < 0.001$)

Callus type	Total phenol content (mg GAE/ g callus DW)	Flavonoid content (mg RE/ g callus DW)	Antioxidant activity (mM TE/g callus DW)
Initial	83.83±5.85[a]	626.06±89.84[a]	610.43±22.70[a]
Optimized	68.88±16.98[b]	167.80±56.76[b]	461.70±128.55[b]
Long term	11.14±1.28[c]	16.09±6.07[c]	19.57±7.19[c]

This culture, efficient in the synthesis of polyphenols and flavonoids, was maintained in the IBB *in vitro* cultures collection for a period of 12 years, but recent determinations in the metabolite's contents have demonstrated the loss of biosynthetic performances. Moreover, the culture, also, showed a different morphological aspect (Figure 5), being white with dehydration zones, clearly differing from the white-brownish appearance of the optimized culture.
Through multiple and repeated subcultivations, the callus lost their biosynthetic performances, maintaining the proliferative capacity, the growth rate varying between 17.5-24.78 after 3 weeks of cultivation.
HP-TLC analyses of *M. officinalis* callus extracts highlighted its capacity to synthesize compounds belonging to the phenolic acid class, as well as flavonoids (Figure 6).



Figure 5. Long-term callus culture of *M. officinalis*

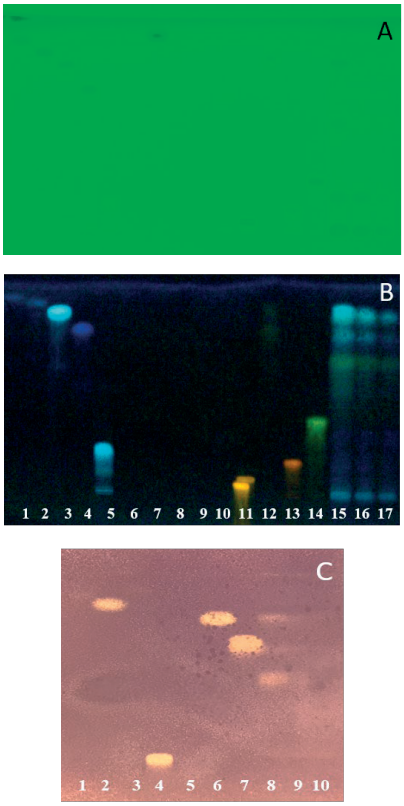


Figure 6. HP-TLC plates of *M. officinalis* callus methanolic extracts migrated in elution system B and visualized under UV light 254 nm **(a)**, derivatized with NP reagent under 366 nm fluorescent lamp (**b**) with lanes (1 – vanillin; 2 – resveratrol; 3- caffeic acid; 4 – gallic acid; 5 – chlorogenic acid; 6 – salicylic acid; 7 – trans cinnamic acid; 8- maslinic acid; 9 – ursolic acid; 10 – catechin; 11 – rutin; 12 – quercetin; 13 – myricetin-3-O-glucoside; 14 – kaempferol 3-O-glucoside; 15-20 – callus extracts) and stained with DPPH reagent for antioxidant activity testing **(c)** with lanes (1– vanillin; 2 – resveratrol; 3 – salicylic acid; 4 – chlorogenic acid; 5 – trans cinnamic acid; 6 – caffeic acid; 7 – gallic acid ; 8-10 – callus extracts)

Among the phenolic acids, the presence of caffeic acid was detected both at 254 nm and 366 nm and post exposure to the NP reagent it displayed a turquoise fluorescence. On TLC plates stained with DPPH solution, its antioxidant activity was also highlighted. Chlorogenic acid was identified on fluorescent TLC plates at 254 and 366 nm, but the staining with the NP and DPPH reagents was weak, this compound probably being present in small quantities. Gallic acid was highlighted at 254 nm and on plates stained with DPPH, revealing a weak antioxidant activity. Among flavonoids, kaempferol 3-O-glucoside was identified both at 254 nm and by staining with the NP reagent and, also, traces of rutin and myricetin 3-O-glucoside. For the flavonoids separated in TLC, no antioxidant activity was determined. The other flavonoid compounds with antioxidant activity remained unidentified due to the lack of standards.

As shown in literature, the main components of *M. officinalis* extracts are phenolic and cinnamic acids (Stini et al., 2024). The most commonly identified phenolic acids were protocatechuic acid, gallic acid and its dimer ellagic acid. Among the cinnamic acids, cinnamic, p-coumaric, caffeic, caftaric and ferulic acids were described. In fact, a significant category in lemon balm is represented by caffeic acid derivatives such as caffeoylquinic esters or caffeoyl-3,4-dihydroxyphenyllactic acid, which form chlorogenic and respectively, rosmarinic acids. Rosmarinic acid is considered the main major compound of lemon balm extracts (Ieri et al., 2017; Kianeresi et al., 2022). Among the flavonoids, quercetin, luteolin, apigenin, hesperin, hesperetin, naringenin, rutin, myricetin (Stini et al., 2024), as well as several glycosylated derivatives including kaempferol 3-O-glucoside (Kuo et al., 2020) were identified. In lemon balm from France, the major flavonoid found was luteolin 3-O-glucoside (Heitz et al., 2000).

Among the compounds identified by in our study, caffeic acid possesses anti-inflammatory, antioxidant and immunomodulatory effects (Anwar et al., 2012), while chlorogenic acid can be an efficient antioxidant, anti-inflammatory, hepatoprotective, cardioprotective, antidiabetic, and antihypertensive agent (Santana-Galvez et al., 2017; Naveed et al., 2018).

CONCLUSIONS

Our results showed a special capacity for the synthesis of secondary metabolites in the first phases of callus culture initiation. The biosynthesis performances were comparable or superior to the results reported in the literature and significantly increased compared to plants grown in pots.

After optimizing the culture growth conditions, the synthesis performances decreased, being in balance with the proliferation and production of callus biomass. The long-term culture lost its biosynthesizing performances, while it maintained the proliferation capacity.

The best solvent proved to be methanol, for the extraction of polyphenols and ethanol, for the extraction of flavonoids.

The present study demonstrates the efficiency of *in vitro* culture techniques, as alternative methods for the production of useful compounds in *M. officinalis*.

ACKNOWLEDGEMENTS

This research work was carried out with the support of the Institute of Biology Bucharest Romanian Academy, Project RO1567-IBB06/2025.

REFERENCES

Alizadeh Behbahani, B., & Shahidi, F. (2019). Melissa officinalis essential oil: Chemical compositions, antioxidant potential, total phenolic content and antimicrobial activity. *Nutrition and Food Sciences Research*, *6*(1), 17-25.

Angelov, P., & Condoret, J. S. (2007). Optimization of operational conditions of ethanol extraction of Rosmarinic acid from lemon balm (*Melissa officinalis* L.). *Chemistry book*, *5*, 71-7.

Anwar J, Spanevello RM, Thomé G, Stefanello N, Schmatz R, Gutierres J, Vieira J, Baldissarelli J, Carvalho FB, da Rosa MM, Rubin MA, Fiorenza A, Morsch VM, Schetinger MR. (2012). Effects of caffeic acid on behavioral parameters and on the activity of acetylcholinesterase in different tissues from adult rats. *Pharmacol Biochem Behav.,* 103(2):386-94.

Barros, L., Dueñas, M., Dias, M. I., Sousa, M. J., Santos-Buelga, C., & Ferreira, I. C. (2013). Phenolic profiles of cultivated, *in vitro* cultured and commercial samples of *Melissa officinalis* L. infusions. *Food chemistry*, *136*(1), 1-8.

Cai, W., Gu, X., & Tang, J. (2010). Extraction, purification, and characterisation of the flavonoids

from *Opuntia milpa alta* skin. *Czech J. Food Sci*, *28*, 108-116.

Coskun, Y., & Kapdan, G. (2025). Silver Nanoparticles (AgNPs) Act as Nanoelicitors in *Melissa officinalis* to Enhance the Production of Some Important Phenolic Compounds and Essential Oils. *Flavour and Fragrance Journal*, *40*(2), 278-288.

Dent, M., Dragović-Uzelac, V., Elez Garofulić, I., Bosiljkov, T., Ježek, D., & Brnčić, M. (2015). Comparison of conventional and ultrasound-assisted extraction techniques on mass fraction of phenolic compounds from sage (*Salvia officinalis* L.). *Chemical and biochemical engineering quarterly*, *29*(3), 475-484.

Doğan, H., Uskutoğlu, T., Baş, H., Stankov, S., Fidan, H., Şenkal, B. C., Stoyanova, A., Petkova, N., Yilmaz, G., & Dincheva, I. (2021). Phytochemical composition of wild lemon balm (*Melissa officinalis* L.) from the flora of Bulgaria. *Anatolian Journal of Botany*, *5*(2), 112-119.

Duda, S. C., Mărghitaş, L. A., Dezmirean, D., Duda, M., Mărgăoan, R., & Bobiş, O. (2015). Changes in major bioactive compounds with antioxidant activity of *Agastache foeniculum, Lavandula angustifolia, Melissa officinalis* and *Nepeta cataria*: Effect of harvest time and plant species. *Industrial Crops and Products*, *77*, 499-507.

Farhat, M. B., Chaouch-Hamada, R., Sotomayor, J. A., Landoulsi, A., & Jordán, M. J. (2014). Antioxidant potential of Salvia officinalis L. residues as affected by the harvesting time. *Industrial Crops and Products*, *54*, 78-85.

Hanganu, D., Vlase, L., Filip, L., Sand, C., Mirel, S., & Indrei, L. L. (2008). The study of some polyphenolic compounds from *Melissa officinalis* L. (Lamiaceae). *Rev Med Chir Soc Med Nat Iasi*, *112*(2), 525-529.

Hassan, R. A., Abotaleb, S. T., Hamed, H. B., & Eldeen, M. S. (2019). Antioxidant and antimicrobial activities of *Melissa officinalis* L. (lemon balm) extracts. *Journal of Agricultural Chemistry and Biotechnology*, *10*(9), 183-187.

Heitz A., Carnat A., Fraisse D., Carnat A.P. and Lamaison J.L. (2000). Luteolin 39-glucuronide, the major flavonoid from *Melissa officinalis* subsp. *officinalis*. *Fitoterapia*, 71, 201–202.

Ieri, F., Cecchi, L., Vignolini, P., Belcaro, M. F., & Romani, A. (2017). HPLC/DAD, GC/MS and GC/GC/TOF analysis of Lemon balm (*Melissa officinalis* L.) sample as standardized raw material for food and nutraceutical uses. *Advances in Horticultural Science*, *31*(2), 141-147.

Jesionek, W., Majer-Dziedzic, B., & Choma, I. M. (2015). Separation, identification, and investigation of antioxidant ability of plant extract components using TLC, LC–MS, and TLC–DPPH•. *Journal of Liquid Chromatography & Related Technologies*, *38*(11), 1147-1153.

Kianersi, F., Amin Azarm, D., Pour-Aboughadareh, A., & Poczai, P. (2022). Change in secondary metabolites and expression pattern of key rosmarinic acid related genes in iranian lemon balm (*Melissa officinalis* L.) ecotypes using methyl jasmonate treatments. *Molecules*, *27*(5), 1715.

Kim, S., Kim, J., Kim, N., Lee, D., Lee, H., Lee, D. Y., & Kim, K. H. (2020). Metabolomic elucidation of the effect of sucrose on the secondary metabolite profiles in Melissa officinalis by ultraperformance liquid chromatography–mass spectrometry. *ACS omega*, *5*(51), 33186-33195.

Koleva, P., Tsanova-Savova, S., Paneva, S., Velikov, S., & Savova, Z. (2021). Polyphenols content of selected medical plants and food supplements present at Bulgarian market. *Pharmacia (0428-0296)*, *68*(4).

Kumar, S., & Pandey, A. K. (2013). Chemistry and biological activities of flavonoids: an overview. *The scientific world journal*, *2013*(1), 162750.

Kuo, T. T., Chang, H. Y., Chen, T. Y., Liu, B. C., Chen, H. Y., Hsiung, Y. C., Hsia, S. M., Chang, C. J., & Huang, T. C. (2020). *Melissa officinalis* extract induces apoptosis and inhibits migration in human colorectal cancer cells. *Acs Omega*, *5*(49), 31792-31800.

Mabrouki, H., Duarte, C. M. M., & Akretche, D. E. (2018). Estimation of total phenolic contents and in vitro antioxidant and antimicrobial activities of various solvent extracts of *Melissa officinalis* L. *Arabian Journal for Science and Engineering*, *43*, 3349-3357.

Manolescu, D., Uță, G. A., Șuțan, A., Ducu, C., Din, A., Moga, S., Negrea, D., Biță, A., Bejenaru, L., Bejenaru, C., & Avram, S. (2022). Biogenic synthesis of noble metal nanoparticles using *Melissa officinalis* L. and *Salvia officinalis* L. extracts and evaluation of their biosafety potential. *Caryologia*, *75*(3), 65-83.

Marxen, K., Vanselow, K. H., Lippemeier, S., Hintze, R., Ruser, A., & Hansen, U. P. (2007). Determination of DPPH radical oxidation caused by methanolic extracts of some microalgal species by linear regression analysis of spectrophotometric measurements. *Sensors*, *7*(10), 2080-2095.

Meftahizade, H., Lotfi, M., & Moradkhani, H. (2010). Optimization of micropropagation and establishment of cell suspension culture in *Melissa officinalis* L. *African Journal of Biotechnology*, *9*(28), 4314-4321.

Mokhtarzadeh, S., Demirci, B., Goger, G., Khawar, K. M., & Kirimer, N. (2017). Characterization of volatile components in *Melissa officinalis* L. under *in vitro* conditions. *Journal of EssEntial oil rEsEarch*, *29*(4), 299-303.

Moradi, M. T., Karimi, A., Alidadi, S., & Hashemi, L. (2016). In vitro anti-adenovirus activity, antioxidant potential and total phenolic compounds of *Melissa officinalis* L. (lemon balm) extract. *International Journal of Pharmacognosy and Phytochemical Research*, *8*(9), 1471-1477.

Mousavi, S. M., & Shabani, L. (2019). Rosmarinic acid accumulation in *Melissa officinalis* shoot cultures is mediated by ABA. *Biol Plant*, *63*, 418-424.

Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia plantarum*, *15*(3).

Naveed, M., Hejazi, V., Abbas, M., Kamboh, A. A., Khan, G. J., Shumzaid, M., Ahmad, F., Babazadeh, D.,

FangFang, X., Modarresi-Ghazani, F., WehHua, L., & XiaoHui, Z. (2018). Chlorogenic acid (CGA): A pharmacological review and call for further research. *Biomedicine & pharmacotherapy*, *97*, 67-74.

Petrova, M., Nikolova, M., Dimitrova, M., & Dimitrova, L. (2021). Assessment of the effect of plant growth regulators on *in vitro* micropropagation and metabolic profiles of *Melissa officinalis* L. (lemon balm). *Journal of microbiology, biotechnology and food sciences*, *11*(3), e4077-e4077.

Phillips, G. C., & Garda, M. (2019). Plant tissue culture media and practices: an overview. *In Vitro Cellular & Developmental Biology-Plant*, *55*, 242-257.

Popova, A., Dalemska, Z., Mihaylova, D., Hristova, I., & Alexieva, I. (2016). *Melissa officinalis* L. - GC profile and antioxidant activity. *Int. J. Pharmacogn. Phytochem. Res*, *8*, 634-638.

Radomir, A. M., Guță, I. C., Buciumeanu, E. C., & Pîrvu, M. L. (2019). Optimization of microwave extraction method of total polyphenols from *Melissa officinalis* L. vitroplants. *Biologie*, *28*, 112-118.

Rahman, R. A. I. A., El-Wakil, H. E. D., Abdelsalam, N. R., Elsaadany, A. (2015). *In-vitro* production of rosmarinic acid from basil (*Ocimum basilicum* L.) and Lemon Balm (*Melissa officinalis* L.). *Sciences*, *5*(01), 47-51.

Santana-Gálvez, J., Cisneros-Zevallos, L., & Jacobo-Velázquez, D. A. (2017). Chlorogenic acid: Recent advances on its dual role as a food additive and a nutraceutical against metabolic syndrome. *Molecules*, *22*(3), 358.

Sellami, I. H., Rebey, I. B., Sriti, J., Rahali, F. Z., Limam, F., & Marzouk, B. (2012). Drying sage (*Salvia officinalis* L.) plants and its effects on content, chemical composition, and radical scavenging activity of the essential oil. *Food and Bioprocess Technology*, *5*(8), 2978-2989.

Shakeri, A., Sahebkar, A., & Javadi, B. (2016). *Melissa officinalis* L.–A review of its traditional uses, phytochemistry and pharmacology. *Journal of ethnopharmacology*, *188*, 204-228.

Spiridon, I., Colceru, S., Anghel, N., Teaca, C. A., Bodirlau, R., & Armatu, A. (2011). Antioxidant capacity and total phenolic contents of oregano (*Origanum vulgare*), lavender (*Lavandula angustifolia*) and lemon balm (*Melissa officinalis*) from Romania. *Natural product research*, *25*(17), 1657-1661.

Stankovic, M. S., Niciforovic, N., Mihailovic, V., Topuzovic, M., & Solujic, S. (2012). Antioxidant activity, total phenolic content and flavonoid concentrations of different plant parts of *Teucrium polium* L. subsp. *polium*. *Acta Societatis Botanicorum Poloniae*, *81*(2).

Stini, E., Tsimogiannis, D., & Oreopoulou, V. (2024). The Valorisation of *Melissa officinalis* Distillation By-Products for the Production of Polyphenol-Rich Formulations. *Molecules*, *29*(2), 377.

Tusevski, O., Kostovska, A., Iloska, A., Trajkovska, L., & Simic, S. G. (2014). Phenolic production and antioxidant properties of some Macedonian medicinal plants. *Central European Journal of Biology*, *9*, 888-900.

Wang, H., Provan, G. J., & Helliwell, K. (2004). Determination of rosmarinic acid and caffeic acid in aromatic herbs by HPLC. *Food Chemistry*, *87*(2), 307-311.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

SCREENING OF GENE EXPRESSION ASSOCIATED WITH SALINITY AND UVB TOLERANCE IN LOCAL TOMATO LANDRACES

Lia ȘERBAN (MLADIN)[1, 2], Oana SICORA[2], Tunde JAKO[2], Cosmin SICORA[2], Monica HÂRȚA[1], Mirela Irina CORDEA[1]

[1]Faculty of Horticulture and Business in Rural Development, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, 3-5 Calea Mănăștur Street, 400372, Cluj-Napoca, Romania
[2]Biological Research Centre Jibou, 16 Wesselenyi M. Street, 455200, Jibou, Romania

Corresponding author email: mcordea@usamvcluj.ro

Abstract

Rapid climate change in recent years may significantly impact future generations through various climate factors affecting crop production. As a result, a primary global concern is developing new crop plant varieties that are more resistant to abiotic stress factors such as high temperatures, salinity, drought, and UVB radiation. These new plant varieties may be selected from local landraces that thrive in the affected soils. Therefore, the present study focuses on several local tomato landraces from northwestern Romania, cultivated in soils impacted by salinity, drought, and high temperatures. This research evaluated the gene expression of selected genes related to abiotic stress, aiming to identify valuable molecular traits among the local landraces that confer resilience to fluctuating environmental conditions. This approach could allow for the breeding of new tomato varieties to meet the food industry and consumers' quantitative and qualitative demands.

Key words*: climatic changes, DREB, HSP70, gene expression, PSIID2, SOD.*

INTRODUCTION

A synthesis report from the Intergovernmental Panel on Climate Change (IPCC) states that human activities, primarily greenhouse gas emissions, undoubtedly cause the warming trend. Current environmental data shows that the global surface temperature has increased by 1.1˚C compared to the levels recorded between 1850 and 1900 from 2011 to 2020 (https://www.ipcc.ch/).

These adverse environmental conditions seriously affect the distribution and growth of crop plants (Guo & Wang, 2011). Therefore, considering the global population is estimated to double by the year 2050, it is imperative to identify viable solutions to ensure food safety and security (www.fao.org).

The discovery and development of new crop plant varieties that have improved resistance to abiotic stresses, such as heat, drought, salinity, and UVB radiation, are major global priorities (Machado & Seralheiro, 2017; Ors & Suarez, 2017; Martinez et al., 2018; Nowicka et al., 2018).

Plants face various abiotic stresses, which force them to develop a complex array of tolerance mechanisms for survival (Koyro, 2012; Zhang et al., 2022). To resist these environmental conditions, such as drought, extreme heat, salinity, and increased UVB radiations, plants generate several physiological and metabolic responses (Knight & Knight, 2001; Hirayama & Shinozaki, 2010; Mantri et al., 2012; Chen et al., 2022).

Plant protective mechanisms against UVB radiation include increasing the expression levels of genes in the oxidative defense system, synthesizing secondary metabolites, repairing DNA, and producing protective compounds that can absorb UVB radiation, such as flavonoids and phenolic compounds. They also accumulate osmoprotectants like proline and glycine betaine (Jordan, 2002; Ballaré, 2003; Ashraf and Foolad, 2007; Zlatev et al., 2012; Sharma et al., 2017; Fraikin, 2018; Chen et al., 2022).

Individual or combined abiotic stress factors differentially induce the expression of the HSP70 (heat shock protein 70) and *PSIID2* (photosystem II D2) genes (Polenta et al., 2020).

These characteristics enhance crop plants' potential to significantly broaden the range of viable growing environments (Ramonell & Somerville, 2002; Broccanello et al., 2023).

Plants can demonstrate phenotypic plasticity under various environmental conditions (Tester & Langridge, 2010). Quantitative analyses of plant traits are essential for quickly selecting crop varieties that thrive in resource-limited environments and challenging soil conditions (Fiorani & Schurr, 2013).

Romania has a significant amount of soil affected by salinization. In Bihor County, located in the northwestern part of the country, approximately 9.11% of the land is impacted by varying degrees of salinity: weak salinity affects 38122 hectares, moderate salinity affects 900 hectares, and strong salinity impacts 400 hectares (http://apmbh.anpm.ro/). Local tomato landraces have been identified growing in these saline-affected soils. These local landraces enable cultivation on soils already impacted by elevated salinity or drought conditions.

By incorporating traits that improve resilience to these stressors, farmers or breeders can continue to develop crops in areas that were previously unsuitable for cultivation.

From a literature review we selected four genes that we consider relevant in investigating resistance to both saline and UVB stress (He et al., 1993; Guo et al., 2011; Gadi, 2018; Ors et al., 2021). After confirming the presence of the target genes: *SOD* (superoxide dismutase), *DREB* (dehydration responsive element-binding protein), *HSP70* (heat shock protein 70), and *PSIID2* (photosystem II D2), a comparative analysis of their expression levels was conducted. This analysis compared the studied landraces with a control variety under varying salt concentrations and UVB stress conditions, aiming at identifying valuable molecular traits in studied landraces that confer resilience to varying environmental conditions.

MATERIAL AND METHODS

1. ***Experimental design and stress treatments***

Four different varieties of tomatoes were used in the experiments, with seeds sourced from local farmers in Bihor County. The landraces were named after their collection sites: 'Ateaș 136', 'Ateaș 37', and 'Cefa 7', while 'Marmande', a conventional cultivar, served as the control group for comparison with local landraces. The seeds were sown in pots (Ø 10 cm) filled with Terra peat soil. After germination, the plants were grown under a 16/8 hours light/dark cycle until they developed their first layer of true leaves.

Four groups of plants, each group with eight plants, were established for the treatments and control group, respectively. The treatments consist of two different salt concentrations and a combination of salt (NaCl) and UV-B radiation as follows: 300 mM NaCl – variant 1 (V1), 300 mM NaCl and UV-B radiation – variant 2 (V2), 450 mM NaCl – variant 3 (V3). The control groups (untreated plants, Ctrl) were regularly watered to maintain soil moisture at 60% of its total water-holding capacity and treated plants were watered in the same manner but with a saline solution. UVB radiation was applied for three consecutive days, two hours per group of plants, with three treatment cycles. The UVB radiation level used (0.45-0.55 mW/cm^2) represented an approximate 30% increase over the UVB radiation levels measured outdoors on a sunny day at noon when solar intensity peaks.

2. ***Determining the expression of target genes in tomato landraces subjected to saline and UVB stress***

To determine the expression of the target genes (the antioxidant gene *SOD*, stress-related genes *DREB* and *HSP70*, and the photosynthesis-associated gene *PSIID2*) in the studied tomato landraces, plant leaves were collected for total RNA isolation, cDNA synthesis, and quantitative Real-Time PCR using a modified protocol from Sicora et al. (2006) and McGinn (2003). Briefly, the leaf material (apx. 80 mg) were homogenized in Trizol (Invitrogen) using a SpeedMill homogenizer (Analitik Jena) and then the RNA extraction protocol was followed as described previously (Sicora et al., 2006, McGinn et al., 2003). For cDNA synthesis was used SensiFAST cDNA Synthesis kit (Meridian Bioscience).

The SsoAdvanced Universal SYBR Green Supermix kit (BioRad) was utilized for RT-PCR, along with the BioRad CFX 96 real-time amplification and detection system with specific fluorophores. All genes were tested with the appropriate primers, using tubulin (*TB*) as the reference gene. The primer sequences employed are similar to those used by Raja et al. (2020) (Table 1).

Table 1. Primer sequences used in genetic characterization experiments

Gene name	Primer sequence (5'– 3)	Product size
SOD		
F	ACT ATC TTC TTC ACC CAG GA	281
R	GAG TTT GGT CCA GTA AGA GG	
DREB		
F	TGG CGT TAG GGT TTT CCG AT	193
R	GCG GGT GCT TTT CGA GTT TT	
HSP 70		
F	GCA CCA TCC ACT TCA CCC AA	220
R	CCC TGA AGT CCA ATG ATC CCA	
PSII D2		
F	TCC TAG GGC GGT TTT GAT GG	205
R	AGT GGC AAA CCT GGA ATC CT	
TB		
F	GAT AAC TGT ACT GGA CTG GAA GG	250
R	GGA TGG CTT CGT TAT CCA AGA G	

Note: F – forward primer, R – reverse primer

After confirming the presence of target genes, the relative abundance of transcripts was mathematically calculated from the gene-specific amplification curves.

A comparative analysis quantified the expression levels of each target gene in the studied landraces and the control variety, which were exposed to different concentrations of salt stress and intensities of UVB radiation.

3. ***Data analysis***

In all experiments three technical replicates were used and an average of the Ct value was plotted.

The gene expression data were presented as (i) expression of the studied genes in different landraces and in the conventional cultivar in relative transcription units and the normalization was done to control sample hence the gene expression of the control samples has the value of 1 (Figures 1-4).

The expression of the studied genes was also presented (ii) relative to the control cultivar, hence the expression of Marmande cultivar always has the value of 1 (Figures 5-8).

For data analysis the Microsoft Excel for MAC 2011 data processing program was used.

RESULTS AND DISCUSSION

Salinity and UVB stress, both alone and in combination, are critical environmental factors in current climate change. When these conditions exceed a plant's protective capacity, they trigger intrinsic molecular protection mechanisms that help mitigate the negative physiological effects of the stressor (Mladin et al., 2018). At the cellular level, numerous genes respond by altering their expression in reaction to these stressful conditions (Agarwal et al., 2007; Rampino et al., 2012; Johnson et al., 2014; Harb et al., 2015; Zandalinas et al., 2017). The level of response and its amplitude is directly correlated with the perceived intensity of the stressor. These protective mechanisms can be broadly categorized into two groups based on their mode of action: some attenuate the impact of the stressor, while others induce metabolic changes within cells to counteract the harmful effects (Reddy et al., 2016). In the present study, in 'Marmande's control variety, the *SOD* gene's expression is minimal under control conditions. Post-treatment gene expression was measured, normalized against the control value. Thus, the highest expression of the *SOD* gene is observed after treatment with 300 mM salt, while the lowest is seen following treatment with 450 mM salt. In the case of the combined salt and UVB treatment, the gene expression level is higher than that observed with the high salt concentration but lower than that observed with 300 mM NaCl treatment (Figure 1a).

The expression of the *DREB* gene follows the same pattern as that of the *SOD* gene, with the lowest expression observed in the control and the highest after treatment with 300 mM NaCl. The combined treatment leads to an overexpression of the *DREB* gene, with a level slightly lower than that observed after treatment with 300 mM NaCl, but much higher than after the 450 mM NaCl treatment (Figure 1b).

The expression of the *HSP70* gene is lowest under control conditions, and with the administration of 300 mM NaCl, a significant increase in its expression is observed. In the case of the combined salt and UVB treatment as well as after 450 mM NaCl, *HSP70* expression is very low, yet still higher than in the control (Figure 1c).

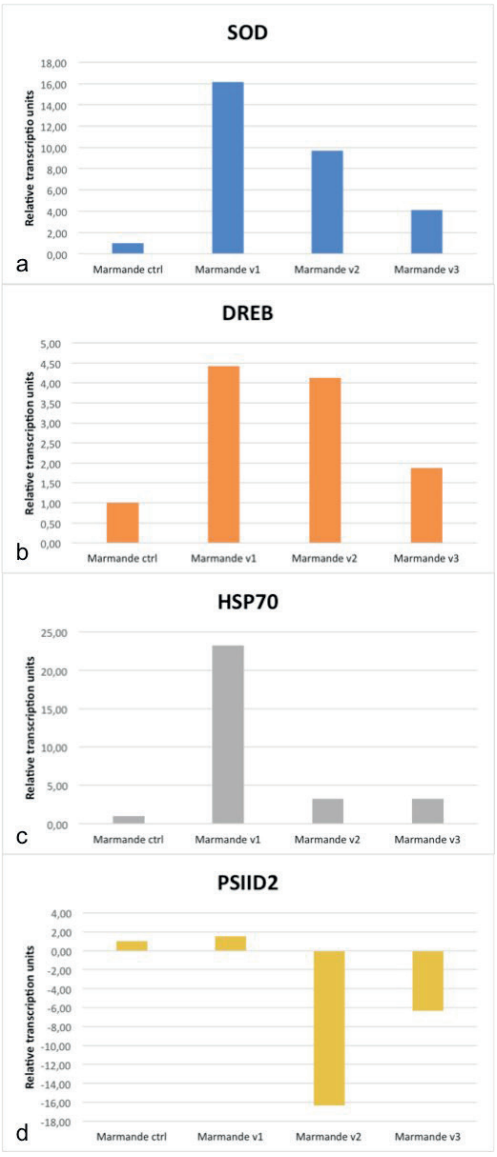


Figure 1. Gene expression of studied genes *SOD*, *DREB*, *HSP70* and *PSIID2*, in the control 'Marmande'; compared to the control plants and the three treatments variants (V1 – 300 mM NaCl, V2 – 300 mM NaCl and UVB, V3 – 450 mM NaCl)

In the case of the PSIID2 gene, following treatment with 300 mM NaCl, its expression is slightly higher than in the control. Under the combined treatment and the higher salt concentration treatment, expression drops dramatically, with the greatest decrease observed in the combined salt and UVB treatment (Figure 1d).

In local landrace 'Ateaș 37', in the control, the expression of the *SOD* gene is the lowest compared to its expression following the treatments. Thus, the highest expression of the *SOD* gene is observed after the 450 mM salt treatment, while the lowest expression is observed after the 300 mM NaCl and UVB treatment (Figure 2a).

The expression of the *DREB* gene follows the same pattern as that of the *SOD* gene, with the lowest expression observed in the control and the highest following treatment with 450 mM NaCl. The treatments with 300 mM NaCl and the combination of 300 mM NaCl and UVB result in similar levels of *DREB* gene (Figure 2b).

The expression of the *HSP70* gene is the lowest in the control, while a significant increase in its expression is observed after treatment with 450 mM NaCl. Under the combined NaCl and UVB treatment, as well as after 300 mM NaCl salt stress, *HSP70* expression is low, but still higher than in the control (Figure 2c).

In the case of the *PSIID2* gene, during the combined salt and UVB treatment, expression is repressed, with higher expression values observed in the 300 mM NaCl and 450 mM NaCl treatments compared to the control (Figure 2d).

In the local landrace 'Ateaș 136', the expression of the *SOD* gene is repressed under the 300 mM saline solution and UVB treatment, as well as the 450 mM saline treatment. However, gene expression following the 300 mM saline treatment is slightly higher than in the control plants (Figure 3a).

The expression of the *DREB* gene is also repressed under the 300 mM NaCl treatment and the 300 mM NaCl combined with UVB. The highest expression, significantly greater than in the control plants, is observed under the 450 mM NaCl treatment (Figure 3b).

For the *HSP70* gene, expression levels are relatively similar across all treatments, yet significantly higher than in the control plants (Figure 3c).

The expression of the *PSIID2* gene is visibly repressed under all treatments compared to the control plants, with consistently low expression levels (Figure 3d).

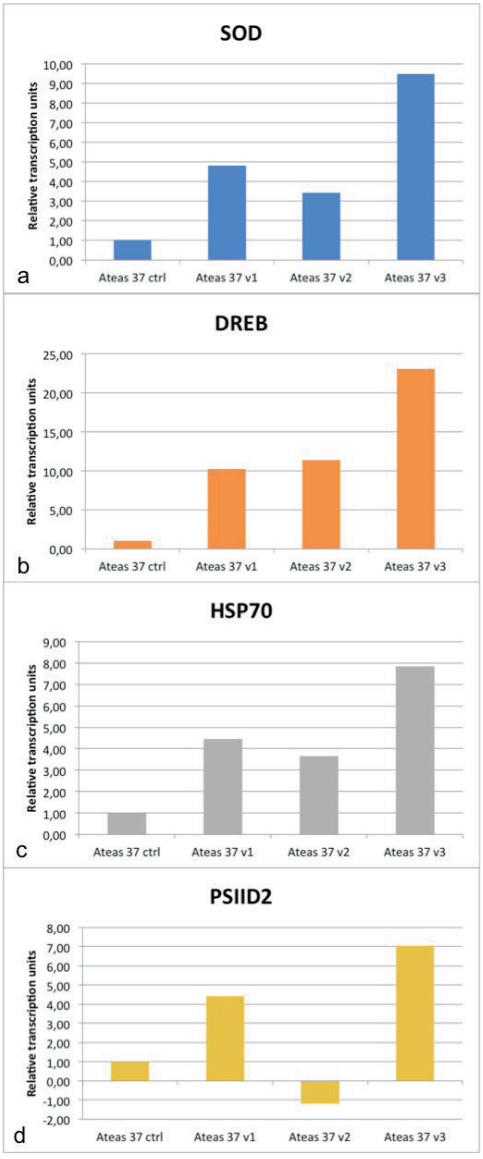


Figure 2. Expression of the studied genes – *SOD*, *DREB*, *HSP70*, and *PSIID2* in the local landrace 'Ateaș 37', compared with the control plants and the three treatment variants (V1 – 300 mM NaCl, V2 – 300 mM NaCl and UVB, V3 – 450 mM NaCl)

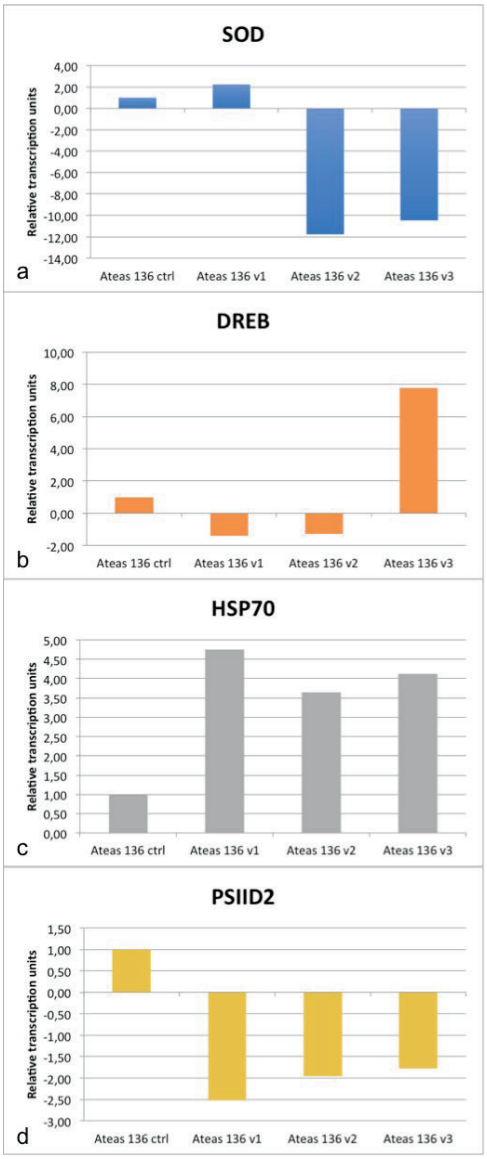


Figure 3. The expression of the studied genes - *SOD*, *DREB*, *HSP70* and *PSIID2* in the local landrace 'Ateaș 136', compared to the control plants and the three treatments variants (V1 – 300 mM NaCl, V2 – 300 mM NaCl and UVB, V3 – 450 mM NaCl)

In the local landrace 'Cefa 7', the highest expression of the *SOD* gene is observed under the 300 mM saline solution combined with UVB treatment.

Meanwhile, in the 300 mM and 450 mM saline treatments, expression is slightly higher than in the control plants (Figure 4a).

The expression of the *DREB* gene follows the same pattern as the *SOD* gene but with higher values. The highest expression is observed under the 300 mM saline solution combined with UVB treatment, while the values resulting from the other two treatments are very close to those of the control plants (Figure 4b).

The expression of the *HSP70* gene is highest under the 300 mM saline solution combined with UVB treatment, while the lowest values, which are very similar, are observed in the 300 mM saline treatment and the control plants (Figure 4c).

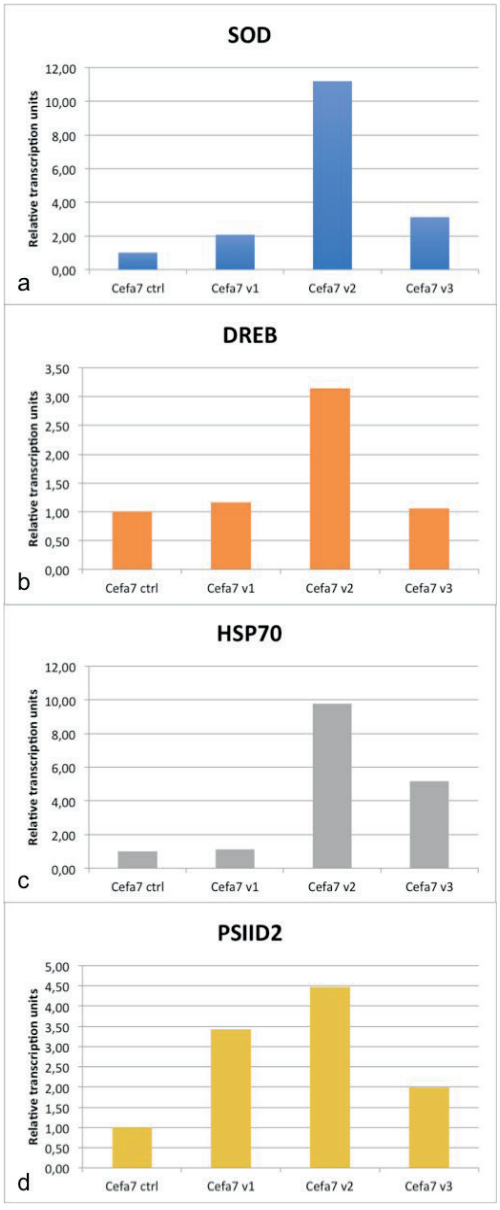


Figure 4. The expression of the studied genes, *SOD*, *DREB*, *HSP70* and *PSIID2* in the local landrace 'Cefa 7' compared to the control plants and the three treatments variants (V1 – 300 mM NaCl, V2 – 300 mM NaCl and UVB, V3 – 450 mM NaCl)

The highest expression of the *PSIID2* gene is observed in plants treated with 300 mM saline solution combined with UVB, followed by those treated with 300 mM saline solution. Plants treated with 450 mM saline solution have gene expression levels similar to those of the control plants (Figure 4d).

The expression of the *SOD* gene (Figure 5) is generally repressed across all treatment variants. The highest expression level is observed in the control plants of the local landrace 'Ateaș 136'. The same landrace, when treated with 300 mM saline solution**,** shows positive expression values similar to those of the local landrace 'Ateaș 37' treated with 450 mM saline solution.

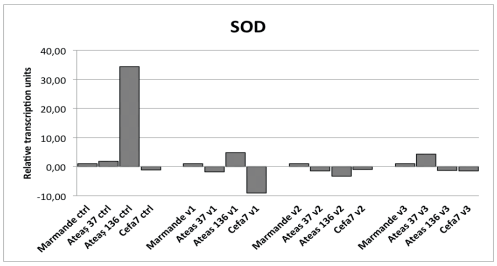


Figure 5. *SOD* gene expression in all studied local landraces including the control cultivar; comparison between all treatment variants (control, V1 – 300 mM NaCl, V2 – 300 mM NaCl and UVB, V3 – 450 mM NaCl)

For the *DREB* gene (Figure 6), expression is repressed in the control plants of the 'Ateaș 37' and 'Ateaș 136' landraces, in all local landraces treated with 300 mM saline solution, as well as in the 300 mM saline + UVB and 450 mM saline treatments for the same landraces.

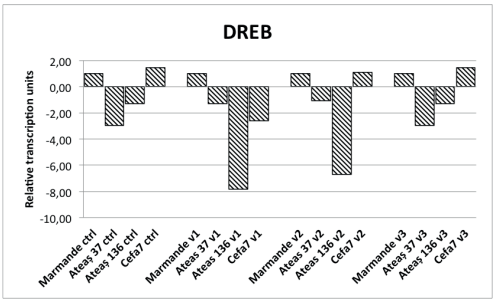


Figure 6. *DREB* gene expression in all studied local landraces including the control cultivar; comparison between all treatment variants (control, V1 – 300 mM NaCl, V2 – 300 mM NaCl și UVB, V3 – 450 mM NaCl)

Low but positive values are observed in the control plants of the 'Marmande' reference variety and the local landrace 'Cefa 7'. Following treatment with 300 mM saline solution, positive gene expression values are recorded in the 'Marmande' variety. The 300 mM saline + UVB treatment induced *DREB* gene expression in both the 'Marmande' variety and the 'Cefa 7' landrace**,** with values similar to those obtained in plants treated with 450 mM saline solution**.**

The *HSP70* gene exhibits low positive values (ranging from 0 to 8 relative transcription units) in nearly all the plants studied. The exception is observed in the repression of the gene in all the plants of the local landraces treated with 300 mM saline solution (Figure 7).

The expression of the *PSIID2* gene shows significant positive values in the local landraces 'Ateaș 136' and 'Cefa 7' treated with 300 mM saline solution and UVB. Minimum positive values are observed in the control plants of the 'Marmande' variety and the local landrace 'Ateaș 136' (Figure 8).

Similar values were obtained in the 'Marmande' variety plants treated with 300 mM saline solution, in the **'**Marmande' variety and 'Ateaș 37' landrace plants treated with 300 mM saline solution and UVB**,** and in the 'Marmande' variety and 'Ateaș 136' landrace plants treated with 450 mM saline solution. The gene is repressed in the control plants of the 'Ateaș 37' and 'Cefa 7' landraces, as well as in the 300 mM saline-treated plants of the 'Ateaș 37', 'Ateaș 136', and 'Cefa 7' landraces**,** and in the 450 mM saline-treated plants of the 'Ateaș 37' and 'Cefa 7' landraces.

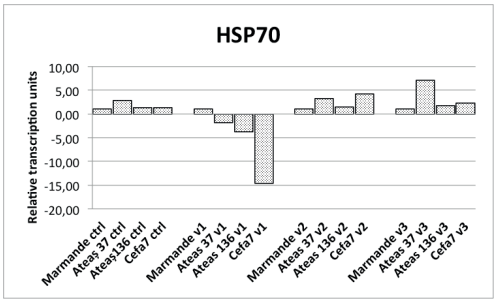


Figure 7. *HSP70* gene expression in all studied local landraces including the control cultivar; comparison between all treatment variants (control, V1 – 300 mM NaCl, V2 – 300 mM NaCl and UVB, V3 – 450 mM NaCl)

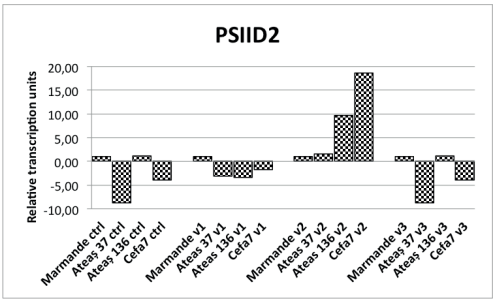


Figure 8. *PSIID2* gene expression in all studied local landraces including the control cultivar; comparison between all treatment variants (control, V1 – 300 mM NaCl, V2 – 300 mM NaCl și UVB, V3 – 450 mM NaCl)

SOD gene (superoxide dismutase) encodes a protein that protects the cell from the effect of abiotic stress (water stress) by protecting the cell structures from the reactive oxygen species (Teixeira et al., 2006; Lata & Prasad, 2011; Zandalinas et al., 2017).

ABA is a phytohormone that regulates a series of metabolic processes and is a signal transductor for stress signaling in plants. *DREB* (dehydration responsive element binding) is a transcription factor that regulates stress signal transduction in an ABA - independent manner and is involved in modulating gene response in plants that are exposed to abiotic stress (Nakashima et al., 2000; Agarwal et al., 2007).

It was shown previously that in plants treated with different abiotic stresses or a combination of stress factors the expression of heat shock proteins (HSPs) along with other enzymes involved in reactive oxygen species detoxification, glycolysis and photosynthesis are induced (Rampino et al., 2012; Johnson et al., 2014; Raja et al., 2020). Elevated levels of *HSP70* gene expression during our study for two of studied tomato landraces is in line with other studies that also reported increased transcript expression of *HSP70* (Rizhsky et al., 2004). In our study, the gene responsive to abiotic stress are induced when the cell enters in stress - mode. At the local landraces, the amount of transcript of stress induced genes is not so high as in the case of control variety 'Marmande'. This can be explained by the higher intrinsic resilience to stress showed by the local varieties. However, when the stress level exceeds the response threshold, the local

varieties respond better than ‘Marmande’ by inducing the stress response specific genes.

Plants exposed to different abiotic stresses showed suppression of many photosynthetic genes. Some studies showed induced expression of *PSIID2* gene under single or combined stress (Raja et al., 2020) while other showed down-regulated expression of genes involved in photosynthetic process (Rampino et al., 2012; Johnson et al., 2014). It was suggested that this variation in the expression profiles of photosynthetic genes is due to the fact that photosynthesis is more affected by stress conditions than other cellular processes. In our study, the expression level of *PSIID2* transcript is downregulated in the case of combined stress and high salt level in ‘Marmande’ variety and ‘Ateaş 37’ only for combined stress, and in ‘Ateaş 136’ landrace the expression is downregulated for all treatments. In ‘Cefa 7’ in all treatment variants and ‘Ateaş 37’ in the case of combined stress, the expression is up regulated. These findings suggest a more sensitivity to applied stresses for ‘Ateaş 136’, ‘Marmande’ and ‘Ateaş 37’, whilst ‘Cefa 7’ is more resilient to stress treatments.

Overall, the local landraces that were cultivated for many years on salt-enriched soil display a higher response threshold to stress as an acclimation effect. When the level of stress triggers mechanisms of response, they also present an increased capacity to genetically induce the genes involved in cellular stress protection.

CONCLUSIONS

Following the comparative analysis of the evolution of the expression of target genes, an increase in expression values is observed for the genes involved in the plant's response to stress.

The ‘Marmande’ variety generally shows a higher level of induction of stress-response genes compared to samples from the local landraces. This effect is likely related to the greater tolerance of these local landraces to saline stress**,** UVB stress, and the combined application of both stress types. Thus, plants from the local landraces perceive the applied stress as less intense and activate the molecular protection mechanisms at a lower level compared to the ‘Marmande’ variety plants.

REFERENCES

Agarwal, P., Agarwal, P. K., Nair, S., Sopory, S. K., & Reddy, M. K. (2007). Stress-inducible DREB2A transcription factor from *Pennisetum glaucum* is a phosphoprotein and its phosphorylation negatively regulates its DNA-binding activity. *Molecular Genetics and Genomics*, *277*, 189-198.

Ashraf M. And Foolad M.R. (2007). Roles of Glycine betaine and Proline in improving plant abiotic stress resistance, *Environmental and Experimental Botany*, Elsevier.

Ballaré, C. L. (2003). Stress under the sun: spotlight on ultraviolet-B responses. *Plant Physiology*, *132*(4), 1725-1727.

Broccanello, C., Bellin, D., DalCorso, G., Furini, A., & Taranto, F. (2023). Genetic approaches to exploit landraces for improvement of *Triticum turgidum* ssp. *durum* in the age of climate change. *Frontiers in Plant Science*, 14, 1101271.

Chen, Z., Dong, Y., Huang, X. (2022). Plant responses to UV-B radiation: Signaling, acclimation and stress tolerance. *Stress Biology*, *2*(1), 51.

Fiorani, F. and Schurr, U. (2013) Future Scenarios for Plant Phenotyping. *Annual Review of Plant Biology*, 64, 267-291.

Fraikin, G. Y. (2018). Signaling mechanisms regulating diverse plant cell responses to UVB radiation. *Biochemistry* (Moscow), 83, 787-794.

Gadi, B. R. (2018). Effect of UV-B radiation on plants. *IJSRST*, 4, 255-260.

Guo, Dong-Gang & Zhang, Xiao-Yang & Shao, Hong-Bo & Bai, Zhong-Ke & Chu, Li-Ye & Shangguan, Tie-Liang & Yan, Kun & Zhang, Li-Hua & Xu, Gang & Sun, Jun-Na, 2011. Energy plants in the coastal zone of China: Category, distribution and development, *Renewable and Sustainable Energy Reviews*, Elsevier, vol. 15(4), 2014-2020.

Guo, J., Wang, M. H. (2011). Expression profiling of the DREB2 type gene from tomato (*Solanum lycopersicum* L.) under various abiotic stresses. *Hortic. Environ. Biotechnol.* 52, 105–111.

Harb, A., Awad, D., & Samarah, N. (2015). Gene expression and activity of antioxidant enzymes in barley (*Hordeum vulgare* L.) under controlled severe drought. *Journal of Plant Interactions*, 10(1), 109-116.

He, J., Huang, L. K., Chow, W. S., Whitecross, M. I., & Anderson, J. M. (1993). Effects of supplementary ultraviolet-B radiation on rice and pea plants. *Functional Plant Biology*, 20(2), 129-142.

Hirayama, T., & Shinozaki, K. (2010). Research on plant abiotic stress responses in the post-genome era: past, present and future. *The plant journal*, 61(6), 1041-1052.

Johnson, S. M., Lim, F. L., Finkler, A., Fromm, H., Slabas, A. R., & Knight, M. R. (2014). Transcriptomic analysis of Sorghum bicolor responding to combined heat and drought stress. *BMC Genomics*, 15, 1-19.

Jordan, B. R. (2002). Molecular response of plant cells to UV-B stress. Functional Plant Biology, *29*(8), 909-916.

Knight, H., & Knight, M. R. (2001). Abiotic stress signalling pathways: specificity and cross-talk. *Trends in plant science*, *6*(6), 262-267.

Koyro, H. W., Ahmad, P., Geissler, N. (2012). Abiotic stress responses in plants: an overview. In book: *Environmental adaptations and stress tolerance of plants in the era of climate change*, Springer, NY Editors: P. Ahmad, M.N.V. Prasad, 1-28.

Lata, C., & Prasad, M. (2011). Role of DREBs in regulation of abiotic stress responses in plants. *Journal of Experimental Botany*, 62(14), 4731-4748.

Machado, R.M.A., Seralheiro, R.P., (2017), Soil salinity effect on vegetable crop growth. Management practices to prevent and mitigate soil salinization, *Horticulture*, 3(2), 30.

Mantri, N., Patade, V., Penna, S., Ford, R., & Pang, E. (2012). Abiotic stress responses in plants: present and future, 1-19, in book: *Abiotic Stress Responses in Plants: Metabolism, Productivity and Sustainability*, P. Ahmad and M.N.V. Prasad (eds.), DOI 10.1007/978-1-4614-0634-1_1, Springer Science+Business Media, LLC.

Martinez, V., Nieves-Cordones, M., Lopez-Delacalle, M., Rodenas, R., Mestre, T. C., Garcia-Sanchez, F., ... & Rivero, R. M. (2018). Tolerance to stress combination in tomato plants: new insights in the protective role of melatonin. *Molecules*, 23(3), 535.

McGinn, P. J., Price, G. D., Maleszka, R., & Badger, M. R. (2003). Inorganic carbon limitation and light control the expression of transcripts related to the CO2-concentrating mechanism in the cyanobacterium *Synechocystis* sp. strain PCC6803. *Plant Physiology*, 132(1), 218-229.

Mladin, Lia, Caprar M., Copaci C., Sicora O., Chis I., Rakosy E., Șumălan R., Sicora C. (2018). Evaluation of salt and UVB stress on tomato landraces from saline zones of Bihor County. *Journal of Horticulture, Forestry and Biotechnology*, 22(1), 88-92.

Nakashima, K., Shinwari, Z. K., Sakuma, Y., Seki, M., Miura, S., Shinozaki, K., Yamaguchi-Shinozaki, K. (2000). Organization and expression of two Arabidopsis DREB2 genes encoding DRE-binding proteins involved in dehydration-and high-salinity-responsive gene expression. *Plant molecular biology*, 42, 657-665.

Nowicka, B., Ciura, J., Szymańska, R., & Kruk, J. (2018). Improving photosynthesis, plant productivity and abiotic stress tolerance–current trends and future perspectives. *Journal of plant physiology*, 231, 415-433.

Ors, S., Suarez, D.L., (2017), Spinach biomass yield and physiological response to interactive salinity and water stress, *Agriculture and Water Management* 190, 31-41

Ors, S., Ekinci, M., Yildirim, E., Sahin, U., Turan, M., & Dursun, A. (2021). Interactive effects of salinity and drought stress on photosynthetic characteristics and physiology of tomato (*Lycopersicon esculentum* L.) seedlings. *South African Journal of Botany*, 137, 335-339.

Polenta, G. A., Guidi, S. M., Ambrosi, V., Denoya, G. I. (2020). Comparison of different analytical methods to evaluate the heat shock protein (HSP) response in fruits. Application to tomatoes subjected to stress treatments. *Current Research in Food Science*, 3, 329-338.

Raja, V., Qadir, S. U., Alyemeni, M. N., Ahmad, P. (2020). Impact of drought and heat stress individually and in combination on physio-biochemical parameters, antioxidant responses, and gene expression in *Solanum lycopersicum*, *3 Biotech*, 10(5), 208.

Ramonell, K. M., & Somerville, S. (2002). The genomics parade of defense responses: to infinity and beyond. *Current opinion in plant biology*, *5*(4), 291-294.

Rampino, P., Mita, G., Fasano, P., Borrelli, G. M., Aprile, A., Dalessandro, G., ... & Perrotta, C. (2012). Novel durum wheat genes up-regulated in response to a combination of heat and drought stress. *Plant Physiology and Biochemistry*, *56*, 72-78.

Reddy, P.S., Chakradhar, T., Reddy, R.A., Nitnavare, R.B., Mahanty, S. and Reddy, M.K., (2016). Role of heat shock proteins in improving heat stress tolerance in crop plants. In: *Heat Shock Proteins and Plants.* Springer International Publishing, Switzerland, 283-307.

Rizhsky, L., Liang, H., Shuman, J., Shulaev, V., Davletova, S., & Mittler, R. (2004). When defense pathways collide. The response of Arabidopsis to a combination of drought and heat stress. *Plant physiology*, 134(4), 1683-1696.

Sharma, S., Chatterjee, S., Kataria, S., Joshi, J., Datta, S., Vairale, M. G., Veer, V. (2017). A review on responses of plants to UV-B radiation related stress. UV-B Radiation: From Environmental Stressor to Regulator of Plant Growth, In book: *UV-B Radiation*, 75-97. DOI: 10.1002/9781119143611.ch5.

Sicora, C. I., Appleton, S. E., Brown, C. M., Chung, J., Chandler, J., Cockshutt, A. M., ... & Campbell, D. A. (2006). Cyanobacterial psbA families in Anabaena and Synechocystis encode trace, constitutive and UVB-induced D1 isoforms. *Biochimica et Biophysica Acta (BBA)-Bioenergetics*, 1757(1), 47-56.

Teixeira, F. K., Menezes-Benavente, L., Galvão, V. C., Margis, R., Margis-Pinheiro, M. (2006). Rice ascorbate peroxidase gene family encodes functionally diverse isoforms localized in different subcellular compartments. *Planta*, 224, 300-314

Tester, M., Langridge, P., Breeding technologies to increase crop production in a changing world (2010). *Science,* 12(327), 818-822

Zandalinas, S. I., Balfagón, D., Arbona, V., & Gómez-Cadenas, A. (2017). Modulation of antioxidant defense system is associated with combined drought and heat stress tolerance in citrus. *Frontiers in Plant Science*, 8, 953.

Zhang, H., Zhu, J., Gong, Z., & Zhu, J. K. (2022). Abiotic stress responses in plants. *Nature Reviews Genetics*, *23*(2), 104-119.

Zlatev, Z. S., Lidon, F. J., & Kaimakanova, M. (2012). Plant physiological responses to UV-B radiation. *Emirates Journal of Food & Agriculture (EJFA)*, *24*(6).

***https://doi.org/10.1007/s13205-020-02206-4

***http://apmbh.anpm.ro/

***www.fao.org

***https://www.ipcc.ch/

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

EFFECTS OF MONTMORILLONITE-ENRICHED HYDROGELS ON LETTUCE MORPHOLOGY UNDER DROUGHT STRESS

Ion NIȚU, Elisabeta Elena POPA, Amalia Carmen MITELUȚ

University of Agronomic Sciences and Veterinary Medicine of Bucharest,
59 Mărăşti Blvd, District 1, Bucharest, Romania

Corresponding author email: nituion.11@yahoo.com

Abstract

Lettuce is a widely cultivated leafy vegetable that is sensitive to drought stress. Drought stress is a significant environmental factor that adversely affects the growth and development of lettuce. Montmorillonite-infused hydrogels are emerging to mitigate the effects of drought by reinforcing soil moisture retention and facilitating plant growth. This study investigates the impact of different montmorillonite-enriched hydrogel samples (0%, 0.25%, 0.5%, and 1% montmorillonite) on lettuce growth under irrigated (every 3 days) and drought-stressed (no watering) conditions. The study examined various morphological characteristics, including total plant length, leaf count, root length, root weight, root volume, stem diameter, and aerial biomass. The results revealed that the hydrogel formulation with the highest montmorillonite content (1%), produced the most favorable results under irrigated and drought-stressed conditions, promoting enhanced root development (11.90% under drought and 4.17% with irrigation compared to the control sample) and leaf production. The findings underline the potential of montmorillonite-enriched hydrogel formulations to improve lettuce growth under different water conditions, presenting a promising approach for sustainable approaches.

Key words*: drought, hydrogel, Lactuca sativa, montmorillonite.*

INTRODUCTION

Lettuce is a widely cultivated leafy vegetable sensitive to drought stress (Chaorlina et al., 2021). Drought is a significant environmental factor that adversely affects lettuce's growth and development, leading to stunted growth, reduced leaf size, and impaired root development (Paim et al., 2020).

In recent years, hydrogels have attracted substantial attention for their potential applications in sustainable agricultural practices (Mazloom et al., 2020)

Hydrogels are polymeric materials that can absorb and retain large amounts of water, which makes them useful for various agricultural applications, such as water-holding agents, controlled-release carriers for nutrients and pesticides, and soil amendments to improve water-holding capacity and nutrient availability (Yu et al., 2017).

Montmorillonite-infused hydrogels are emerging to mitigate the effects of drought by reinforcing soil moisture retention and facilitating plant growth (Uddin, 2018).

The incorporation of montmorillonite, a clay mineral with high water absorption capacity, into hydrogel formulations has shown promising results in enhancing the water retention properties of the hydrogels and improving plant growth under water-limited conditions (Montesano et al., 2015).

Hydrogels with different montmorillonite contents may have varying effects on the morphological characteristics of lettuce under irrigated and drought-stressed conditions. On lettuce, as with many crops, identifying optimal hydrogel formulations can help improve plant performance and yield under different water regimes. The effects on the lettuce morphology with varying montmorillonite-enriched hydrogels remain to be thoroughly explored.

Root length, volume, and weight are crucial indicators of a plant's ability to access water and nutrients (Zhang et al., 2020). Changes in these parameters under drought stress can reveal the effectiveness of hydrogels in promoting root development. Drought stress often leads to reduced leaf area, affecting photosynthesis and overall plant growth. Measuring leaf area, length, width, and the number of leaves can provide insights into the hydrogel's impact on mitigating these effects (Seleiman et al., 2021). Stem diameter and height can be indicators of

overall plant vigor. (Kim et al.., 2010). Measuring both above-ground (aerial) and below-ground (root) biomass can provide a comprehensive assessment of plant growth (Madec et al., 2017).

Previous studies have examined the effects of hydrogels on various crops, including lettuce, but the specific impact of montmorillonite-enriched hydrogels on lettuce morphology under drought stress remains understudied. The objective of this study was to investigate the effects of montmorillonite-enriched hydrogels on the morphological characteristics of lettuce under irrigated and drought-stressed conditions.

MATERIALS AND METHODS

HYDROGEL SYNTHESIS

The hydrogel samples utilized in this study were synthesized at the National Institute for Laser, Plasma and Radiation Physics, Măgurele, employing the electron beam radiation method with potassium persulfate as the activator.

Four distinct hydrogel formulations were developed, varying in their compositions of sodium alginate, acrylic acid, polyethylene oxide, and montmorillonite at concentrations of 0%, 0.25%, 0.5%, and 1%, respectively, as presented in Table 1.

Table 1. Sample codification used in this study

Sample code		Montmorillonite dose (%)
Bead Hydrogel	Granular Hydrogel	
Hb1	Hg1	0
Hb2	Hg2	0.25
Hb3	Hg3	0.5
Hb4	Hg4	1
C		Control sample with no hydrogel

Two methods of hydrogel administration were evaluated in this study: granular form (Hg) and beads (Hb). Each hydrogel composition was applied in 10 replicates, with the weight of a single hydrogel bead maintained at 0.2 g, which was also the weight used for the granular form. The hydrogels were incorporated into 50 g of soil, and 100 mL of water was added to each sample to facilitate the swelling of the polymeric material.

Biological material

The lettuce (*Lactuca sativa*) seedlings placed in alveolar tray were acquired from VDRS Buzău (Figure 1).



Figure 1. Lettuce seedling

Working protocol

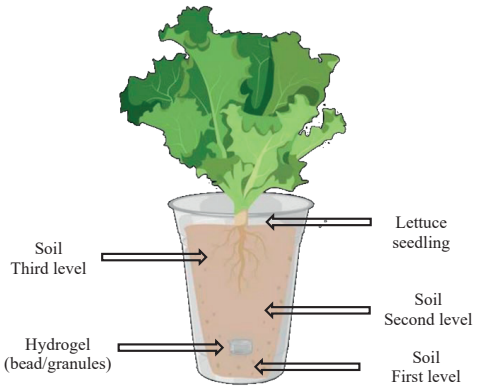


Figure 2. Working protocol graphic illustration
Created with BioRender.com

For this experiment, cups were used, in which soil (first level) and the hydrogel samples as beads (a) and granules (b), weighing approximately 0.2 g (Figures 2 and 3) were placed.



(a) (b)

Figure 3. Application of hydrogel: (a) Beads and (b) Granules

The second level of soil was then added, and hydration was carried with 100 ml water/each sample to assure the maximum swelling degree of the hydrogels. Further, after 24h, lettuce seedlings were transplanted, and the third level of soil was then added (Figure 4). Ten repetitions for each sample were prepared.



Figure 4. Lettuce seedlings in cups with hidrogels

After 3 weeks, the seedlings were transplanted from cups into pots (Figure 5).



Figure 5. Lettuce plants in pots with hydrogels

The samples were monitored for approximately 5 weeks, after which the seedlings were transplanted from pots into a greenhouse setting (Figure 6). The lettuce samples were transplanted according to a predetermined protocol with 2 equidistant rows established with a drip irrigation system. The samples were planted in the soil at a distance of approximately 50 cm, with 4 replications of each sample at the level of each row. One row consisted of lettuce samples maintained under irrigated conditions throughout the experimental period. The other with lettuce plants maintained for 1 week under irrigation conditions, after which drought conditions were imposed by shutting off the irrigation system for the entire experimental period.



Figure 6. Lettuce plants in greenhouse

MORPHOLOGICAL CHARACTERISTICS ANALYSIS

TOTAL PLANT LENGTH

The method involved measuring with a ruler at 7-day intervals to determine the total plant length. The measurement consisted of determining the distance from the soil level to the total length of the tallest leaf.

The mature size of lettuce (*Lactuca sativa*) can vary depending on the cultivar and growing conditions. Mature lettuce plants might reach a height of about 15-30 cm (Concepcion et al., 2020).

LEAF COUNT

The total number of leaves was determined over 40 days. The counting of lettuce leaves was carried out after removing any dried leaves.

Quantifying the total number of leaves on lettuce plants offers insights into the progress and density of the foliage. This metric aids in evaluating the overall health and vigor of the plant, as a greater leaf count often indicates enhanced growth and productivity (Singh et al., 2018).

ROOT LENGTH

The root length was determined by measuring the root system from the top of the root to the apical meristem.

The roots play a critical role in providing water and nutrients to the lettuce plant, and their morphological characteristics, such as length, volume, and weight, are key indicators of the plant's overall vigor and ability to withstand stress (Gregory & Kirkegaard, 2016).

ROOT WEIGHT AND AERIAL BIOMASS

After separating the root and aerial portions of the plants, which consisted of the stem and the rosette of leaves, the root and aerial biomass were determined using a technical balance.

The observed changes in root and aerial biomass under drought stress and the different hydrogel treatments can reveal the effectiveness of the hydrogels in supporting plant growth and development (Tomadoni et al., 2020).

ROOT VOLUME

The root volume was determined by immersing the root system in a graduated cylinder containing a known volume of water and calculating the root volume based on the difference in water level observed compared to the initial level.

The root volume is a useful indicator of the plant's ability to absorb water and nutrients from the soil, which is crucial for maintaining growth and productivity under limited water conditions (Raviteja et al., 2021).

STEM DIAMETER

The stem diameter was measured using a ruler following the separation of the aerial portion from the root system.

The stem diameter can provide insights into the overall vigor and health of the lettuce plant, as it reflects the plant's ability to transport water and nutrients effectively (Zhang et al., 2020).

RESULTS AND DISCUSSIONS

TOTAL PLANT LENGTH

All tested samples, including the control, exhibited relatively uniform total plant lengths (~40-42 cm) prior to the drought stress treatment, suggesting that the hydrogels did not significantly influence early plant growth.

The results demonstrated that the hydrogel bead samples consistently exhibited superior performance in supporting lettuce growth under both drought-stressed and irrigated conditions (Figure 7). Although the control sample performed well under irrigation, the **Hb1**, **Hg2**, and **Hb4** hydrogel formulations demonstrated superior results under drought conditions compared to the control sample. Consequently, the **Hb1** hydrogel can be considered the most suitable overall choice for promoting stable and resilient growth in lettuce plants across varying water availability scenarios.

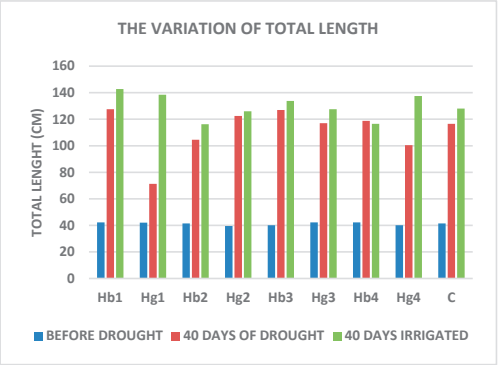


Figure 7. Total length of the lettuce samples after 40 days of testing

TOTAL NUMBER OF LEAVES

The results demonstrate that the hydrogel formulations, in both bead and granular forms, substantially enhanced lettuce leaf production (>50 leaves) under both drought-stressed and well-irrigated conditions. Importantly, the efficacy of the bead and granular hydrogel samples exhibited minimal variation, implying that both formulations were equally capable of supporting leaf development under environmental stress conditions. Considering the superior initial leaf count and sustained performance across both drought-stressed and well-irrigated conditions, the **Hb4** and **Hb2** samples could be considered the most dependable choices for reliably supporting abundant leaf production in lettuce plants under variable water availability conditions (Figure 8).

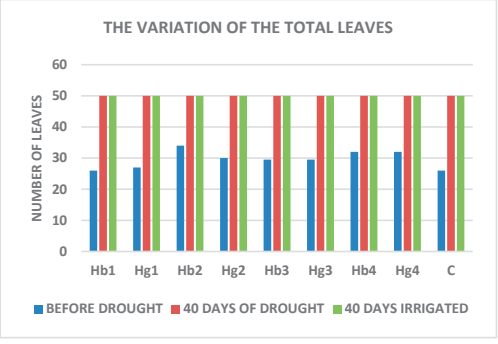


Figure 8. Total number of leaves of the lettuce samples after 40 days of testing

ROOT LENGTH

The results indicate that root length diminished across all samples under drought stress compared to the pre-drought period (Figure 9). The **Hb4** sample demonstrated the most effective performance, with a root length of 23.5 cm, outperforming the control samples at 21 cm under drought stress. This represents an 11.90% increase, suggesting enhanced root resilience under water-limited conditions. Furthermore, under irrigated conditions, the **Hb4** hydrogel maintained a root length of 25 cm compared to the control's 24 cm, indicating a 4.17% improvement with the hydrogel application. The **Hb4** sample consistently produced the longest root systems, providing the most effective support for root growth and development under both drought-stressed and irrigated conditions.

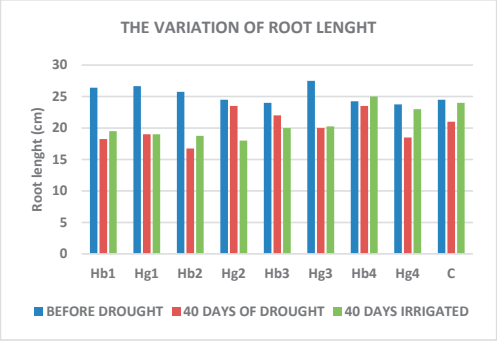


Figure 9. Root length of the lettuce samples after 40 days of testing

ROOT WEIGHT AND AERIAL BIOMASS

The lettuce sample **Hg1** exhibited the highest aboveground biomass of 265.23 g under drought stress, exceeding the control sample's 232.12 g by 14.26% (Figure 10). However, under irrigated conditions, the **control** sample attained the greatest aboveground biomass of 314.45 g, surpassing the 304.67 g achieved by the **Hg1** sample, a 3.11% decrease compared to the control. While the control demonstrated superior aboveground biomass production under optimal irrigation, the **Hg1** sample proved to be the most effective in supporting robust plant growth and biomass accumulation under water-limited conditions.

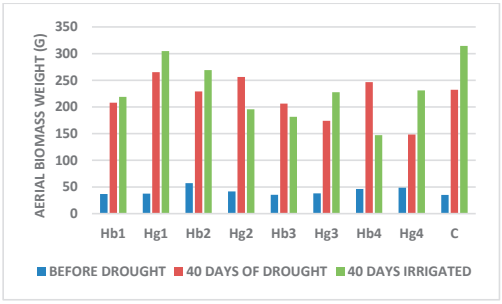


Figure10. Aerial biomass of the lettuce samples after 40 days of testing

The **Hg1** sample demonstrated the most favorable root weight performance under drought stress, with a root weight of 23.82 g, which exceeded the control sample's 20.35 g by 17.02% (Figure 11). Under irrigation, the **Hg1** hydrogel treatment exhibited the highest root weight performance, with a root weight of 30.43 g, which exceeded the control sample's 29.58 g by 2.87%. The **Hg1** sample demonstrated the most favorable performance, consistently surpassing the control sample in terms of root biomass under both drought-stressed and well-irrigated conditions. This suggests that the **Hg1** hydrogel formulation is the most effective choice for fostering resilient root growth under diverse water conditions.

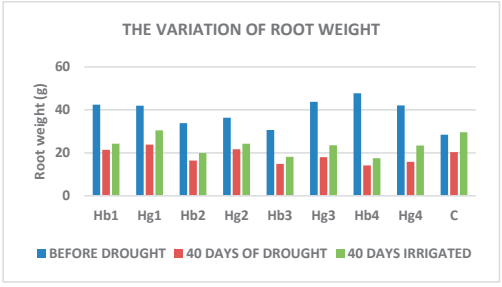


Figure 11. Root weight of the lettuce samples after 40 days of testing

ROOT VOLUME

For the root volume of the lettuce samples, the **Hg1** sample exhibited the most favorable root volume performance under drought stress conditions, with a root volume of 27.5 cm^3, which surpassed the control sample at 20 cm^3, representing a 37.5% increase (Figure 12).

However, under well-irrigated conditions, the **control** sample demonstrated the highest root volume at 30 cm³, exceeding the **Hg1**'s 25.3 cm³ by 15.67%. These results suggest that while the control sample provided superior root volume under optimal water availability, the **Hg1** sample was the most effective in enhancing root growth and resilience in drought-stressed environments.

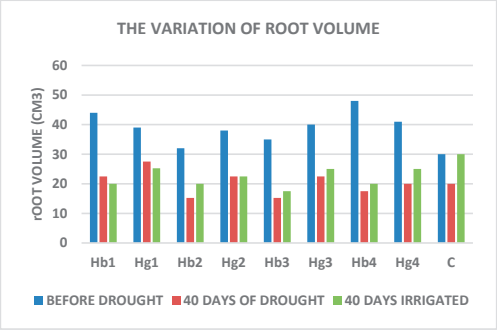


Figure 12. Root volume of the lettuce samples after 40 days of testing

STEM DIAMETER

The lettuce sample with hydrogel bead formulation **Hb2** exhibited the most favorable root diameter under drought stress, with a value of 1.6 cm compared to the control at 1.3 cm, representing a 23.08% improvement (Figure 13). Conversely, under irrigated conditions, the hydrogel granule **Hg1** demonstrated the highest root diameter at 1.5 cm, surpassing the control's 1.3 cm by 15.38%. Overall, **Hg1** provided the most consistent enhancement in root diameter across both drought and irrigation.

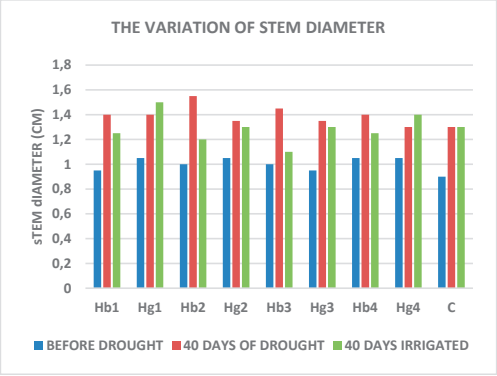


Figure 13. Stem diameter of the lettuce samples after 40 days of testing

CONCLUSIONS

The results of the study indicate that the incorporation of montmorillonite-enriched hydrogels into the soil substrate positively impacted the growth and development of lettuce plants under both drought stress and optimal irrigation conditions, outperforming the control samples.

The lettuce samples with hydrogel beads (**Hb4)** exhibited the most favorable outcomes for total plant length and root length and the number of leaves, suggesting their effectiveness in supporting overall plant development under water-limited conditions. **Hg1** hydrogel granules excelled in promoting aerial biomass, root weight, and root volume, demonstrating their capability to uphold root and biomass-related parameters in drought-stressed plants. Under consistent watering, the control samples generally outperformed the hydrogel treatments in terms of total plant length and root volume, indicating that regular water availability can be more beneficial for these morphological factors. However, the **Hg1** sample maintained the highest root weight and stem diameter among the treatments, suggesting their potential to enhance these specific characteristics even under optimal moisture conditions.

In summary, the **Hb4** and **Hg1** hydrogel formulations appear to be the most balanced and effective in supporting lettuce growth and resilience across varying water availability scenarios. While the control samples exhibited the best overall performance under irrigation, the hydrogel treatments, particularly **Hg1**, demonstrated the capacity to consistently improve key morphological parameters, highlighting their value in enhancing the sustainability and productivity of lettuce cultivation, especially in regions prone to drought.

ACKNOWLEDGEMENTS

This work was supported by a grant of the Ministry of Research, Innovation and Digitization, CCCDI – UEFISCDI, project number PN-III-P2-2.1-PED-2021-2151, within PNCDI III, Contract No. 663PED/2022 (HYDROBIOGEL).

REFERENCES

Chaorlina, A., Setyaningsih, M., & Faruq, H. (2021). The Utilization of Tofu Waste Water as An Addition of Nutrition in Hydroponic Media to Lettuce Growth (*Lactuca sativa* L.). In IOP Conference Series Earth and Environmental Science (Vol. 755, Issue 1, p. 12049). IOP Publishing. https://doi.org/10.1088/1755-1315/755/1/012049.

Concepcion, R., Lauguico, S., Alejandrino, J., Dadios, E. P., & Sybingco, E. (2020). Lettuce Canopy Area Measurement Using Static Supervised Neural Networks Based on Numerical Image Textural Feature Analysis of Haralick and Gray Level Co-Occurrence Matrixs. In *AGRIVITA Journal of Agricultural Science* (Vol. 42, Issue 3). Brawijaya University. https://doi.org/10.17503/agrivita.v42i3.2528.

Gregory, P., & Kirkegaard, J. A. (2016). *Growth and Function of Root Systems*. In Elsevier eBooks (p. 230). Elsevier BV. https://doi.org/10.1016/b978-0-12-394807-6.00127-1.

Kim, S., Iyer, G., Nadarajah, A., Frantz, J. M., & Spongberg, A. L. (2010). Polyacrylamide Hydrogel Properties for Horticultural Applications. In *International Journal of Polymer Analysis and Characterization* (Vol. 15, Issue 5, p. 307). Taylor & Francis. https://doi.org/10.1080/1023666x.2010.493271.

Madec, S., Baret, F., Solan, B. de, Thomas, S., Dutartre, D., Jézéquel, S., Hemmerlé, M., Colombeau, G., & Comar, A. (2017). High-Throughput Phenotyping of Plant Height: Comparing Unmanned Aerial Vehicles and Ground LiDAR Estimates. In *Frontiers in Plant Science* (Vol. 8). Frontiers Media. https://doi.org/10.3389/fpls.2017.02002.

Mazloom, N., Khorassani, R., Zohury, G. H., Emami, H., & Whalen, J. K. (2020). Lignin-based hydrogel alleviates drought stress in maize. In *Environmental and Experimental Botany* (Vol. 175, p. 104055). Elsevier BV. https://doi.org/10.1016/j.envexpbot.2020.104055.

Montesano, F. F., Parente, A., Santamaria, P., Sannino, A., & Serio, F. (2015). Biodegradable Superabsorbent Hydrogel IncreasesWater Retention Properties of Growing Media and Plant Growth. In *Agriculture and Agricultural Science Procedia* (Vol. 4, p. 451). Elsevier BV. https://doi.org/10.1016/j.aaspro.2015.03.052.

Paim, B. T., Crizel, R. L., Siebeneichler, T. J., Rodrigues, V. R., Rombaldi, C. V., & Galli, V. (2020). Mild drought stress has potential to improve lettuce yield and quality. In *Scientia Horticulturae* (Vol. 272, p. 109578). Elsevier BV. https://doi.org/10.1016/j.scienta.2020.109578.

Raviteja, M. S. V., Laxman, R. H., Rashmi, K., Kannan, S. P., Namratha, M. R., & Reddy, K. M. (2021). Effect of container size and types on the root phenotypic characters of *Capsicum*. In *Journal of Horticultural Sciences* (Vol. 16, Issue 2, p. 261). Society for Promotion of Horticulture. https://doi.org/10.24154/jhs.v16i2.1606.

Seleiman, M. F., Al-Suhaibani, N., Ali, N., Akmal, M., Alotaibi, M. A., Refay, Y., Dindaroğlu, T., Abdul-Wajid, H. H., & Battaglia, M. L. (2021). Drought Stress Impacts on Plants and Different Approaches to Alleviate Its Adverse Effects [Review of Drought Stress Impacts on Plants and Different Approaches to Alleviate Its Adverse Effects]. *Plants,* 10(2), 259. Multidisciplinary Digital Publishing Institute. https://doi.org/10.3390/plants10020259.

Singh, M. C., Singh, K., & Singh, J. (2018). Indirect method for measurement of leaf area and leaf area index of soilless cucumber crop. In *Advances in Plants & Agriculture Research* (Vol. 8, Issue 2). MedCrave Group. https://doi.org/10.15406/apar.2018.08.00311.

Tomadoni, B., Salcedo, M. F., Mansilla, A. Y., Casalongué, C. A., & Álvarez, V. A. (2020). Macroporous alginate-based hydrogels to control soil substrate moisture: Effect on lettuce plants under drought stress. In *European Polymer Journal* (Vol. 137, p. 109953). Elsevier BV. https://doi.org/10.1016/j.eurpolymj.2020.109953.

Uddin, F. (2018). Montmorillonite: An Introduction to Properties and Utilization. In *InTech eBooks*. https://doi.org/10.5772/intechopen.77987.

Yu, J., Shi, J., Ma, X., Dang, P., Yan, Y., Mamedov, A. I., Shainberg, I., & Levy, G. J. (2017). Superabsorbent Polymer Properties and Concentration Effects on Water Retention under Drying Conditions. In *Soil Science Society of America Journal* (Vol. 81, Issue 4, p. 889). Wiley. https://doi.org/10.2136/sssaj2016.07.0231.

Zhang, Y., He, Q., Xie, F., & Li, M. (2020a). Effects of Drought Stress on Growth of Lettuce Seedlings.

Zhang, Y., He, Q., Xie, F., & Li, M. (2020b). Effects of Drought Stress on Growth of Lettuce Seedlings. https://doi.org/10.23977/fbb2020.016.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

INFLUENCE OF CONVENTIONAL AND BIOLOGICAL CONTROL ON POSTHARVEST QUALITY OF TOMATOES

Mihaela PARASCHIV, Gabriela ȘOVĂREL

Research and Development Institute for Vegetable and Flower Growing Vidra,
22 Calea Bucureşti, Vidra, 077185, Ilfov, Romania

Corresponding author email: gabriela_sovarel@yahoo.com

Abstract

The objective of this experiment was to evaluate the efficacy of some chemical and biological products used in the control of foliar pathogens in tomato crop in greenhouse and their influence on the quality and shelf life of tomato fruits. Conventional products ensured a much higher efficay (>90%) compared to biological ones (<67%). The high moisture content of the fruits contributes to their relatively short shelf life. Water loss not only accelerates the aging process of the fruits but also impacts their quality. This research systematically examines variations in water content, total dry matter, total soluble solids, organic acids, and firmness in tomatoes stored at temperatures of 6°C and 22°C, starting from the time of harvest and extending for 3, 5, 7, and 10 days. Observations indicated that water loss, evidenced by reduced firmness and fruit weight, was associated with an increase in soluble solids and total dry weigt per unit mass. Additionally, the degradation of organic acids in tomatoes was influenced by the storage duration, with increasing temperatures accelerating these transformations.

Key words: *Solanum lycopersicum, biological and conventional control, storage temperatures, postharvest quality.*

INTRODUCTION

Tomatoes (*Solanum lycopersicum*) are among the most consumed vegetables worldwide, playing an important role in human nutrition due to their high content of nutrients and bioactive phytochemicals, such as vitamins C and E and lycopene (Ebert, 2020).

By 2030, the European Union aims to reduce the use of chemical pesticides by up to 50% (Bremmer et al., 2021; Pânzaru et al., 2023).

Organic agriculture is a dynamic sector in Romania that has seen an upward evolution in recent years. Romanian agricultural research gives special importance to the development of technologies for the cultivation of vegetables grown in conventional and/or ecological agriculture. Good results were obtained in biological control of disases and pests on tomatoes (Bratu et al., 2015, Hogea, 2020), pepper (Călin et al., 2017; 2020), eggplants (Iosob & Cristea, 2022), cucumbers (Cenușă et al., 2016), melons (Șovărel et al., 2024), cabbage (Iosob et al., 2023), zucchini (Șovărel et al., 2024) and onion (Călin et al., 2016).

Global tomato production has increased by approximately 13% in the last decade, according to FAOSTAT (2025). The demand for tomatoes with superior organoleptic qualities continues to grow, highlighting the need for appropriate management of this crop (Fenech et al., 2019; Wang et al., 2023). Organoleptic parameters are influenced by growing conditions, ripening stage, and post-harvest processing technologies (Cause et al., 2010; Chaïb et al., 2007; Zushi & Higashijima, 2022). As climacteric fruits, tomatoes continue to ripen after harvest, with a tendency to quickly become overripe and vulnerable to spoilage (Bourne, 2006). Their shelf life is usually between 2 and 3 weeks, and after harvest, they undergo significant chemical changes (Anyasi et al., 2016; Thole et al., 2020; Tolasa et al., 2021; Vunnam et al., 2014). Quality deterioration is influenced by bacteria, yeasts, molds, and viruses (Fiddler, 1982). Although global production has increased, post-harvest losses remain a major challenge, with estimates ranging from 20% to 50%, depending on storage and handling (Kader, 1985; Albornoz et al., 2019). Thus, extending shelf life and improvingquality are essential objectives that impact farmers' incomes and global food security.

Post-harvest quality deterioration of tomatoes is influenced by temperature and storage time. Low-temperature storage is crucial for

maintaining quality (Bourne, 2006). The optimal temperature for storing tomatoes is close to 0 °C; an increase of 10 °C can triple the rate of deterioration. However, Chira (1999) indicates that the ideal refrigeration temperature varies between 0.5 and 13°C, depending on the stage of ripening. Immediate post-harvest cooling is essential for quality preservation (Kader et al., 1985), helping to maintain organoleptic characteristics that are crucial for consumer satisfaction (Colantonio et al., 2022; Wang et al., 2023). Proper management of temperature and relative humidity is also essential to prevent rapid spoilage of tomatoes and to ensure global food security (Mrema & Rolle, 2002; Hatami et al., 2013; Morgan, 2021). Implementing effective temperature and humidity management strategies can maintain product quality and reduce the economic impact associated with spoilage (Alia-Tejacal et al., 2007; Kabir et al., 2020; Morgan, 2021). Therefore, optimizing storage conditions is essential to improve tomato shelf life.

The objective of this experiment was to evaluate the efficacy of chemical and biological products in controlling foliar pathogens in tomato crops grown in greenhouses, as well as to assess their effects on fruit quality and shelf life.

MATERIALS AND METHODS

The experience was conducted in two greenhouses at the Research Development Institute for Vegetable and Flower Growing Vidra, in 2024. Planting was made on 10 July, using tomato (*Solanum lycopersicum*) variety 'Prekos F1', arranged according to the method of randomized blocks, 4 replications. The treatements were applied preventively for *Alternaria solani* and *Fulvia fulva* and *Botrytis cinerea* control.

In the conventional system were made 2 applications to control the pathogens *A. solani* and *F. fulva* (July 19, 26) and 1 treatment for *B. cinerea* (September 6).

The experiment for conventional pathogen control consists on 4 variants treated and untreated control.

Conv. 1: Ortiva Top (azoxistrobin 200 g/L + difenoconazol 125 g/l) 1 l/ha (produced by Syngenta Crop Protection AG, Switzerland);

Conv. 2: Cidely Top (difenoconazol 125 g/l + ciflufenamid 15 g/l) 1 l/ha; Botrefin (cyprodinil 375 g/l + fludioxonil 250 g/l) 0.8 kg/ha (produced by Syngenta Crop Protection AG, Switzerland);

Conv. 3: Amistar (azoxistrobin 250 g/L) 0.75 l/ha; Sygnum (boscalid 26,7% + piraclostrobin 6,7%) 1.5 kg/ha (produced by Syngenta Crop Protection AG, Switzerland);

Conv. 4: Dagonis (difenoconazol 50 g/l + fluxapiroxad 75 g/l) 1 l/ha; Switch (fludioxonil 25% + ciprodinil 37,5%) 0.8 kg/ha (produced by BASF Agr B.V., Arnhem (NL) - Freienbach Branch, Switzerland);

Conv. 5: Untreated control.

Also, in the ecological pathogen control, the experience also includes 5 experimental variants, to which 6 treatments were applied: July 19 (T1), July 26 (T2), August 2 (T3), September 7 (T4), September 14 (T5) and September 21 (T6), as follows:

Biol. 1: Cavaler 600SL (microorganisms *Bacillus pumillus* and *Bacillus subtilis*) 0.3% (produced by Chromosome Dnamics S.A. Bucharest, Romania);

Biol. 2: Amulet (microorganisms *Bacillus thuringiensis, Bacillus subtilis, Bacillus megatherium*) 40 l/ha (produced in the U.E.);

Biol. 3: Zytron (citrus seed extract 20%) 0.15% (produced by Atlantica Agricola S.A. Spain);

Biol. 4: Mimoten (*Mimosa tenuifolia* 80% extract) 0.3% (produced by Atlantica Agricola S.A. Spain);

Biol. 5: Untreated control.

Observations and determinations were made on the leaves (5 plants/variant) regarding the frequency and severity of the *Alternaria solani*, *Fulvia fulva* and *Botrytis cinerea* pathogens, based on which the effectiveness of the products was calculated.

Climate data monitoring in greenhouse was done with the help of thermohygrometers, which record air temperature and humidity at hourly intervals.

The atmospheric humidity in the greenhouse was greatly influenced by the amount of precipitation that fell during this period. Thus, the precipitation that fell on July 17, 20 and 21, in the form of torrential rains, in amounts of 11, 18 and 22 l/sqm, caused an increase in atmospheric humidity to values of over 88%, between July 17 and 29.

In September, atmospheric humidity began to increase, with values between 85 and 92.4%

recorded in 19 days, with a maximum average of 84.1% for the month (Figure 1).
Tomato harvesting took place in Phase VI, according to the USDA tomato ripening stages (Bertin, 2018). The fruits were stored at temperatures of 6°C and 22°C, with a relative humidity of 70%.

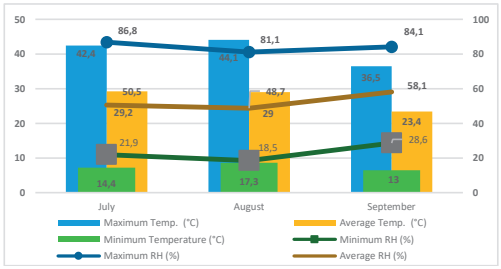


Figure 1. Greenhouse climate data
for the period July - september 2024

Fruit quality characteristics were evaluated at harvest and after 3, 5, 7, and 10 days of storage. The analyses included weight loss, dry weight, total soluble solids, firmness, pH and ash content. The percentage of weight loss was calculated based on the duration of storage, following the methodology presented by Tefera et al. (2007). The formula used was: Weight loss (%) = M_0 (M_0–M_1) × 100, where M_0 represents the initial mass of the fruits (g) and M_1 represents the mass of the fruits after storage (g).
Total dry matter content (DW), expressed as a percentage (%), was determined gravimetrically by heating at 105°C to constant weight (Krełowska-Kułas, 1993).
Total soluble solids (TSS) were measured by extracting tomato juice from 5 tomatoes per group using a food blender, followed by filtration. TSS was measured using a portable digital refractometer (Model: HI 96800, Hanna Instruments, USA), with the results expressed as % Brix (PN-90/A-75101/04. 1990).
Fruit firmness was assessed at four equidistant points along each tomato using a Force Gauge PCE-FM 200 firmness measuring instrument. The results obtained were expressed in Newtons (N) and averaged over a sample of ten fruits (n = 10).
The pH of each juice sample was measured using an electronic pH meter (Model: EUTECH Cyberscan pH 11, Singapore) equipped with a glass electrode. Ash content was determined by calcining the samples at 450°C and expressed as a percentage of fresh weight (% FW) (Horwitz, 2000).

Statistical analysis

All data were analyzed using SPSS 26.0 (International Business Machines Corp., Chicago, IL, USA). Results are presented as mean ± standard deviation (S.D.). A three-way analysis of variance (ANOVA) was performed to evaluate the effects of the independent variables-genetic background, storage condition and duration-on the dependent variables (chemical and physical parameters), at a significance level of 5% ($P < 0.05$).

RESULTS AND DISCUSSIONS

In conventional system, very good results in controling the pathogen *Alternaria solani* were obtained in all treated variants (Figure 2). The products Ortiva Top 1 l/ha, Cidely Top 1 l/ha, Amistar 0.75 l/ha and Dagonis 1 l/ha ensured an efficacy between 96.85% and 98.39% in controling this pathogen on leaves. The same products were also more than 99% efficacy in controlling the pathogen *Fulvia fulva* (Figure 2). In the specialized literature, it is mentioned that fungicides containing difenoconazole and azoxystrobin (such as Score 25% SC and Amistar 25% SC) significantly reduce the incidence and severity of early blight disease in tomatoes, subsequently contributing to increasing the yield of tomato fruits (Ramadan et al., 2021). Strobilurin compounds, used as fungicides are very effective for a number of fungi including early blight (Bartlett et al., 2002; Pasche et al., 2004; Zafar and Shaukat, 2018). Strobilurins (azoxystrobin, pyraclostrobin and boscalid) are fungicides with beneficial physiological effects on crop yield due to promotion of net carbon assimilation, nitrate reductase enzyme activity, stress tolerance and hormonal balance (Esteves et al., 2018).
Azoxystrobin are known to break down this resistance because of their different mode of action as compared to other fungicides. Excellent control, curative, translaminar and systemic properties of azoxystrobin enables it to be used efficiently against leaf blight of tomato at very low application rates (Hewitt, 1998; Mejia Arreaza and Hernandez, 2001; Anand et al., 2010). Azoxystrobin shows a unique spectrum of disease control and is active against

Oomycetes, Ascomycetes, Basidiomycetes and Deuteromycetes (Anand et al., 2010).
The atmospheric humidity in the greenhouse over 88%, between July 17 and 29, creates favorable conditions for the evolution of the pathogens *Alternaria solani* and *Fulvia fulva*.
In the biological control of the pathogen *Alternaria solani*, good results were obtained with the treated variants, with an efficacy between 58.79% (Amulet) and 66.30% (Mimoten) in controling this pathogen on leaves, and 66.67% (Cavaler 600SL) and 70% (Amulet, Zytron) on fruits. Also, low efficacy was recorded in the control of the pathogen *Fulvia fulva* with values between 44.05% (Amulet) and 54.54% (Cavaler 600SL).

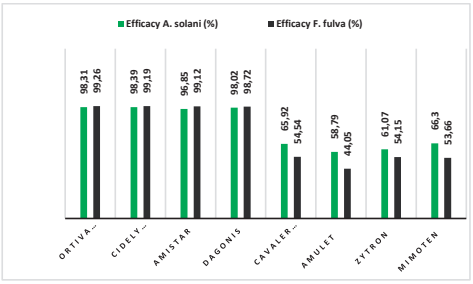


Figure 2. The efficacy of conventional and biological products on *A.solani* and *F. fulva*

The products Ortiva Top 1 l/ha, Botrefin 0.8 l/ha, Sygnum 1.5 kg/ha and Switch 0.8 l/ha, used to control gray rot, produced by the pathogen *Botrytis cinerea*, had a very good efficacy, between 97.89 and 98.67%. Among the treated variants, *Botrytis cinerea* attack was manifested on the fruits only in the variant treated with the product Ortiva Top 1 l/ha (Figure 3).
The efficacy of biological products was between 43.61% (Zytron) and 56.53% (Cavaler 600SL) in controlling the pathogen *Botrytis cinerea* on leaves and 63.10% (Amulet) – 70.23% (Zytron) on tomato fruits.
Specific strains of various *Bacillus* species have proven effective in controlling fungal pathogens, including *Fusarium, Rhizoctonia, Oidium, Septoria, Macrophomina, Botrytis, Pythium, Verticillium, Phytophthora, Sclerotium*, and *Alternaria. Bacillus* species possess the capability to decrease populations of fungal pathogens through the production of a substantial quantity of antibiotics (Iqbal et al., 2023).
Bacillus subtilis is one of the most potential biological control agents, because of the broad-spectrum activity of their antibiotics. Foliar application of *B. subtilis* alone and in combination with the plant nutrients managed early blight disease significantly (Awan & Shoaib, 2019).

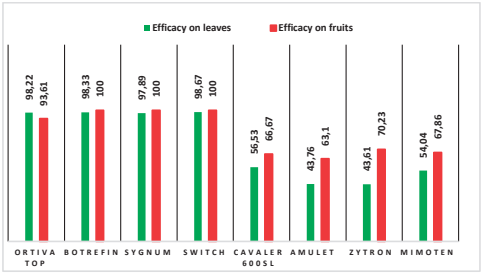


Figure 3. The efficacy of conventional and biological products on *B. cinerea*

In all variants treated with different protection products for *Alternaria solani*, *Fulvia fulva* and *Botrytis cinerea* pathogens control, yield increases with values between 7.33 and 9.01 t/ha for conventional and between 2.14 and 3.04 t/ha for biological (Figure 4).

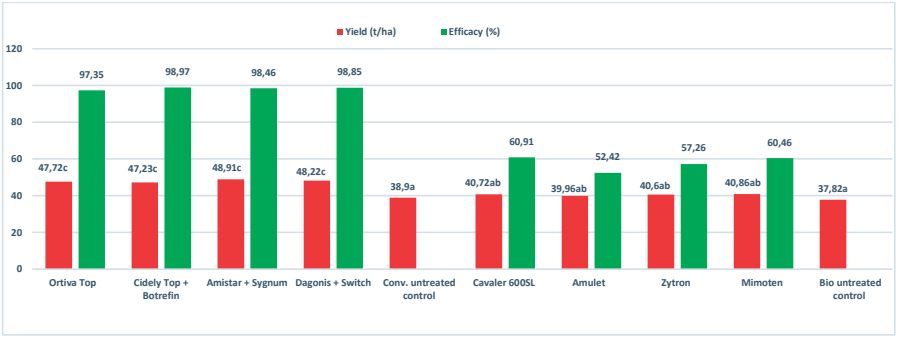


Figure 4. The influence of conventional and biological products on the yield of tomatoes grown in greenhouses

Results on quality maintenance during storage

Weight loss

In general, weight loss increases as the storage period is prolonged, suggesting a progressive degradation of product quality. Studies conducted over 10 days have revealed a significant decrease in tomato weight, attributable to respiration and transpiration processes (Anyasi et al., 2016; Moneruzzaman et al., 2008; Vunnam et al., 2014). At a temperature of 22°C, weight losses are significantly higher compared to those at 6°C, indicating that higher temperatures favor deterioration.

Weight loss also varies depending on the phytosanitary treatments applied during the growing season. For conventional products, losses ranged from 3.08% (Dagonis, Switch) to 3.59% (Amistar, Sygnum) with a control loss of 4.21% at 6°C, and from 4.40% (Ortiva Top) to 5.98% (Cidely Top, Btrefin) with a control loss of 6.91% at 22°C (Table 1). In the case of organic treatments, losses were between 2.31% (Zytron) and 4.31% (Mimoten) at 6°C and between 5.36% (Cavaler 600L) and 6.81% (Mimoten) at 22°C (Table 2). ANOVA analysis showed a significant influence of phytosanitary treatments on weight loss, with an impact of 62.0%.

The percentage of weight loss during storage is influenced by the stage of maturity of the product (Moneruzzaman et al., 2009). In general, fruits and vegetables are considered unmarketable if they lose between 5% and 10% of their initial weight, a phenomenon associated with quality deterioration, including wilting and shriveling (Ben-Yehoshua & Rodov, 2002). Excessive water loss not only reduces weight but also affects the texture and flavor of the products (Ben-Yehoshua & Rodov, 2002; Caleb et al., 2012). Water loss accelerates senescence and membrane degradation (Ben-Yehoshua et al., 1983). Additionally, factors such as pre-harvest conditions (including product type and orchard practices), harvest parameters (such as injuries and damages, maturity stage, weather conditions, harvesting method, and crown position), and post-harvest management (covering storage conditions and pre-treatments) significantly influence water loss (Tyagi et al., 2017; Lufu et al., 2020).

Dry weight content

Tomatoes contain between 92.5% and 95% water, with the remaining 5% to 7.5% consisting of total dry weight (Davies & Hobson, 1981). The composition of the dry weight includes sugars, particularly carbohydrates such as glucose and fructose, which constitute approximately 48% of the total sugars, as well as lipids, proteins, minerals, vitamins, organic acids, and phenolic compounds (Kader, 2002). The dry weight of tomatoes can be influenced by various factors, including genotype, environmental conditions, cultivation methods, and the amount of water used in irrigation. Water loss and the total dry weight remaining were not affected by the phytosanitary protection treatments applied during the growing season. However, storage temperature and storage time had a significant impact on dry weight, accounting for 14.1% and 66.8% of the variation, respectively, according to ANOVA analysis. The highest values of this biochemical indicator were observed after 10 days of storage at ambient temperature - 6.55% for conventional treatments, 6.85% for organic treatments, and 7.02% for the control. These values were similar to the observed weight losses of 4.95%, 6.03%, and 6.96%, respectively (Table 3).

Total soluble solids content

Regarding the total soluble solids (TSS) content, no variations were observed during cold storage or at ambient temperature, depending on the type of phytosanitary treatments applied during the growing period. Instead, a progressive increase in TSS was noted over the storage duration. The average soluble solids content of tomatoes varied between 4.27°Brix (Dagonis, Switch) and 4.31°Brix (Cidely Top, Botrefin) at harvest (conventional variant) and increased to between 5.20° Brix (Ortiva Top) and 5.47° Brix (Amistar, Sygnum) at 6°C. At 22°C, TSS varied between 6.12° Brix (Ortiva Top) and 6.78°Brix (Dagonis, Switch). The organic variant recorded higher TSS values, reaching a maximum of 5.84°Brix (Amulet) at 6°C and 6.54°Brix (Amulet) at 22°C. From the perspective of Duncan's test, TSS was significantly influenced only by storage temperature and storage time. Similar results were obtained by Al-Dairi et al. (2021). Islam et al. (2012) noted an increase in TSS in tomatoes during storage, rising from 4.20% to 5.00% after 7 days at room temperature. They also observed a variation from 4.10% to 4.90% in tomatoes stored under low-temperature conditions after 17 days of storage. Rab et al. (2013) reported that, in the

case of tomatoes not subjected to pre-cooling, the average TSS content reached 8.66%.

Firmness

Tomato firmness is a fundamental criterion in the marketing process. Statistical analysis revealed a decrease in fruit firmness during the ten days of storage. At the beginning of this period, the average firmness of tomatoes treated with conventional phytosanitary products ranged from approximately 15.44 N (Dagonis, Switch) to 16.73 N (Amistar, Sygnum).

Table 1. Characteristics of 'Prekos F1' hybrid tomato treated with conventional phytosanitary products during storage: weight loss (%), dry weight (%), total soluble solids (TSS, °Brix), firmness (N), pH and ash (%)

Storage temperature	Storage time (days)	Variant	Weight loss (%)	Dry weight (%)	TSS (°Brix)	Firmness (N)	pH	Ash (%)
6°C	At harvest	Conv. 1	-	5.97±0.29^{a}	4.30±0.17^{a}	16.22±1.35^{a}	3.97±0.18^{a}	0.44±0.01^{a}
		Conv. 2	-	4.60±0.28^{c}	4.31±0.08^{a}	15.76±1.69^{a}	4.02±0.11^{a}	0.44±0.01^{a}
		Conv. 3	-	5.61±0.08^{b}	4.28±0.23^{a}	16.73±1.12^{a}	4.04±0.09^{a}	0.43±0.01^{a}
		Conv. 4	-	4.91±0.09^{c}	4.27±0.42^{a}	15.44±0.98^{a}	4.00±0.11^{a}	0.43±0.03^{a}
		Conv. 5	-	4.75±0.01^{c}	4.23±0.32^{a}	15.45±1.32^{a}	4.02±0.47^{a}	0.44±0.01^{a}
	3 days	Conv. 1	0.69±0.28^{a}	5.75±0.26^{a}	4.41±0.19^{a}	15.52±1.67^{a}	4.00±0.21^{a}	0.44±0.01^{ab}
		Conv. 2	0.88±0.31^{a}	6.11±0.47^{a}	4.37±0.15^{a}	15.24±1.66^{a}	4.05±0.19^{a}	0.46±0.01^{a}
		Conv. 3	0.67±0.44^{a}	5.15±0.14^{b}	4.47±0.26^{a}	16.11±2.09^{a}	4.09±0.14^{a}	0.43±0.01^{b}
		Conv. 4	0.79±0.69^{a}	5.61±0.84^{ab}	4.59±0.44^{a}	14.79±1.96^{b}	4.03±0.21^{a}	0.44±0.01^{ab}
		Conv. 5	1.07±0.35^{a}	5.27±0.32^{b}	4.40±0.62^{a}	13.68±2.00^{b}	4.07±0.38^{a}	0.45±0.01^{ab}
	5 days	Conv. 1	1.50±0.49^{b}	6.14±0.20^{ab}	4.62±0.22^{a}	14.44±1.96^{a}	4.08±0.27^{a}	0.45±0.01^{ab}
		Conv. 2	1.62±0.38^{b}	6.34±0.30^{ab}	4.62±0.35^{a}	14.11±1.60^{a}	4.11±0.32^{a}	0.48±0.04^{ab}
		Conv. 3	1.23±0.33^{c}	5.33±0.17^{c}	4.57±0.29^{a}	15.02±1.75^{a}	4.11±0.18^{a}	0.43±0.01^{b}
		Conv. 4	1.42±0.71^{bc}	5.81±0.28^{b}	4.65±0.45^{a}	12.00±2.85^{b}	4.07±0.22^{a}	0.46±0.01^{ab}
		Conv. 5	2.63±0.61^{a}	5.86±0.12^{b}	4.47±0.38^{a}	12.08±1.33^{b}	4.10±0.27^{a}	0.49±0.02^{a}
	7 days	Conv. 1	2.01±0.56^{b}	6.21±0.13^{ab}	5.13±0.91^{a}	12.97±1.11^{a}	4.12±0.14^{a}	0.46±0.01^{bc}
		Conv. 2	2.25±0.23^{b}	6.35±0.42^{a}	5.23±0.21^{a}	12.44±1.32^{a}	4.15±0.37^{a}	0.49±0.02^{a}
		Conv. 3	2.28±0.66^{b}	6.41±0.29^{a}	5.10±0.75^{a}	13.00±1.21^{a}	4.14±0.25^{a}	0.46±0.02^{c}
		Conv. 4	1.97±0.57^{b}	5.95±0.21^{b}	4.95±0.40^{a}	11.08±0.97^{b}	4.17±0.37^{a}	0.49±0.01^{ab}
		Conv. 5	3.12±0.56^{a}	6.08±0.06^{b}	5.05±0.64^{a}	11.07±1.14^{b}	4.19±0.22^{a}	0.51±0.01^{a}
	10 days	Conv. 1	3.15±0.97^{b}	6.45±0.38^{a}	5.28±0.75^{a}	10.41±0.85^{a}	4.18±0.20^{a}	0.47±0.01^{c}
		Conv. 2	3.27±0.35^{b}	6.54±0.23^{a}	5.33±0.55^{a}	10.11±1.33^{a}	4.21±0.19^{a}	0.56±0.02^{a}
		Conv. 3	3.59±1.31^{ab}	6.56±0.24^{a}	5.47±0.34^{a}	10.63±1.40^{a}	4.19±0.24^{a}	0.50±0.02^{bc}
		Conv. 4	3.08±0.93^{b}	6.44±0.45^{a}	5.38±0.42^{a}	9.77±1.35^{b}	4.22±0.24^{a}	0.51±0.01^{b}
		Conv. 5	4.21±0.86^{a}	6.32±0.33^{a}	5.25±0.27^{a}	9.81±0.99^{b}	4.27±0.40^{a}	0.53±0.01^{b}
22°C	At harvest	Conv. 1	-	5.81±0.31^{a}	4.28±0.15^{a}	16.19±1.31^{a}	4.00±0.15^{a}	0.46±0.01^{a}
		Conv. 2	-	4.63±0.35^{a}	4.34±0.28^{a}	15.78±1.75^{a}	4.03±0.20^{a}	0.44±0.02^{b}
		Conv. 3	-	5.57±1.60^{a}	4.27±0.19^{a}	16.70±1.48^{a}	4.04±0.11^{a}	0.44±0.01^{b}
		Conv. 4	-	4.88±0.26^{a}	4.24±0.32^{a}	15.45±0.93^{a}	4.05±0.09^{a}	0.43±0.01^{b}
		Conv. 5	-	5.78±0.45^{a}	4.22±0.40^{a}	15.46±1.47^{a}	4.06±0.26^{a}	0.49±0.00^{a}
	3 days	Conv. 1	1.53±0.37^{b}	5.84±0.46^{b}	5.29±0.73^{a}	14.97±1.82^{a}	4.08±0.44^{a}	0.48±0.01^{a}
		Conv. 2	1.72±0.27^{b}	5.95±0.18^{a}	5.64±0.43^{a}	13.85±1.42^{a}	4.11±0.17^{a}	0.46±0.01^{b}
		Conv. 3	1.62±0.36^{b}	5.85±0.04^{ab}	5.10±0.75^{a}	14.01±1.73^{a}	4.11±0.20^{a}	0.46±0.01^{b}
		Conv. 4	2.86±1.18^{a}	6.10±0.31^{a}	4.95±0.80^{a}	11.98±1.55^{b}	4.17±0.23^{a}	0.43±0.01^{c}
		Conv. 5	3.12±1.52^{a}	6.19±0.10^{a}	4.72±0.64^{a}	11.26±2.03^{b}	4.16±0.31^{a}	0.45±0.01^{bc}
	5 days	Conv. 1	2.11±0.18^{b}	6.05±0.24^{a}	5.55±0.61^{a}	12.02±1.35^{a}	4.15±0.19^{a}	0.51±0.01^{a}
		Conv. 2	2.56±0.28^{b}	6.33±0.47^{a}	5.71±0.44^{a}	11.01±1.17^{a}	4.19±0.27^{a}	0.47±0.02^{ab}
		Conv. 3	2.44±1.22^{b}	6.27±0.07^{a}	5.37±0.21^{a}	12.31±1.15^{a}	4.16±0.22^{a}	0.47±0.02^{ab}
		Conv. 4	2.63±1.55^{b}	6.54±0.41^{a}	5.63±0.54^{a}	9.96±1.36^{b}	4.23±0.31^{a}	0.44±0.02^{b}
		Conv. 5	3.81±0.76^{a}	6.26±0.34^{a}	5.27±0.77^{a}	9.78±1.41^{b}	4.35±0.25^{a}	0.47±0.03^{ab}
	7 days	Conv. 1	3.12±0.85^{b}	6.37±0.32^{a}	5.72±0.59^{a}	11.17±1.22^{a}	4.28±0.18^{a}	0.52±0.02^{a}
		Conv. 2	4.40±1.24^{a}	6.66±0.28^{a}	5.94±0.63^{a}	11.05±1.16^{ab}	4.32±0.29^{a}	0.49±0.01^{ab}
		Conv. 3	2.82±1.22^{b}	6.26±0.19^{a}	5.62±0.31^{a}	12.11±1.37^{a}	4.27±0.28^{a}	0.47±0.02^{b}
		Conv. 4	3.31±1.48^{b}	6.56±0.16^{a}	6.31±0.45^{a}	9.08±0.83^{b}	4.37±0.41^{a}	0.47±0.02^{b}
		Conv. 5	5.18±1.88^{a}	6.97±0.24^{a}	5.55±0.37^{a}	9.14±1.26^{b}	4.40±0.34^{a}	0.50±0.02^{ab}
	10 days	Conv. 1	4.40±1.58^{b}	6.63±0.27^{a}	6.12±0.47^{a}	8.32±1.15^{a}	4.36±0.27^{a}	0.54±0.04^{a}
		Conv. 2	5.98±1.26^{a}	6.94±0.22^{a}	6.24±0.58^{a}	8.24±1.40^{a}	4.39±0.33^{a}	0.58±0.08^{a}
		Conv. 3	4.72±1.41^{b}	6.93±0.18^{a}	6.24±0.44^{a}	8.00±1.15^{a}	4.37±0.25^{a}	0.52±0.01^{a}
		Conv. 4	4.69±1.57^{b}	6.88±0.14^{a}	6.78±0.55^{a}	7.28±0.87^{b}	4.48±0.47^{a}	0.51±0.05^{a}
		Conv. 5	6.91±1.00^{a}	7.05±0.44^{a}	6.07±0.29^{a}	7.44±0.93^{b}	4.56±0.41^{a}	0.57±0.01^{a}

*Duncan test: Mean values in a column that do not share the same letter (a, b, c) indicate significant differences ($p \leq 0.05$).

Table 2. Characteristics of 'Prekos F1' hybrid tomato treated with organic phytosanitary products during storage: weight loss (%), dry weight (%), total soluble solids (TSS, °Brix), firmness (N), pH and ash content (%)

Storage temperature	Storage time (days)	Variant	Weight loss (%)	Dry weight. (%)	TSS (°Brix)	Firmness (N)	pH	Ash (%)
6°C	At harvest	Biol. 1	-	5.19±0.29^{a}	4.47±0.23^{a}	16.26±1.40^{a}	3.89±0.24^{a}	0.45±0.01^{a}
		Biol. 2	-	4.93±0.28^{a}	4.55±0.17^{a}	15.79±1.24^{a}	3.95±0.08^{a}	0.46±0.01^{a}
		Biol. 3	-	5.05±0.08^{a}	4.39±0.31^{a}	16.65±1.38^{a}	4.03±0.16^{a}	0.43±0.01^{a}
		Biol. 4	-	4.90±0.09^{a}	4.26±0.12^{a}	15.51±1.15^{a}	4.02±0.22^{a}	0.43±0.03^{a}
		Biol. 5	-	4.76±0.01^{a}	4.25±0.44^{a}	15.44±0.99^{a}	4.02±0.33^{a}	0.44±0.01^{a}
	3 days	Biol. 1	0.91±0.18^{a}	5.22±0.46^{a}	4.93±0.45^{a}	15.64±1.23^{a}	3.99±0.23^{a}	0.48±0.01^{a}
		Biol. 2	0.75±0.09^{a}	5.07±0.15^{a}	5.05±0.17^{a}	15.38±1.35^{a}	4.04±0.20^{a}	0.47±0.01^{a}
		Biol. 3	0.72±0.20^{a}	5.08±0.28^{a}	4.82±0.31^{a}	16.20±2.04^{a}	4.07±0.18^{a}	0.45±0.01^{b}
		Biol. 4	1.30±0.47^{a}	5.40±0.81^{a}	4.75±0.12^{a}	13.88±1.42^{b}	4.08±0.28^{a}	0.44±0.01^{b}
		Biol. 5	1.08±0.35^{a}	5.27±0.33^{a}	4.41±0.44^{a}	13.65±1.58^{b}	4.06±0.35^{a}	0.45±0.01^{b}
	5 days	Biol. 1	1.74±0.62^{ab}	5.47±0.32^{a}	5.43±0.31^{a}	14.50±1.77^{a}	4.07±0.31^{a}	0.50±0.01^{a}
		Biol. 2	1.69±0.51^{ab}	5.38±0.48^{a}	5.10±0.10^{a}	14.18±1.81^{a}	4.09±0.32^{a}	0.49±0.03^{a}
		Biol. 3	1.19±0.55^{b}	5.22±0.37^{a}	5.40±0.17^{a}	14.91±1.28^{a}	4.11±0.25^{a}	0.46±0.01^{b}
		Biol. 4	2.58±0.83^{a}	5.75±0.40^{a}	5.00±0.54^{a}	12.15±2.43^{b}	4.09±0.22^{a}	0.44±0.01^{b}
		Biol. 5	2.62±0.76^{a}	5.86±0.48^{a}	4.50±0.73^{a}	12.10±1.38^{b}	4.09±0.19^{a}	0.48±0.02^{a}
	7 days	Biol. 1	2.23±0.49^{b}	5.59±0.09^{b}	5.48±0.53^{a}	13.12±1.55^{a}	4.11±0.38^{a}	0.50±0.03^{ab}
		Biol. 2	2.25±0.50^{b}	5.60±0.25^{b}	5.46±0.19^{a}	12.62±1.17^{a}	4.14±0.26^{a}	0.50±0.01^{ab}
		Biol. 3	1.69±0.46^{c}	5.47±0.25^{b}	5.42±0.25^{a}	13.09±1.31^{a}	4.17±0.31^{a}	0.48±0.02^{b}
		Biol. 4	3.04±0.75^{a}	6.01±0.11^{a}	5.51±0.68^{a}	11.21±0.83^{b}	4.17±0.35^{a}	0.51±0.01^{a}
		Biol. 5	3.13±0.82^{a}	6.07±0.44^{a}	5.07±0.49^{a}	11.05±1.29^{b}	4.20±0.29^{a}	0.51±0.02^{a}
	10 days	Biol. 1	2.96±0.50^{b}	5.88±0.34^{b}	5.61±0.38^{a}	10.30±1.14^{a}	4.16±0.20^{a}	0.51±0.01^{b}
		Biol. 2	3.44±0.76^{b}	6.20±0.27^{a}	5.84±0.26^{a}	10.06±1.27^{a}	4.19±0.19^{a}	0.50±0.03^{b}
		Biol. 3	2.31±0.82^{c}	5.60±0.46^{b}	5.50±0.18^{a}	10.21±1.34^{a}	4.23±0.25^{a}	0.53±0.02^{a}
		Biol. 4	4.31±0.59^{a}	6.22±0.28^{a}	5.67±0.47^{a}	9.44±1.08^{b}	4.23±0.37^{a}	0.53±0.03^{a}
		Biol. 5	4.20±0.41^{a}	6.31±0.30^{a}	5.25±0.60^{a}	9.80±0.86^{b}	4.26±0.40^{a}	0.54±0.04^{a}
22°C	At harvest	Biol. 1	-	5.17±0.46^{a}	4.44±0.31^{a}	16.21±1.16^{a}	3.90±0.18^{a}	0.46±0.01^{a}
		Biol. 2	-	5.89±0.35^{a}	4.58±0.23^{a}	15.73±1.81^{a}	3.94±0.32^{a}	0.45±0.02^{a}
		Biol. 3	-	5.07±1.60^{a}	4.42±0.15^{a}	16.67±1.55^{a}	4.05±0.29^{a}	0.44±0.01^{b}
		Biol. 4	-	4.94±0.26^{a}	4.22±0.25^{a}	15.46±0.99^{b}	4.01±0.17^{a}	0.44±0.01^{b}
		Biol. 5	-	4.80±0.45^{a}	4.21±0.39^{a}	15.46±1.38^{b}	4.04±0.22^{a}	0.43±0.02^{b}
	3 days	Biol. 1	1.52±0.34^{b}	5.97±0.19^{a}	5.08±0.51^{a}	15.01±1.77^{a}	4.07±0.19^{a}	0.50±0.03^{a}
		Biol. 2	1.37±0.24^{b}	5.90±0.08^{a}	5.17±0.32^{a}	13.88±1.56^{a}	4.14±0.28^{a}	0.46±0.01^{b}
		Biol. 3	1.70±0.55^{b}	6.03±0.32^{a}	5.02±0.46^{a}	14.22±1.47^{a}	4.11±0.31^{a}	0.49±0.01^{a}
		Biol. 4	2.59±0.14^{a}	6.14±0.19^{a}	4.67±0.27^{a}	12.08±1.33^{b}	4.22±0.27^{a}	0.45±0.02^{b}
		Biol. 5	3.12±0.65^{a}	6.21±0.35^{a}	4.71±0.28^{a}	11.27±1.88^{b}	4.17±0.33^{a}	0.45±0.02^{b}
	5 days	Biol. 1	2.25±0.42^{b}	5.98±0.21^{a}	5.39±0.27^{a}	11.97±1.28^{a}	4.22±0.44^{a}	0.50±0.02^{a}
		Biol. 2	2.33±0.43^{b}	6.01±0.09^{a}	5.77±0.22^{a}	10.63±1.03^{a}	4.19±0.27^{a}	0.48±0.03^{b}
		Biol. 3	2.49±0.40^{b}	6.17±0.28^{a}	5.62±0.45^{a}	11.58±1.03^{a}	4.27±0.38^{a}	0.50±0.02^{a}
		Biol. 4	2.95±0.44^{ab}	6.21±0.31^{a}	5,02±0.50^{a}	9.68±1.21^{b}	4.24±0.25^{a}	0.47±0.02^{b}
		Biol. 5	3.82±0.92^{a}	6.24±0.31^{a}	5.30±0.44^{a}	9.77±1.29^{b}	4.33±0.37^{a}	0.47±0.02^{b}
	7 days	Biol. 1	2.83±0.97^{c}	6.05±0.38^{a}	5.56±0.33^{a}	10.20±1.51^{a}	4.34±0.29^{a}	0.51±0.01^{a}
		Biol. 2	4.27±1.12^{b}	6.38±0.44^{a}	5.92±0.18^{a}	10.47±1.17^{ab}	4.40±0.34^{a}	0.50±0.02^{a}
		Biol. 3	4.33±0.40^{b}	6.47±0.22^{a}	6.08±0.29^{a}	10.18±1.22^{a}	4.35±0.22^{a}	0.52±0.02^{a}
		Biol. 4	5.83±0.78^{a}	6.62±0.33^{a}	6.12±0.41^{a}	9.35±0.68^{b}	4.41±0.36^{a}	0.52±0.03^{a}
		Biol. 5	5.19±1.19^{a}	6.55±0.18^{a}	5.58±0.46^{a}	9.12±1.04^{b}	4.40±0.35^{a}	0.51±0.01^{a}
	10 days	Biol. 1	5.36±0.75^{b}	6.11±0.25^{b}	5.88±0.20^{a}	7.51±1.18^{a}	4.47±0.36^{a}	0.54±0.02^{b}
		Biol. 2	6.37±1.62^{a}	6.67±0.27^{a}	6.54±0.32^{a}	7.37±1.33^{a}	4.44±0.28^{a}	0.55±0.01^{b}
		Biol. 3	5.57±0.90^{ab}	6.53±0.22^{ab}	6.22±0.17^{a}	7.22±1.21^{a}	4.50±0.37^{a}	0.58±0.06^{a}
		Biol. 4	6.81±1.08^{a}	6.88±0.35^{a}	6.43±0.28^{a}	6.89±0.74^{b}	4.57±0.44^{a}	0.59±0.03^{a}
		Biol. 5	6.90±1.74^{a}	6.99±0.46^{a}	6.10±0.31^{a}	7.42±1.15^{b}	4.55±0.33^{a}	0.58±0.04^{a}

*Duncan test: Mean values in a column that do not share the same letter (a, b, c) indicate significant differences ($p \leq 0.05$).

After ten days, this value decreased to a range of 7.28 N (Dagonis, Switch) to 8.32 N (Ortiva Top). In tomatoes treated with organic phytosanitary products, the average firmness at harvest was between 15.51 N (Mimoten) and 16.65 N (Zytron). After ten days of storage at 22°C, fruit firmness ranged from 6.89 N (Mimoten) to 7.51 N (Cavaler 600SL), which is lower than that of the conventional variety. Significant differences in fruit firmness were observed after five days of storage at 6°C and after three days at 22°C due to the phytosanitary treatments applied pre-harvest. These results suggest that pre-harvest agricultural mana-

gement conditions can significantly influence tomato firmness during the post-harvest period. Paull et al. (1999) noted that during tomato fruit ripening, hemicelluloses and pectin become more soluble, leading to damage and weakening of the cell walls, which results in softer fruit. According to previous research, for tomatoes to maintain their commercial characteristics after the storage period, their firmness should not fall below 4-5 N (Kader, 2002).

Table 3. Comparison of the effects of phytosanitary protection methods on the quality of stored tomatoes at different temperatures

Storage temperature	Storage time (days)	Phytosanitary product	Weight loss (%)	Dry weight. (%)	TSS (% Brix)	Firmness (N)	pH	Ash (%)
6°C	At harvest	Conventional	-	5.27±0.63^{a}	4.29±0.02^{b}	15.80±0.96^{a}	4.01±0.03^{a}	0.44±0.01^{a}
		Biological	-	5.02±0.13^{a}	4.42±0.12^{a}	15.80±0.93^{a}	3.97±0.07^{a}	0.43±0.10^{a}
		Control	-	5.18±0.48^{a}	4.24±0.04^{b}	14.45±0.04^{b}	4.03±0.02^{a}	0.42±0.02^{a}
	3 days	Conventional	0.76±0.10^{b}	5.66±0.39^{a}	4.46±0.10^{b}	15.17±0.99^{a}	4.04±0.04^{a}	0.44±0.01^{a}
		Biological	0.92±0.27^{ab}	5.19±0.15^{a}	4.89±0.13^{a}	15.28±0.99^{a}	4.05±0.04^{a}	0.46±0.02^{a}
		Control	1.13±0.05^{a}	5.72±0.49^{a}	4.84±0.30^{a}	13.67±0.06^{b}	4.07±0.03^{a}	0.45±0.01^{a}
	5 days	Conventional	1.44±0.16^{b}	5.91±0.44^{ab}	4.62±0.03^{a}	13.89±1.31^{a}	4.09±0.08^{a}	0.46±0.02^{b}
		Biological	1.80±0.58^{b}	5.46±0.22^{b}	5.23±0.22^{a}	13.94±1.22^{a}	4.09±0.02^{a}	0.47±0.03^{a}
		Control	2.63±0.06^{a}	6.14±0.30^{a}	4.84±0.39^{a}	12.09±0.08^{b}	4.08±0.03^{a}	0.49±0.01^{a}
	7 days	Conventional	2.13±0.16^{b}	6.23±0.20^{b}	5.10±0.12^{b}	12.37±0.90^{a}	4.15±0. 02^{a}	0.48±0.02^{b}
		Biological	2.30±0.56^{b}	5.67±0.24^{a}	5.47±0.04^{a}	12.51±0.90^{a}	4.15±0.03^{a}	0.50±0.01^{a}
		Control	3.11±0.03^{a}	6.31±0.27^{a}	5.30±0.30^{ab}	11.03±0.12^{b}	4.20±0.06^{a}	0.51±0.01^{a}
	10 days	Conventional	3.27±0.23^{b}	6.50±0.16^{a}	5.37±0.08^{b}	10.23±0.37^{a}	4.20±0.02^{a}	0.51±0.04^{a}
		Biological	3.25±0.85^{b}	4.43±2.96^{a}	5.86±0.14^{a}	10.45±1.97^{a}	4.20±0.03^{a}	0.52±0.02^{a}
		Control	4.22±0.38^{a}	6.50±0.22^{a}	5.65±0.12^{a}	9.81±0.06^{b}	4.27±0.06^{a}	0.53±0.03^{a}
22°C	At harvest	Conventional	-	5.22±0.56^{a}	4.28v0.04^{b}	15.78±0.96^{a}	4.03±0.02^{ab}	0.44±0.01^{a}
		Biological	-	5.27±0.43^{a}	4.42±0.15^{a}	15.77±0.95^{a}	3.98±0.09^{b}	0.44±0.03^{a}
		Control	-	5.23±0.47^{a}	4.24±0.05^{b}	14.48±0.13^{b}	4.05±0.05^{a}	0.44±0.01^{a}
	3 days	Conventional	1.93±0.62^{b}	5.94±0.12^{b}	5.25±0.30^{a}	13.70±1.25^{a}	4.12±0.04^{b}	0.46±0.02^{a}
		Biological	1.80±0.55^{b}	6.01±0.10^{b}	4.99±0.22^{ab}	13.80±1.23^{a}	4.13±0.05^{b}	0.48±0.02^{a}
		Control	2.15±0.10^{a}	6.20±0.12^{a}	4.87±0.18^{b}	11.27±0.17^{b}	4.17±0.04^{a}	0.45±0.02^{a}
	5 days	Conventional	2.44±0.23^{b}	6.30±0.20^{b}	5.57±0.15^{ab}	11.33±1.07^{a}	4.17±0.05^{b}	0.47±0.03^{a}
		Biological	2.51±0.31^{b}	6.39±0.11^{ab}	5.45±0.33^{b}	10.97±1.02^{a}	4.23±0.03^{b}	0.49±0.02^{a}
		Control	3.80±0.06^{a}	6.43±0.24^{a}	5.82±0.18^{a}	9.78±0.18^{b}	4.34±0.04^{a}	0.47±0.01^{a}
	7 days	Conventional	3.41±0.69^{b}	6.36±0.18^{a}	5.90±0.31^{a}	10.85±1.27^{a}	4.31±0.05^{b}	0.48±0.02^{a}
		Biological	4.32±1.22^{ab}	6.48±0.24^{a}	5.92±0.26^{a}	10.05±0.49^{ab}	4.36±0.05^{ab}	0.51±0.01^{a}
		Control	5.16±0.07^{a}	6.63±0.17^{a}	6.12±0.31^{a}	9.13±0.09^{b}	4.40±0.07^{a}	0.51±0.02^{a}
	10 days	Conventional	4.95±0.70^{c}	6.55±0.15^{a}	6.35±0.30^{a}	7.96±0.47^{a}	4.40±0.05^{b}	0.54±0.03^{a}
		Biological	6.03±0.68^{b}	6.85±0.33^{b}	6.22±0.24^{a}	7.24±0.27^{b}	4.50±0.06^{a}	0.57±0.02^{a}
		Control	6.96±0.11^{a}	7.02±0.09^{a}	6.57±0.23^{a}	7.43±0.13^{b}	4.56±0.05^{a}	0.58±0.02^{a}
Total		Conventional	2.03±1.55^{c}	6.15±0.62^{a}	6.35±0.30^{a}	12.71±2.66^{a}	4.15±0.12^{b}	0.47±0.04^{a}
		Biological	2.29±1.88^{b}	6.06±0.51^{a}	6.21±0.24^{a}	12.28±1.48^{a}	4.17±0.17^{b}	0.47±0.09^{a}
		Control	3.02±1.11^{a}	6.30±0.86^{a}	6.57±0.23^{a}	11.31±2.26^{b}	4.21±0.17^{a}	0.48±0.08^{a}

*Duncan test: Mean values in a column that do not share the same letter (a, b, c) indicate significant differences ($p \leq 0.05$).

pH

Organic acids in fruits are derived from stored carbohydrates (Sakiyama & Stevens, 1976), although some may be transported from leaves and roots to the fruit (Davies & Maw, 1972). Tomatoes with high acid and sugar content are valued for their superior flavor, while those with low acidity are often considered tasteless (Etissa et al., 2014). The study did not reveal significant differences in the pH of fruits treated with various phytosanitary methods, whether organic or conventional. However, a slight trend towards an increase in organic acid content was noted, as indicated by lower pH values. The specialized literature mentions that phytosanitary treatments applied during tomato cultivation result in changes in cellular metabolism and variations in the production of secondary compounds, including acids (Kumar et al., 2021). The ripening process of fruits leads to a decrease in acidity, which is revealed by an increase in pH, a phenomenon that is more pronounced at ambient temperatures. Refrigeration helps maintain acidity. Previous studies suggest that fruit acidity decreases due to the utilization of acids as an energy source, a process through which they are converted into sugars (Karadeniz, 2004). Given that organic acids

serve as substrates for respiration in enzymatic reactions, it is expected that total acidity will be lower in the post-harvest period (Shokrollahfam et al., 2012, cited by Zeraatgar et al., 2018).

Ash content

The ash content of foods reflects the total raw minerals present. The 'Prekos F1' hybrid exhibited an ash content of 0.43-0.46% at harvest, which aligns with the range reported by Agbemafle et al. (2015). Notably, the ash concentration was not influenced by the phytosanitary treatments applied; rather, it increased proportionally with the weight loss of the fruit. Specialized studies confirm that mineral concentrations in fruits typically remain constant during storage, except in cases of water loss or metabolic activity (Garuba et al., 2018).

The analysis of Table 3 highlights significant differences between conventional and organic (Eco) phytosanitary protection methods compared to the control group (without treatment) in terms of preserving fruit quality during storage. The organic method maintains superior tomato quality for up to 10 days, but only at a low temperature of 6°C, resulting in lower weight losses. In contrast, the conventional method effectively preserves tomato quality at ambient temperatures of 22°C for the same duration.

Table 4 presents the relationships among the studied indicators. The results revealed strong linear correlations, both positive and negative, particularly between storage time and various quality indicators. Significant correlations were observed with weight loss (r=0.849), firmness (r=-0.826), fruit pH (r=0.766), dry weight content (r=0.505), and ash content (r=0.323).

Table 4. Correlation matrix among storage temperature (°C), storage time (days), phytosanitary product, weight loss (%), dry weight (%), total soluble solids (TSS, °Brix), firmness (N), pH, and ash content (%)

	Storage temperature	Storage time	Phytosanitary product	Weight loss (%)	Dry weight (%)	TSS (°Brix)	Firmness (N)	pH	Ash (%)
Storage temperature	1								
Storage time	0.000	1							
Phytosanitary product	0.000	0.000	1						
Weight loss (%)	0.346(**)	0.849(**)	0.217(**)	1					
Dry weight (%)	0.295(**)	0.505(**)	0.083	0.634(**)	1				
TSS (°Brix)	0.159	0.114	0.089	0.118	0.184(*)	1			
Firmness (N)	-0.289(**)	-0.826(**)	-0.212(*)	-0.874(**)	-0.538(**)	-0.173(*)	1		
pH	0.464(**)	0.766(**)	0.172(*)	0.928(**)	0.605(**)	0.156	-0.847(**)	1	
Ash (%)	0.164	0.323(**)	0.032	0.601(**)	0.382(**)	0.104	-0.551(**)	0.545(**)	1

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.01 level (2-tailed).

Additionally, a significant linear interdependence was found between storage temperature and the following indicators: weight loss (r=0.346), dry weight content (r=0.295), firmness (r=-0.289), and pH (r=0.464). Notably, significant correlations were also identified between the phytosanitary products applied during the growing season and weight loss (r=0.346), dry weight content (r=0.295), firmness (r=-0.289), and pH (r=0.464). These data suggest that the weight loss, firmness, and pH of fruits during storage may be influenced by the phytosanitary protection practices used during the growing season. Furthermore, significant positive linear relationships were observed between weight loss and dry weight (r=0.634), pH (r=0.928), and ash content (r=0.601). In contrast, the relationship between firmness and weight loss was negative, exhibiting a high intensity (r=-0.874). This is expected, as the turgor of fruits is determined by the water content of the plants; thus, water loss leads to softening of the fruits and a subsequent reduction in commercial quality. Additionally, total soluble solids (TSS) increase as firmness decreases (r=0.173). A significant negative linear relationship was also noted between tomato firmness and ash content (r=-0.551), with the latter increasing due to water loss. Moreover, a decrease in firmness was observed

as fruit pH increased, indicating a reduction in organic acid levels within the fruit. This reduction is typical, as organic acids play a crucial role in the post-harvest respiration processes of fruit.

CONCLUSIONS

1. Conventional products (Ortiva Top 0.1%, Cidely Top 0.1%, Amistar 0.1% and Dagonis 0.08%) ensure over 90% efficacy in controlling the pathogens *Alternaria solani*, *Fulvia fulva* and *Botrytis cinerea*, in the tomato crop in greenhouses.
2. Biological products (Cavaler 600SL 3 l/ha, Amulet 4 l/ha, Zytron 1.5 l/ha and Mimoten 3 l/ha) have an efficacy of between 43 and 67% in the control of the pathogens *Alternaria solani, Fulvia fulva* and *Botrytis cinerea*, as a result their use is recommended in case of a low attack or preventive when conditions are favorable for the attack.
3. Phytosanitary treatments applied on the vegetation period significantly influence the firmness and quality of tomatoes during storage. Refrigeration plays a crucial role in preserving these qualities. For organically treated tomatoes, these attributes were superior to those of conventional tomatoes, but only under refrigeration after a storage period of 10 days.
4. Higher temperatures tend to accelerate the deterioration of product quality. Tomatoes treated with conventional phytosanitary products show greater resistance to high temperatures during storage compared to those treated with organic products. Among the conventional treatments, Ortiva Top and Amistar ensured the best results. In the organic category, Cavaler 600SL, Amulet, and Zytron proved to be the most effective.
5. A thorough analysis of the long-term effects of phytosanitary treatments on the nutritional and organoleptic quality of tomatoes is necessary.

ACKNOWLEDGEMENTS

This research work was carried out with the support of the Ministry of Agriculture and Rural Development, financed by Project ADER 6.3.15/2023 – Integrated management for the control of pest agents in the main vegetable species grown in greenhouses in conventional and organic systems.

REFERENCES

Agbemafle, R., Owusu-Sekyere, J. D., & Bart-Plange, A. (2015). Effect of deficit irrigation and storage on the nutritional composition of tomato (*Lycopersicon esculentum* Mill. cv. Pectomech). *Hrvatski časopis za prehrambenu tehnologiju, biotehnologiju i nutricionizam, 10*(1-2), 59-65.

Albornoz, K., Cantwell, M. I., Zhang, L., & Beckles, D. M. (2019). Integrative analysis of postharvest chilling injury in cherry tomato fruit reveals contrapuntal spatio-temporal responses to ripening and cold stress. *Scientific reports*, *9*(1), 2795.

Al-Dairi, M., Pathare, P. B., & Al-Yahyai, R. (2021). Chemical and nutritional quality changes of tomato during postharvest transportation and storage. *Journal of the Saudi Society of Agricultural Sciences*, *20*(6), 401-408.

Alia-Tejacal, I., Villanueva-Arce, R., Pelayo-Zaldívar, C., Colinas-León, M. T., López-Martínez, V., & Bautista-Baños, S. (2007). Postharvest physiology and technology of sapote mamey fruit (*Pouteria sapota* (Jacq.) HE Moore & Stearn). *Postharvest Biology and Technology*, *45*(3), 285-297.

Amaro Amanda Cristina Esteves, Anamaria Ribeiro Pereira Ramos, Ana Claudia Macedo, Elizabeth Orika Ono, João Domingos Rodrigues (2018) Effects of the fungicides azoxystrobin, pyraclostrobin and boscalid on the physiology of *Japanese cucumber*. *Scientia Horticulturae*, *Volume 228*, 26 January 2018, Pages 66-75.

Anand, T., Chandrasekaran, A., Kuttalam, S. and Samiyappan, R. (2010) Evaluation of azoxystrobin (Amistar 25 SC) against early leaf blight and leaf spot diseases of tomato. *Journal of Agricultural Technology*, *Vol. 6*(3): 469-485

Anyasi, T. A., Aworh, C. O., & Jideani, A. I. (2016). Effect of packaging and chemical treatment on storage life and physicochemical attributes of tomato (*Lycopersicon esculentum* Mill cv. Roma). *African Journal of Biotechnology*, *15*(35), 1913-1919.

Bartlett, D.W., J.M. Clough, J.R. Godwin, A.A. Hall, M. Hamer and B. Parr- Dobrzanski (2002). The strobilurin fungicides. *Pest Management Science*, 58: 649-662.

Ben-Yehoshua, S., & Rodov, V. (2002). Transpiration and water stress. In *Postharvest Physiology and Pathology of Vegetables* (pp. 143-197). CRC Press.

Ben-Yehoshua, S., Shapiro, B., Chen, Z. E., & Lurie, S. (1983). Mode of action of plastic film in extending life of lemon and bell pepper fruits by alleviation of water stress. *Plant Physiology, 73*(1), 87-93.

Bertin, N. (2018). Fruit quality. In *Tomatoes* (pp. 137-179). Wallingford UK: CABI.

Bourne, M. C. (2006). Selection and use of postharvest technologies as a component of the food chain. *Journal of food science*, *69*(2), 43-46.

Bratu, E., Petcuci, A. M., & Sovarel, G. (2015). Efficacy of the product Spinosad an insecticide used in the control of tomato leafminer (*Tuta absoluta*-Meyrick, 1917), *Bulletin of the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Horticulture, Vol. 72,* No. 1: 209-210.

Bremmer, J., Gonzalez-Martinez, A., Jongeneel, R., Huiting, H., Stokkers, R., & Ruijs, M. (2021). Impact assessment of EC 2030 Green Deal Targets for sustainable crop production (No. 2021-150). Wageningen Economic Research.

Caleb, O. J., Mahajan, P. V., Opara, U. L., & Witthuhn, C. R. (2012). Modelling the respiration rates of pomegranate fruit and arils. *Postharvest Biology and Technology, 64*(1), 49-54.

Călin Maria, T.O. Cristea, P.M. Brezeanu, S. Ambarus, C. Brezeanu, S.P. Muscalu, F. Sova, C.A. Antal, M. Prisecaru, M. Costache, G. Sovarel, L. Bratu, F. Burnichi, G. Sbîrciog (2020). Biological control of pepper pests in organic agriculture – Proc. IHC 2018 *Symposium 28 – II Int. Symp. on Innovative Plant Protection in Horticulture, Turcia, Acta Hortic.1269*.22, pag. 161-168.

Călin, M., Cristea, T. O., Ambarus, S., Brezeanu, C., Marian, P., Brezeanu, M. C., Costache M., Șovărel G. & Bratu, E. (2016). The study on the effectiveness of amblyseius swirskii at.-h predator in control of onion thrips thrips tabaci in pepper crops. *Romanian Journal for Plant Protection*, *9*: 35-39.

Călin, M., Cristea, T. O., Ambăruș, S., Brezeanu, C., Brezeanu, P. M., Costache, M., Șovărel G. & Bratu, L. (2017). Biological control of two-spotted spider mite in pepper and melon crops cultivated in tunnels. *Scientific Papers. Series B, Horticulture. Vol. LXI*: 347-350.

Cenușă, A. E., Șovărel, G., Costache, M., Bratu, E., & Velea, M. (2016). Effect of some "bio-insecticides" used against two spotted spider mites (*Tetranychus urticae* Koch.) in the cucumber crop under plastic tunnel conditions. *Scientific Papers. Series B, Horticulture. Vol. LX*: 139-143.

Chaïb, J., Devaux, M. F., Grotte, M. G., Robini, K., Causse, M., Lahaye, M., & Marty, I. (2007). Physiological relationships among physical, sensory, and morphological attributes of texture in tomato fruits. *Journal of Experimental Botany*, *58*(8), 1915-1925.

Chira A., (1999) Some aspects concerning influence of post-harvest techniques on the storage ability of some vegetable species. Valorization of Vegetable Products, *ICDLF Annals, XVI*: 213-217.

Colantonio, V., Ferrão, L. F. V., Tieman, D. M., Bliznyuk, N., Sims, C., Klee, H. J., Klee, P. M. & Resende Jr, M. F. (2022). Metabolomic selection for enhanced fruit flavor. *Proceedings of the National Academy of Sciences*, *119*(7), e2115865119.

Davies, J. N., & Maw, G. A. (1972). Metabolism of citric and malic acids during ripening of tomato fruit. *Journal of the Science of Food and Agriculture, 23*(8), 969-976.

Davies, J. N., Hobson, G. E., & McGlasson, W. B. (1981). The constituents of tomato fruit - the influence of environment, nutrition, and genotype. Critical Reviews in *Food Science & Nutrition, 15*(3), 205-280.

Ebert, A. W. (2020). The role of vegetable genetic resources in nutrition security and vegetable breeding. *Plants*, *9*(6), 736.

Elkoly, Ramadan & El-Samadesy, A & Helalia, A & Elballat, Ehab. (2021). Efficacy of certain chemical Fungicides and Biofungicides on early blight disease in tomato under field conditions. *Al-Azhar Journal of Agricultural Research*, 46, 20-82. 10.21608/ajar.2021.245632.

Etissa, E., Dechassa, N., Alamirew, T., Alemayehu, Y., & Dessalegne, L. (2014). Response of fruit quality of tomato grown under varying inorganic N and P fertilizer rates under furrow irrigated and rainfed production conditions. *Int. J. Dev. Sustain, 3*(2), 371-387.

Fenech, M., Amaya, I., Valpuesta, V., & Botella, M. A. (2019). Vitamin C content in fruits: Biosynthesis and regulation. *Frontiers in plant science*, *9*, 2006, 10.3389/fpls.2018.02006.

Garuba, T., Mustapha, O. T., & Oyeyiola, G. P. (2018). Shelf life and proximate composition of tomato (*Solanum lycopersicum* L.) fruits as influenced by storage methods. *Ceylon Journal of Science*, *47*(4).

Hatami M., Kalantari S., Delshad M., (2013). Responses of different maturity stages of tomato fruit to different storage conditions. *Acta Horticulturae, 101*: 857-864.

Hewitt, H.G. (1998). Strobilurins. Fungicides in Crop Protection. CAB International, New York, pp. 128-129.

Hina Zafar and S. Shahid Shaukat (2018) Evaluation of some fungicides for the control of early blight disease (*Alternaria solani*) of tomato. *Int. J. Biol. Biotech.*, *15*(1): 129-140.

Hogea, S. Ș. (2020). *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae)–biology, ecology, prevention and control measures and means in greenhouse tomato crops. A review. *Current Trends in Natural Sciences, 9*(17), 222-231.

Horwitz W. (2000). Official methods of analysis of AOAC International (17thed). *Association of Official Analytical Chemists*, Gaithersburg, MD, USA

Iosob, G. A., & Cristea, T. O. (2022). The study of *Perillus bioculatus* F. (Heteroptera, Pentatomidae) population as a potential key factor in the development of biological management strategies for *Leptinotarsa decemlineata* Say at *Solanum melongena* L. *Romanian Journal of Horticulture*, *3*, 91-98.

Iosob, G. A., Cristea, T. O., & Bute, A. (2023). Future perspectives for pest and disease management in brassica: a review. *Scientific Studies & Research. Series Biology/Studii si Cercetari Stiintifice. Seria Biologie*, *32*(1).

Islam, M. P., Morimoto, T., & Hatou, K. (2012). Storage behavior of tomato inside a zero energy cool chamber. Agricultural Engineering International: *CIGR Journal, 14*(4), 209-217.

Kabir, M. S. N., Ali, M., Lee, W. H., Cho, S. I., & Chung, S. O. (2020). Physicochemical quality changes in tomatoes during delayed cooling and storage in a controlled chamber. *Agriculture*, *10*(6), 196.

Kader A. A. (2002). Methods of gas mixing, sampling and analysis. *Postharvest technology of horticultural crops, 3*, 145-148.

Kader, A. A. (1985). Effects of postharvest handling procedures on tomato quality. In *Symposium on Tomato Production on Arid Land 190* (pp. 209-222).

Karadeniz, F. (2004). Distribuția principală a acidului organic a sucurilor de citrice autentice în Turcia. *Revista turcă de agricultură și silvicultură*, *28*(4), 267-271.

Krełowska-Kułas M. (1993). .Badanie jakości produktów spożywczych. *PWE, Warszawa* [In Polish].

Kumar, S., Singh, T. B., Agnihotri, R. K., & Chaturvedi, P. (2021). Comparative effect of NaCl and PEG on physiological and biochemical attributes during vegetative stage of tomato. Ind. *J. Plant Physiol, 4*, 14-23.

Lufu, R., Ambaw, A., & Opara, U. L. (2020). Water loss of fresh fruit: Influencing pre-harvest, harvest and postharvest factors. *Scientia Horticulturae, 272*, 109519.

Mejia Arreaza, J. and Hernandez, M.M. (2001). Evaluation of azoxystrobin on the early blight control (*Alternaria solani*) in tomatoes. *Revista de la faculated de Agronomia, Universidad del zulia*, *18*(2): 106-116.

Moneruzzaman, K. M., Hossain, A. B. M. S., Sani, W., & Saifuddin, M. (2008). Effect of stages of maturity and ripening conditions on the physical characteristics of tomato. *American Journal of Biochemistry and Biotechnology*, *4*(4), 329-335.

Moneruzzaman, K. M., Hossain, A. B. M. S., Sani, W., & Saifuddin, M. (2009). Effect of stages of maturity and ripening conditions on the biochemical characteristics of tomato. *American Journal of Biochemistry and Biotechnology*, *4*(4), 336-344.

Morgan, L. (2021). Greenhouse produce quality and assessment. In *Hydroponics and protected cultivation: a practical guide* (pp. 246-267). Wallingford UK: CABI.

Mrema, C.G., & Rolle, S.R. (2002). Status of the postharvest sector and its contribution to agricultural development and economic growth. *9th JIRCAS International Symposium - Value Addition to Agricultural Product*, 13-20.

Owais Iqbal, Chengyun Li, Nasir Ahmed Rajput, Abdul Mubeen Lodhi (2023) Management of Phytopathogens by Antagonistic *Bacillus* spp. in Tomato Crop DOI: http://dx.doi.org/ 10.5772/ intechopen.112439

Paull, R. E., Gross, K., & Qiu, Y. (1999). Changes in papaya cell walls during fruit ripening. *Postharvest Biology and Technology, 16*(1), 79-89.

Pasche, J.S., C.M. Wharam and N.C. Gudmestad (2004). Shift insensitivity of Alternaria solani in response to QoI fungicides. *Plant Disease*, 88: 181-187

Pânzaru, R. L., Firoiu, D., Ionescu, G. H., Ciobanu, A., Medelete, D. M., & Pîrvu, R. (2023). Organic Agriculture in the Context of 2030 Agenda Implementation in European Union Countries. *Sustainability*, *15*(13), 10582.)

Rab, A., Rehman, H., Haq, I., Sajid, M., Nawab, K., & Ali, K. (2013). Harvest stages and pre-cooling influence the quality and storage life of tomato fruit. *The Journal of Animal & Plant Sciences, 23*(5): 1347-1352

Sakiyama, R., & Stevens, M. A. (1976). Organic acid accumulation in attached and detached tomato fruits. *Journal of American Society Horticulture Science 101*, 394-396.

Șovărel G., Scurtu I., Hogea S., Sbirciog G. (2024) Biological and conventional control of *Sphaerotheca fuliginea* pathogen on zucchini crops. *Scientific Papers. Series B, Horticulture, Vol. LXVIII*, Issue 2, Print ISSN 2285-5653, 588-593;

Șovărel G., Scurtu I., Hogea S., Sbirciog G. (2024) Biological control of main diseases and pests on melon crops in the field. *Scientific Papers. Series B, Horticulture, Vol. LXVIII,* Issue 1, Print ISSN 2285-5653, 521-526; WOS:001368682800066, JCR - Q4, IF =0,4;

Tefera, A., Seyoum, T., & Woldetsadik, K. (2007). Effect of disinfection, packaging, and storage environment on the shelf life of mango. *Biosystems Engineering*, *96*(2), 201-212.

Thole, V., Vain, P., Yang, R. Y., Almeida Barros da Silva, J., Enfissi, E. M., Nogueira, M. & Martin, C. (2020). Analysis of tomato post-harvest properties: Fruit color, shelf life, and fungal susceptibility. *Current protocols in plant biology*, *5*(2), e20108.

Tolasa, M., Gedamu, F., & Woldetsadik, K. (2021). Impacts of harvesting stages and pre-storage treatments on shelf life and quality of tomato (*Solanum lycopersicum* L.). *Cogent food & agriculture*, *7*(1), 1863620.

Tyagi, S., Sahay, S., Imran, M., Rashmi, K., & Mahesh, S. S. (2017). Pre-harvest factors influencing the postharvest quality of fruits: A review. Curr. *J. Appl. Sci. Technol, 23*(1), 12.

Vunnam, R., Hussain, A., Nair, G., Bandla, R., Gariepy, Y., Donnelly, D. J. & Raghavan, G. S. V. (2014). Physico-chemical changes in tomato with modified atmosphere storage and UV treatment. *Journal of food science and technology,* 51, 2106-2112.

Wang, S., Qiang, Q., Xiang, L., Fernie, A. R., & Yang, J. (2023). Targeted approaches to improve tomato fruit taste. *Horticulture Research*, *10*(1), uhac229.

Zeraatgar H., Davarynejad G. H., Moradinezhad F. & Abedi B. (2018). Effect of salicylic acid and calcium nitrate spraying on qualitative properties and storability of fresh jujube fruit (*Ziziphus jujube* Mill.). *Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 46*(1), 138-147.

Zoia Arshad Awan, Amna Shoaib (2019) Combating early blight infection by employing Bacillus subtilis in combination with plant fertilizers, *Current Plant Biology, Volume 20*, 100125.

Zushi, K., & Higashijima, M. (2022). Correlation network analysis identified the key interactions of texture profiles with the sensory, physical, and organoleptic properties of cherry tomato cultivars grown under salt stress. *Scientia Horticulturae*, *293*, 110754.

PN-90/A-75101/04. 1990. Przetwory owocowe i warzywne. Przygotowanie próbek i metody badań fizykochemicznych. Oznaczanie zawartości suchej masy metodą wagową [Fruit products and vegetable. The preparation of samples and method of physico-chemist investigations. Marking the content of dry mass with weight method]. PKN, MiJ Warszawa

FAOSTAT, Value of Agricultural Production, https://www.fao.org/faostat/en/#data/QV, accessed January 2025.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

ALLIUM CEPA SEEDLINGS RESPONSE TO STRESS GENERATED BY CHEMICALS

Andreea Adriana PETCOV[1], Florin SALA[1, 2]

[1]University of Life Sciences "King Mihai I" from Timişoara, 119 Calea Aradului Street, 300645, Timişoara, Romania
[2]Agricultural Research and Development Station Lovrin, 200 Principală Street, 307250, Lovrin, Timiş County, Romania

Corresponding author email: florin_sala@usvt.ro

Abstract

This research analyzed the response induced by certain chemicals in Allium cepa seedlings under laboratory conditions, University of Life Sciences "King Mihai I" from Timişoara. Onion bulbs with roots were treated with water (V1), copper sulfate ($CuSO_4$ 300 mg L^{-1} – V2), Faster insecticide (0.3 ml L^{-1} – V3, 0.4 ml L^{-1} – V4, 0.5 ml L^{-1} – V5) and Karate zeon insecticide (0.15 mg L^{-1} – V6; 0.20 mg L^{-1} – V7; 0.25 mg L^{-1} – V8). Morphological parameters (root length, RL; leaf number, LN; leaf length, LL) were determined. The results of the experimental trials presented statistical safety ($p<0.001$). For RL morphological parameter, significant differences from the mean ($\overline{RL}$ = 4.62 cm) were shown by the variants V1 (ΔRL = 4.09 cm; $p<0.01$), and variant V2 (ΔRL = -3.32 cm; $p<0.01$). In the case of the LN parameter, significant differences compared to the mean ($\overline{LN}$ = 0.99) were presented by variant V1 (ΔLN = 0.91, $p<0.001$), and variants V2 and V3 (ΔLN = -0.55, ΔLN = -0.50, $p<0.05$). For LL morphological parameter, compared to the mean ($\overline{LL}$ = 4.41), significant differences were presented by variant V1 (ΔLL = 6.07, $p<0.001$), and variants V2 and V3 (ΔLL = -3.39, ΔLL = -2.77, $p<0.05$).

Key words: *Allium cepa, pesticides, morphological indices, root system, seedlings, stress response.*

INTRODUCTION

Vegetables are important in human nutrition due to their fiber, nutrient content and a wide range of active principles (Bassey et al., 2021; Barbu et al., 2022; Drăghici et al., 2023).

The root system of vegetable plants is diverse in typology, in relation to the plant species and specific influencing factors, and is important for the relationship of plants with the soil, or growing substrate, for the absorption of water and nutrients (Nziengui & Ditengou, 2023).

Vegetable plants develop specific activities (enzymatic, microbiological, ion exchange, etc.) at the level of the root system (root hairs, rhizosphere), in relation to the plant species and the culture system (Mladenova et al., 2024).

The use of chemicals (e.g. pesticides) in horticultural ecosystems has utility for plant protection, according to the cropping system, but has also been associated with various negative effects on the environment and, sometimes, on plants and the quality of plant products (Ahmadi et al., 2024). Pesticides are inputs that indirectly influence the yield of horticultural crops, through plant protection (Wan et al., 2023).

The use of pesticides was analyzed in relation to the category of substances and the share used, in relation to the crops (vegetables), horticultural systems, farmer training, horticultural practices and technologies, protection and control aspects (weeds, diseases, pests) as well as from an economic perspective (Ngowi et al., 2007; Wan et al., 2023).

The relationship of horticultural plants with different chemical substances (contaminants) was analyzed in different plant organs (Li, 2022). The author reported different patterns of presence of the evaluated chemical elements, at the root level and at the leaf level. Reductions in plant root growth have been recorded in conditions of cadmium presence in the soil, in the rhizosphere area (Lux et al., 2011). Experimental supplementation of some mineral elements such as cobalt (Co), copper (Cu) and

zinc (Zn) led to their differentiated accumulation in the experimentally tested vegetable plants (Zakharova et al., 2023).

Among vegetables, onion is a plant of great importance worldwide (Bettoni et al., 2014; Cho et al., 2024). The onion root has a specific structure, with the role of fixing the plant, providing water and nutrients, and has a variable development in relation to the substrate and different influencing factors (Bailey et al., 2002). Morphological and genetic parameters at the root level in Allium plants were analyzed to evaluate the response of plants to different factors (Michalska-Kacymirow et al., 2014; Karasinski et al., 2017; Pokluda et al., 2023; Solouki et al., 2023; Maity et al., 2024). Self-defense mechanisms have been identified in onions in relation to biotic stress (Cho et al., 2024).

This study evaluated, in laboratory conditions, the response of *Allium cepa* plants to different chemical substances, based on morphological parameters of the root and leaf system.

MATERIALS AND METHODS

The research was organized and conducted under laboratory conditions, within the Genetics discipline, Universsity of Life Sciences "King Mihai I" from Timișoara, 2023. Onion bulbs, *Allium cepa* L., were placed in Petri dishes for vegetation, in distilled water. At the beginning of the experiment (T0, date February 21, 2023) a humid environment was provided with distilled water, in equal amounts. A control variant, with distilled water, was maintained (V1), for comparisons during the experiment. On February 24 (T1), observations were made on roots and coleoptiles. After the first observations, different chemicals were added, which differentiated the experimental variants; distilled water (V1), copper sulfate - $CuSO_4$ – 300 mg L^{-1} – V2), Faster insecticide (0.3 ml L^{-1} – V3, 0.4 ml L^{-1} – V4, 0.5 ml L^{-1} – V5) and Karate zeon insecticide (0.15 mg L^{-1} – V6; 0.20 mg L^{-1} – V7; 0.25 mg L^{-1} – V8). Morphological parameters (root length, RL; leaf number, LN; leaf length, LL) were determined. The determinations were made at five times (T2-T6), until March 12, 2023.

The values of the morphological parameters were recorded, and the experimental data set was analyzed by general statistical analysis, as well as by correlation, regression, multivariate analysis (Hammer et al., 2001; JASP, 2022), in order to quantify the response of the plants in the growth conditions generated by the chemical substances supplemented.

RESULTS AND DISCUSSIONS

Under the influence of the chemicals added to the growth vessels, morphological parameters of onion plants evolved differently. Starting from the moment T2, differentiations between variants were recorded for each monitored parameter (Table 1).

Table 1. Parameter values in *Allium cepa* in relation to experimental variants

Parameters	MU	V1	V2	V3	V4	V5	V6	V7	V8	±SE
BW	(g)	5.51	5.52	5.66	6.21	4.99	4.84	5.24	5.77	±0.16
BD	(mm)	22.61	22.55	22.54	22.91	22.05	21.39	21.94	22.50	±0.17
BH	(mm)	28.46	26.17	25.61	28.17	25.84	23.36	23.92	23.72	±0.69
RNT1	(no)	18.90	18.70	18.30	22.20	18.30	20.20	17.50	16.30	±0.63
RLT1	(cm)	1.41	1.30	1.08	1.48	1.38	1.51	1.28	1.05	±0.06
CLT1	(cm)	0.00	0.00	0.00	0.15	0.00	0.21	0.00	0.00	±0.03
RLT2	(cm)	4.34	1.35	1.10	1.49	1.47	2.08	1.81	1.64	±0.36
LLT2	(cm)	3.19	0.00	0.00	0.53	0.03	0.49	0.54	0.21	±0.38
LNT2	(no)	1.60	0.00	0.00	0.40	0.00	0.60	0.40	0.00	±0.19
RLT3	(cm)	7.96	1.48	1.28	2.08	5.29	4.50	2.64	4.80	±0.81
LLT3	(cm)	8.60	0.45	0.56	1.54	3.49	2.85	3.20	3.68	±0.91
LNT3	(no)	2.50	0.40	0.20	1.00	0.90	1.30	1.40	1.20	±0.25
RLT4	(cm)	7.96	1.48	1.28	2.08	5.29	4.50	2.64	4.80	±0.81
LLT4	(cm)	14.21	0.93	1.69	3.86	4.25	5.66	5.54	5.15	±1.43
LNT4	(no)	2.90	0.60	0.80	1.30	1.10	1.70	1.50	1.40	±0.25
RLT5	(cm)	16.68	1.66	5.18	4.64	8.30	12.40	11.62	9.28	±1.72
LLT5	(cm)	19.66	2.07	3.45	6.89	9.02	14.48	9.16	6.93	±2.03
LNT5	(no)	2.90	1.00	1.20	1.80	1.90	2.80	1.80	1.40	±0.24
RLT6	(cm)	18.49	1.70	7.68	7.16	9.97	14.09	13.81	10.80	±1.82
LLT6	(cm)	27.68	3.72	5.80	10.38	14.67	20.44	13.25	8.18	±2.81
LNT6	(no)	3.40	1.10	1.20	2.30	2.90	3.00	2.10	1.40	±0.31

Lower values of the parameters were recorded in variant V2, which indicates a higher level of stress in the case of this variant. The values of the parameters RL, LL, and LN, on the experimental variants, are shown in Table 1. The results reliability, as well as the presence of variance, were confirmed based on ANOVA Test (Table 2).

Different correlation levels were observed between *Allium* bulb parameters, as well as at the level of morphological parameters at time T1 (Figure 1). In contrast, positive correlations were recorded at the level of plant morphological parameters over the observation interval T2-T6 (Figure 1).

Table 2. Results from the ANOVA Test

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	8988.22	20	449.411	49.201	6.66E-55	1.6424
Within Groups	1342.73	147	9.134			
Total	10330.95	167				

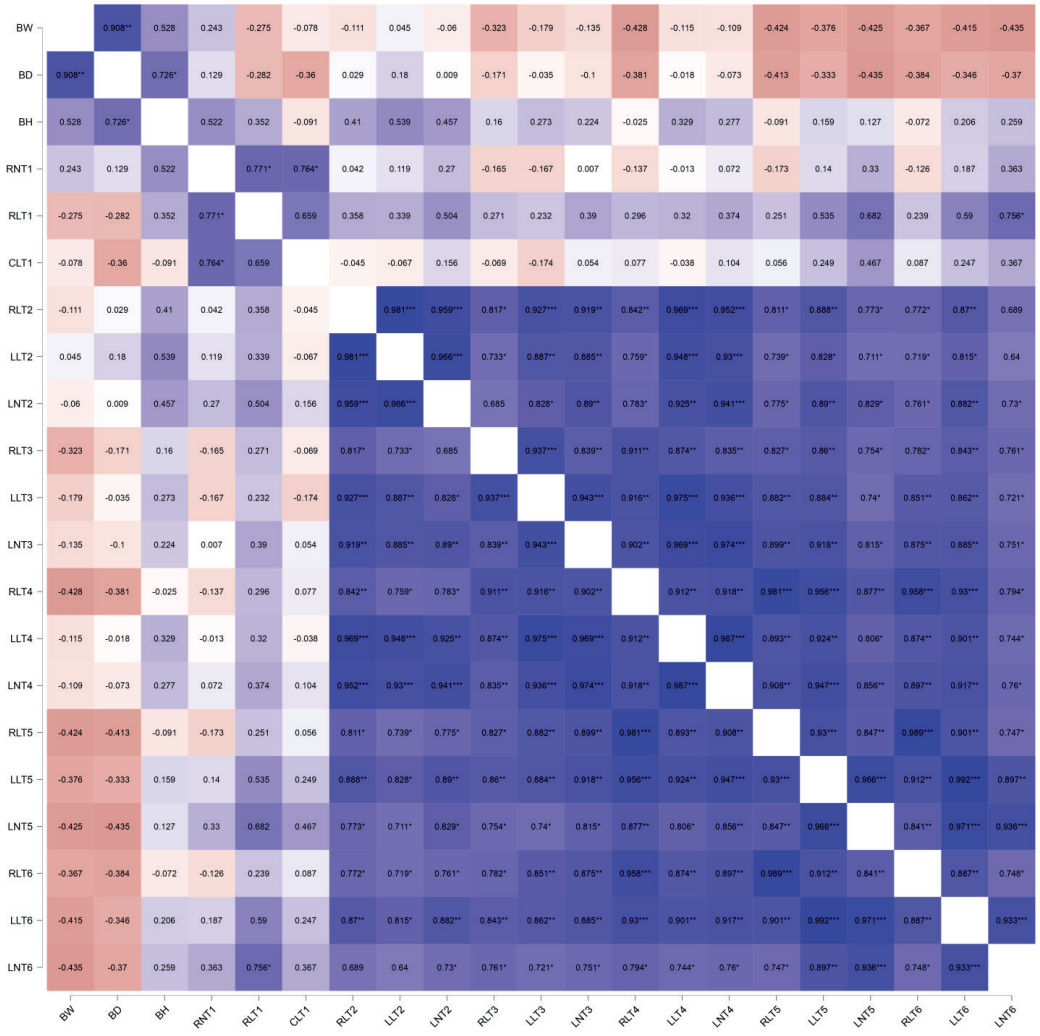


Figure 1. Correlations at the level of morphological parameters, *Allium cepa*

The control variant (V1) benefited from normal plant growth conditions, without chemical treatments. In this variant, high values of the parameters were recorded at the evaluation times, T1-T6. In the case of the RL parameter, the value $\overline{RL}$ =4.62 cm resulted, as the average of the experimental variants (Table 3). Compared to $\overline{RL}$, variant V1 presented positive differences, and variant V2 negative differences ($p<0.01$). In the case of the LL parameter, the value $\overline{LL}$ =4.41 resulted, as the average of the experimental variants. Compared to $\overline{LL}$, the control variant (V1) presented positive differences ($p<0.001$), and the variants V2 and V3 negative differences ($p<0.05$), Table 3. In the case of the LN parameter, the result was $\overline{LN}$ =0.99, as the average value of the experimental variants. Compared to $\overline{LN}$, the control variant (V1) presented positive differences ($p<0.001$), and the variants V2 and V3 presented negative differences ($p<0.05$), (Table 3).

Table 3. Significance of differences in analyzed parameters

Variant	Parameters					
	RL	Diff	LL	Diff	LN	Diff
V1	8.71	4.09^{**}	10.48	6.07^{***}	1.90	0.91^{***}
V2	1.30	-3.32^{oo}	1.02	-3.39^{o}	0.44	-0.55^{o}
V3	2.81	-1.81^{ns}	1.64	-2.77^{o}	0.49	-0.50^{o}
V4	2.89	-1.73^{ns}	3.34	-1.07^{ns}	0.97	-0.02^{ns}
V5	4.71	0.09^{ns}	4.49	0.08^{ns}	0.97	-0.02^{ns}
V6	6.22	1.60^{ns}	6.30	1.89^{ns}	1.34	0.35^{ns}
V7	5.41	0.79^{ns}	4.53	0.12^{ns}	1.03	0.04^{ns}
V8	4.91	0.29^{ns}	3.45	-0.96^{ns}	0.77	-0.22^{ns}

Under the influence of the applied chemicals, the variation of Allium plant parameters was described by polynomial models, with variable distribution in relation to the time factor (T, days).

The average growth rate ($\overline{AGR}$) was calculated for each parameter and experimental variant (Table 4). The $\overline{AGR}$ values for variants V2 to V8, under stress conditions generated by the chemicals used, were below the level of the control variant (V1).

The root system ensures the supply of water and nutrients to plants, and the reduction in the functioning of the root system influences the growth and development of plants (Macias-Leon and Leskovar, 2017; Atif et al., 2020; Solano et al., 2023).

Table 4. Average growth rate of parameters in *Allium cepa*

Experimental Variant	Average growth rate ($\overline{AGR}$)		
	$\overline{AGR_{RL}}$	$\overline{AGR_{LL}}$	$\overline{AGR_{LN}}$
V1	0.6603	0.3742	0.0679
V2	0.0607	0.0366	0.0158
V3	0.2743	0.0587	0.0173
V4	0.2557	0.1191	0.0347
V5	0.3561	0.1605	0.0347
V6	0.5032	0.2252	0.0480
V7	0.4932	0.1617	0.0367
V8	0.3857	0.1232	0.0276
SE	±0.065	±0.037	±0.006

The average growth rate of leaf length $\overline{AGR_{LL}}$ was described in relation to $\overline{AGR_{RL}}$, equation (1), with $R^2 = 0.915$, $p = 0.0021$, $F = 27.053$, and the average growth rate of leaf number $\overline{AGR_{LN}}$ was described in relation to $\overline{AGR_{RL}}$, equation (2), with $R^2 = 0.855$, $p = 0.0079$, $F = 14.758$.

$$\overline{AGR_{LL}} = 0.8683x^2 - 0.1044x + 0.04964 \quad (1)$$

$$\overline{AGR_{LN}} = 0.1272x^2 - 0.01103x + 0.01796 \quad (2)$$

where: x – average root growth rate ($\overline{AGR_{RL}}$)

$\overline{AGR_{LL}}$ parameter variation, based on $\overline{AGR_{LN}}$ values, was given by equation (3), with $R^2 = 0.982$, $F = 139.85$, $p<0.001$.

$$\overline{AGR_{LL}} = 29.51x^2 + 3.783x - 0.02047 \quad (3)$$

where: x – average rate of growth of the number of leaves ($\overline{AGR_{LN}}$)

Polynomial models and graphical models described the variation of the parameters RL, LL, LN in relation to time (T). The distribution of the control variant (V1) is presented compared to the variant V2, which recorded high level stress (Figures 2-4).

The variance was explained differentially, in relation to the principal components and morphological parameters, according to the values in Table 5. For example, the PCA diagram for the LL parameter was presented, in which the first two principal components (PC1, PC2) explained 97.617% of variance.

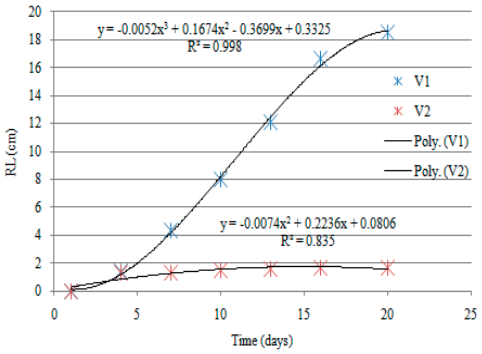


Figure 2. Root length (RL) parameter variation in relation to time

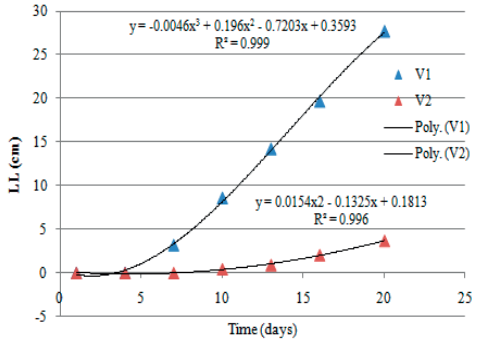


Figure 3. Leaf length (LL) parameter variation in relation to time

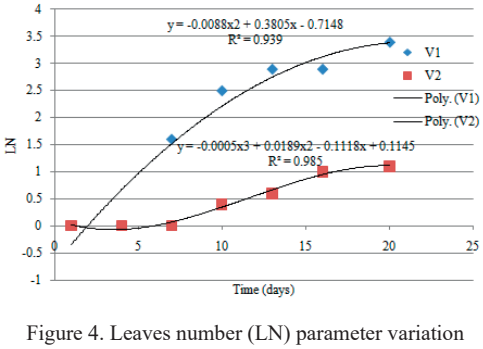


Figure 4. Leaves number (LN) parameter variation in relation to time

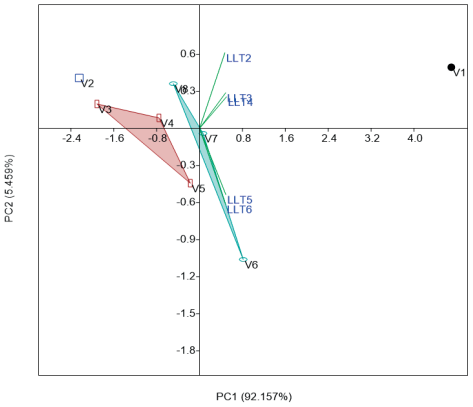


Figure 5. Principal component analysis (PCA) diagram in relation to the leaf length parameter, moments T2-T6

Table 5. Level of variance in relation to the principal components and morphological parameters

Principal Component (PC)	Parameters					
	Root length		Leaf length		Leaves number	
	Eigenvalue	% variance	Eigenvalue	% variance	Eigenvalue	% variance
1	4.371	87.426	4.608	92.157	4.397	87.932
2	0.391	7.823	0.273	5.460	0.423	8.466
3	0.230	4.601	0.107	2.140	0.118	2.363
4	0.007	0.150	0.011	0.213	0.050	1.003
5	6.87E-34	1.37E-32	0.002	0.030	0.012	0.236

The experimental variants were grouped into clusters (Figure 6), according to level of similarity, based on parameters values (RL, LL, LN), moments T1-T6 (Coph.corr. = 0.811). In stress-free conditions, plants from variant V1 recorded high values of the parameters RL, LL, LN in the test conditions. Thus, variant V1 was positioned independently of the other variants (Figure 6). The other variants were grouped into two sub-clusters. Variants V2 and V3, with similar levels of response to the stress generated by the chemicals, followed by variant V4, were grouped within a sub-cluster. The other variants, with intermediate values to the stress generated, were grouped into sub-cluster C2-B. The chemicals used, in the tested concentrations, differentially influenced morphological parameters in onions, under laboratory conditions. The substances with the most pronounced repressive effect were $CuSO_4$ (300 mg L^{-1}) in variant V2, followed by Faster (0.3 ml L^{-1}) in variant V3.

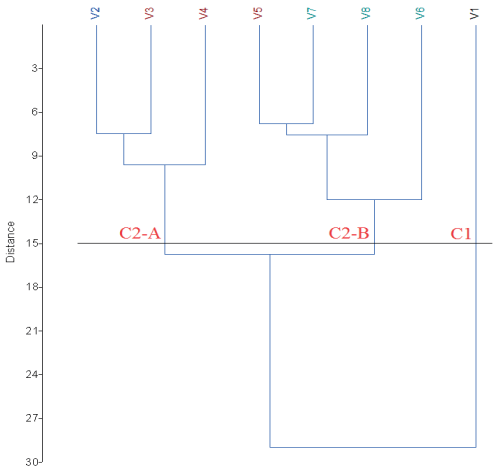


Figure 6. Cluster dendrogram based on Euclidean distances

The effect of Cu toxicity in onion roots was recorded depending on the concentration of Cu added under control, and the exposure time of the plants (Qin et al., 2015). Cu detection in onion leaves was studied in relation to different Cu concentrations provided in controlled germination medium (Jabbar et al., 2019). Copper supplementation to onions in different forms (nanoparticles, chelates, sulfate) showed that although $CuSO_4$ is the more accessible form, better effects were recorded in the case of Cu nanoparticles (Mottaleb et al., 2021). In a controlled environment, onion plant tolerance to low doses of Cu was recorded, and plant compromise with increasing concentration (Silva et al., 2022).

Within variant V4, the Faster product (0.4 ml L^{-1}), more pronounced repressive effects were recorded on the RL parameters, followed by LL, and the LN parameter recorded values at the average level of the experiment.

In the case of the Karate zeon product, variant V6 had values above the average of the experiment for all parameters, Variant V7 presented values above the average (RL parameter), and values at the average level (LL, LN parameters). In the case of variant V8 (0.25 mg L^{-1}) the Karate zeon product generated a stronger stress effect on the foliar parameters (LL, LN).

The RL and LL parameters showed higher sensitivity to $CuSO_4$ (300 mg L^{-1}) in variant V2, and to Faster (0.3 ml L^{-1}) in variant V3.

Under conditions of soils with high metal content (e.g. Cd), different levels of Cd in vegetable plants and mechanisms associated with Cd in plants were identified (Xiao et al., 2023). Onion and garlic showed lower Cd content in plants, as a result of mechanisms associated with Cd in the root (Xiao et al., 2023).

The average root growth rate (ΔRL) recorded the lowest values in variants V3 and V8, interval T1-T2. Variant V2 maintained low values of ΔRL throughout the observation interval.

In the case of the LL parameter, the average growth rate ΔLL recorded the lowest level in variants V2 and V3, the interval T1-T2. Variant V2 maintained low values of ΔLL throughout the observation interval.

In the case of the LN parameter, the average growth rate ΔLN recorded the lowest level in variants V2, V3 and V8 over the T1-T2 interval. Variant V2 maintained low values of ΔLN over the entire observation interval.

CONCLUSIONS

Differential effects were recorded in onions associated with chemical substances supplemented under controlled conditions.

A pronounced repressive effect was recorded in the case of $CuSO_4$ (300 mg L^{-1}, V2) on the RL parameter. The Faster product (0.3 ml L^{-1}, and 0.4 ml L^{-1}, V3, V4) generated more pronounced negative effects on RL, followed by LL and LN.

The Karate zeon product generated obvious repressive effects at a concentration of 0.25 mg L^{-1} (V8) on the LL and LN parameters.

The RL and LL parameters showed higher sensitivity to $CuSO_4$ (300 mg L^{-1}) in variant V2, and to the Faster product (0.3 ml L^{-1}) in variant V3.

The average growth rate of the studied parameters (ΔRL, ΔLL, ΔLN) was influenced by the substances added to the plant growth vessels. The lowest level was recorded in variant V2, under the influence of $CuSO_4$ throughout the study period. During the T1-T2 interval, variants V2, V3 and V8 presented reduced growth rate values.

The variation of the values of the parameters RL, LL, LN with respect to time was described

by polynomial equations ($p<0.001$). Multivariate analysis explained the variance and association of variants based on similarity.

ACKNOWLEDGEMENTS

The authors wish to thank to University of Life Sciences "King Mihai I" from Timișoara to facilitate this study.

REFERENCES

Ahmadi, S., Khazaei, S., Mehri, F. (2024). The concentration of pesticide residues in vegetables: A systematic review and meta-analyses. *Journal of Agriculture and Food Research, 15*, 101027.

Atif, M.J., Ahanger, M.A., Amin, B., Ghani, M.I., Ali, M., Cheng, Z. (2020). Mechanism of allium crops bulb enlargement in response to photoperiod: A review. *International Journal of Molecular Sciences*, *21*(4), 1325.

Bailey, P.H., Currey, J.D., Fitter, A.H. (2002). The role of root system architecture and root hairs in promoting anchorage against uprooting forces in *Allium cepa* and root mutants of *Arabidopsis thaliana. Journal of Experimental Botany*, *53*(367), 333–40.

Barbu, A., Ion, V.A., Frîncu, M., Petre, A., Bădulescu, L. (2022). Nutritional composition of fresh organic vegetables. *Scientific Papers. Series B, Horticulture*, *LXVI* (2), 240–244.

Bassey, E.J., Cheng, J.H., Sun, D.W. (2021). Novel nonthermal and thermal pretreatments for enhancing drying performance and improving quality of fruits and vegetables. *Trends in Food Science & Technology*, *112*, 137–148.

Bettoni, M.M., Mogor, Á.F., Pauletti, V., Goicoechea, N. (2014). Growth and metabolism of onion seedlings as affected by the application of humic substances, mycorrhizal inoculation and elevated CO_2. *Scientia Horticulturae*, *180*, 227–235.

Cho, H., Park, J.Y., Kim, D.H., Han, J., Natesan, K., Choi, M.-S., Lee, S.-K., Kim, J., Cho, K., Ahn, B.O. (2024). Understanding the defense mechanism of Allium plants through the onion isoallicin-omics study. *Frontiers in Plant Science*, *15*, 1488553.

Drăghici, M.C., Geicu-Cristea, M., Popa, E.E., Miteluț, A.C., Popescu, P.A., Tylewicz, U., Rosa, M.D., Popa, M.E. (2023). Research on blanching pretreatment and freezing technology effect on selected vegetables. *AgroLife Scientific Journal*, *12*(2), 69–76.

Hammer, Ø., Harper, D.A.T., Ryan, P.D. (2001). PAST: Paleontological Statistics software package for education and data analysis. *Palaeontologia Electronica*, *4*(1), 1–9.

Jabbar, A., Akhtar, M., Mehmmod, S., Iqbal, M., Ahmed, R., Baig, M.A. (2019). Quantification of copper remediation in the *Allium cepa* L. leaves using electric field assisted laser induced breakdown spectroscopy. *Spectrochimica Acta Part B: Atomic Spectroscopy*, *162*, 105719.

JASP Team (2022). JASP (Version 0.16.2) [Computer software].

Karasinski, J., Wrobel, K., Escobosa, A.R.C., Konopka, A., Bulska, E., Wrobel, K. (2017). *Allium cepa* L. response to sodium selenite (Se(IV)) studied in plant roots by a LC-MS-Based proteomic approach. *Journal of Agricultural and Food Chemistry*, *65*(19), 3995–4004.

Li, Z. (2022). Modeling plant uptake of organic contaminants by root vegetables: The role of diffusion, xylem, and phloem uptake routes. *Journal of Hazardous Materials*, *434*, 128911.

Lux, A., Martinka, M., Vaculík, M., White, P.J. (2011). Root responses to cadmium in the rhizosphere: A review. *Journal of Experimental Botany*, *62*(1), 21–27.

Macias-Leon, M.A., Leskovar, D.I. (2017). Plant growth regulator effects on germination and root traits of 'Lambada' and 'Don Victor' onion cultivars. *HortScience*, *52*(12), 1759–1764.

Maity, S., Guchhait, R., Pramanick, K. (2024). Role of aquaporins in micro-plastics accumulation in onion roots and the effects of micro-plastics on microtubules stability and organization under heat and salinity stress. *Environmental and Experimental Botany*, *220*, 105692.

Michalska-Kacymirow, M., Kurek, E., Smolis, A., Wierzbicka, M., Bulska, E. (2014). Biological and chemical investigation of *Allium cepa* L. response to selenium inorganic compounds. *Analytical and Bioanalytical Chemistry*, *406*(15), 3717–22.

Mladenova, G., Malcheva, B., Yordanova, M. (2024). Rhizoplane and rhizosphere microbial activity in ecologically cultivated vegetables and other crops. *Scientific Papers. Series B, Horticulture*, *LXVIII*(2), 495–502.

Mottaleb, S.A., Hassan, A.Z.A., El-Bahbohy, R., Mahmoud, A.W.M. (2021). Are copper nanoparticles toxic to all plants? A case study on onion (*Allium cepa* L.). *Agronomy*, *11*(5), 1006.

Ngowi, A.V., Mbise, T.J., Ijani, A.S., London, L., Ajayi, O.C. (2007). Pesticides use by smallholder farmers in vegetable production in Northern Tanzania. *Crop Protection*, *26*(11), 1617–1624.

Nziengui, H., Ditengou, F.A. (2023). Editorial: In tune with their environment: how plant roots cope with environmental signals. *Frontiers in Plant Science*, *14*, 1234996.

Pokluda, R., Ragasová, L.N., Jurica, M., Kalisz, A., Komorowska, M., Niemiec, M., Caruso, G., Gąstoł, M., Sekara, A. (2023). The shaping of onion seedlings performance through substrate formulation and co-inoculation with beneficial microorganism consortia. *Frontiers in Plant Science*, *14*, 1222557.

Qin, R., Wang, C., Chen, D., Björn, L.O., Li, S. (2015). Copper-induced root growth inhibition of Allium cepa var. agrogarum L. involves disturbances in cell division and DNA damage. *Environmental Toxicology and Chemistry*, *34*(5), 1045–55.

Silva, G.M., Oliveira, I.doN., Fernandes, M.C.deC., Garrido, F.deS.R.G. (2022). Physicochemical patterns of copper toxicity in *Allium cepa* roots. *Research, Society and Development*, *11*(14), e327111436285.

Solano, C., Artola, A., Barrena, R., Ballardo, C., Sánchez, A. (2023). Effect of the exogenous application of different concentrations of indole-3-acetic acid as a growth regulator on onion (*Allium cepa* L.) cultivation. *Agronomy*, *13*(9), 2204.

Solouki, A., Berna-Sicilia, J.Á., Martinez-Alonso, A., Ortiz-Delvasto, N., Bárzana, G., Carvajal, M. (2023). Onion plants (*Allium cepa* L.) react differently to salinity levels according to the regulation of aquaporins. *Heliyon*, *9*(3), e13815.

Wan, B., Zhou, E., Xiao, P., Sun, X., Yang, J. (2023). Pesticide overuse in vegetable production: a case study of urban agriculture in city X, China. *Environmental Research Communications*, *5*, 085012.

Xiao, Q., Wang, S., Chi, Y. (2023). Accumulation and chemical forms of cadmium in tissues of different vegetable crops. *Agronomy*, *13*(3), 680.

Zakharova, M., Baliuk, S., Vorotyntseva, L. (2023). Influence of micronutrient content optimization on the vegetables quality and yield. *Scientific Papers. Series B, Horticulture*, *LXVII*(2), 574–580.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

CONTENT OF MICROELEMENTS IN FRENCH BEAN PODS FROM DIFFERENT CULTIVATIONS

Marko PETEK[1], Ema KLARIĆ[2], Sanja FABEK UHER[2], Antun ŠOKEC[2], Szilvia VERES[3], Šic Žlabur JANA[2]

[1]University of Zagreb, Faculty of Agriculture, Department of Plant Nutrition, Svetošimunska cesta 25, Zagreb, Croatia
[2]Univesity of Zagreb, Faculty of Agriculture, Svetošimunska cesta 25, Zagreb, Croatia
[3]University of Debrecen, Egyetem tér 1, Debrecen, Hungary

Corresponding author email: sfabek@agr.hr

Abstract

The aim of this work was to determine how the cultivation method affects the microelements content of the French bean (Phaseolus vulgaris L.*) pods. The pod samples were collected in the city of Zagreb at three markets, three retail chains and three stores selling organic products. The concentrations of iron, zinc, manganese and copper were determined using an atomic absorption spectrometer. The value of the dry matter in the pods was between 6.74 and 12.54%. The proportion of microelements in the dry matter of the pods (mg/kg ST) was Fe 49.86-188.9, Zn 23.43-43.47, Mn 2.53-42.3, Cu 6.57-9.74, and in the fresh matter (mg/100 g fresh matter) Fe 0.49-1.96, Zn 0.19-0.42, Mn 0.03-0.32 and Cu 0.05-0.11. According to the Regulation on the provision of food information to consumers, it can be concluded that the consumption of 100 g of the studied french beans tested covers 4.21-8.0% of the daily human requirement of iron, 2.5-3.8% of the daily requirement of zinc, 4.0-13.0% of the daily requirement of manganese and 7.0-8.0% of the daily requirement of copper.*

Key words*: copper, iron, manganese, Phaseolus vulgaris* L.*, zinc.*

INTRODUCTION

A French bean pods are the fruits of *Phaseolus vulgaris* L., commonly known as the bean or common bean or filed bean, an annual herbaceous species within the legume family (Fabaceae) (Carović-Stanko et al., 2022). French bean pods are typically preserved through methods such as sterilization or freezing to ensure availability throughout the year, although they may also be consumed fresh (Lešić et al., 2016; Carović-Stanko et al., 2022). Microelements are essential nutrients required by plants in trace amounts, yet they play a critical role in plant growth and development, comparable to that of macroelements which plants need in larger amounts. Despite their low concentrations in plant tissues, they are indispensable for various physiological and biochemical processes.

Iron (Fe) is predominantly bound within plant tissues, with approximately 80-90% existing in tightly bound forms, making it moderately to poorly mobile. Its movement within plants is hindered when complexed with hydroxycarboxylic acids, amino acids, phenols, thiols, or polysaccharides, particularly in the presence of $Ca(HCO_3)$ (Vukadinović and Vukadinović, 2011). Structurally, iron is an integral component of numerous prosthetic groups in enzymes, including cytochromes, peroxidases, and catalases. Cytochromes, which share structural similarities with chlorophyll, contain an iron atom within the porphyrin core and function as coenzymes in electron transport (Lazarević and Poljak, 2019). Additionally, iron is crucial in oxidoreduction reactions, particularly within Fe-S proteins such as ferredoxin, which facilitates the interconversion of Fe^{2+} and Fe^{3+} within photosystem I (Vukadinović et al., 2014). Iron also regulates chlorophyll synthesis and photosynthesis by modulating the concentration of α-aminolevulinic acid (ALA), a key precursor of heme and chlorophyll (Bahat and Stepinac, 2011).

Zinc (Zn), although required in trace amounts, is essential for plant physiological processes. It is the only metal involved in all major enzyme classes, including oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases

(Sadeghzadeh, 2013). Zinc is necessary for the synthesis of RNA polymerase, thereby influencing DNA and RNA synthesis as well as overall protein synthesis. It also protects ribosomal RNA from degradation by ribonuclease enzymes (Rudani et al., 2018). Furthermore, zinc is vital for auxin metabolism, particularly in the biosynthesis of indoleacetic acid (IAA), an essential plant growth hormone. It plays a crucial role in the synthesis of tryptophan, a precursor of IAA (Lazarević and Poljak, 2019; Hafeez et al., 2013). Manganese (Mn) is an essential element involved in a variety of biochemical and oxidetion-reduction processes within plants. It serves as an enzymatic cofactor, particularly for enolase and carboxylase, and is integral to the Krebs cycle, where it activates decarboxylase and dehydrogenase enzymes. Manganese is indispensable for photosynthesis and nitrogen metabolism, acting as a cofactor in photosystem II electron transport. It also plays a pivotal role in water photolysis, a process essential for oxygen evolution during photosynthesis (Vukadinović and Vukadinović, 2011; Lazarević and Poljak, 2019).
Copper (Cu) is another essential microelement involved in plant physiological and biochemical processes. It functions as a cofactor in numerous enzymes responsible for redox reactions, given its ability to reversibly transition between Cu^{+} and Cu^{2+} oxidation states (Lazarević and Poljak, 2019). Copper is crucial in cell wall metabolism, protein metabolism, photosynthetic electron transport, hormone signaling, oxidative stress responses, and mitochondrial respiration. It also serves as an ethylene sensor, with its most critical function being electron transfer in mitochondrial and chloroplast reactions (Pradeep and Aishwarya, 2023; Chen et al., 2022; Miljković et al., 2018; La Torre et al., 2018). Copper is a key component of enzymes such as ribulose diphosphate carboxylase and cytochrome c oxidase, both of which are essential for photosynthesis and cellular respiration. Its deficiency leads to impaired chlorophyll synthesis, thylakoid membrane formation, and overall photosynthetic efficiency, resulting in chlorosis as a primary symptom (Rusjan, 2012).
The objective of this study is to determine the microelement content in French bean pods purchased from different sales channels, including markets, retail chains, and organic products stores. The findings will be analyzed to assess whether the cultivation method and point of purchase influence the microelement composition of the beans.

MATERIALS AND METHODS

Sampling of French bean pods was conducted on October 17, 2022, in Zagreb to determine phosphorus and iron concentrations. Samples were collected from three distinct sales channels, with three separate sampling locations within each channel:
Markets (M): Dolac (M1), Kvatrić (M2), and Voćarna Agrovir (M3)
Retail Chains (RC): Eurospin (RC1), Kaufland (RC2), and Spar (RC3)
Organic Product Stores (OPS): Garden (OPS1), bio & bio (OPS2), and Mrkvica (OPS3)
Each sampling was conducted in triplicate to ensure reliability.
Label declarations at sales points provided insights into the cultivation methods of the sampled French bean pods. As the samples from retail chains were not labeled as organic, they were presumed to be conventionally cultivated using mineral fertilizers. Verbal inquiries with market vendors did not yield specific information regarding fertilization practices. In contrast, all samples obtained from organic product stores were confirmed to be organically grown, as these stores exclusively sell certified organic products.
Following collection, composite samples were transported to the analytical laboratory of the Department of Plant Nutrition at the Faculty of Agriculture, University of Zagreb, for chemical analysis. The French bean pods were processed by peeling, slicing, and drying at 105°C. The dried samples were then ground, homogenized, and digested using concentrated nitric acid (HNO_3) and perchloric acid ($HClO_4$) in a microwave-assisted digestion system. Iron, zinc, manganese and copper concentration was determined via atomic absorption spectrometry (AOAC, 2023). Dry matter content was assessed gravimetrically by drying samples to a constant mass.
Statistical analysis was performed using analysis of variance (ANOVA) with the SAS System for Windows, version 9.1 (SAS Institute Inc.). The Tukey test (SAS, 2002-2003) was employed to assess significant differences among the results.

RESULTS AND DISCUSSIONS

Table 1 presents the dry matter content of French bean pods samples analyzed according to their sales chanell. The dry matter content in French bean pods obtained from market samples ranged from 8.28 to 12.54%, while samples from retail chains exhibited dry matter content values between 6.74 and 10.50%. In contrast, French bean pods from organic products stores showed dry matter contents ranging from 7.83 to 10.39%. The lowest dry matter content was observed in French bean pods sampled from the 'Spar' retail chain (6.74% dry matter), whereas the highest value was recorded in French bean pods from the 'Dolac' market (12.54% dry matter). Statistically, the highest dry matter content was found in the samples from the 'Dolac' market, while the lowest values were observed in French bean pods from the retail chains 'Spar' (6.74% dry matter) and 'Kaufland' (7.31% dry matter).

Table 1. Dry matter (% DM) content determined in French bean pods samples collected from, markets, retail chains and organic products stores

Sales channels		% dry matter			
Markets (M)	M1	12.54	a	10.38	a
	M2	10.32	b		
	M3	8.28	d		
Retail chains (RC)	RC1	10.50	b	8.18	b
	RC2	7.31	ef		
	RC3	6.74	f		
Organic products stores (OPS)	OPS1	10.39	b	9.31	ab
	OPS2	7.83	de		
	OPS3	9.71	c		

Different letters represent significantly different values according to Tukey's test, p≤0.05. The non-letter values are not significantly different.

The average iron content (mg Fe/kg dry matter) in French bean pods sampled from markets and retail chains did not exhibit a statistically significant difference. However, the average iron content in organic products stores was statistically higher (Table 2). The iron content in the dry matter of French bean pods across the different sales chanells ranged from 49.86 to 188.90 mg Fe/kg dry matter. The highest statistically significant iron content was recorded in samples from the 'Garden' organic products store (188.90 mg Fe/kg dry matter), while the lowest value was observed in French bean pods from the 'Kvatrić' market (49.86 mg Fe/kg dry matter).

Table 2. Iron content in dry matter of French bean pods determined in samples collected from, markets, retail chains and organic products stores

Sales channels		mg Fe/kg dry matter			
Markets (M)	M1	74.14	c	63.42	b
	M2	49.86	d		
	M3	66.25	c		
Retail chains (RC)	RC1	70.58	c	72.94	b
	RC2	75.25	c		
	RC3	73.00	c		
Organic products stores (OPS)	OPS1	188.90	a	115.82	a
	OPS2	70.23	c		
	OPS3	88.33	b		

Different letters represent significantly different values according to Tukey's test, p≤0.05. The non-letter values are not significantly different.

Iron content in fresh French bean pods sampled from organic products stores was consistently higher than that in French bean pods from markets and retail chains (Table 3).

Table 3. Iron content in fresh matter of French bean pods determined in samples collected from, markets, retail chains and organic products stores

Sales channels		mg Fe/100 g in fresh matter			
Markets (M)	M1	0.93	b	0.66	b
	M2	0.51	d		
	M3	0.55	d		
Retail chains (RC)	RC1	0.74	c	0.59	b
	RC2	0.55	d		
	RC3	0.49	d		
Organic products stores (OPS)	OPS1	1.96	a	1.12	a
	OPS2	0.55	d		
	OPS3	0.86	b		

Different letters represent significantly different values according to Tukey's test, p≤0.05. The non-letter values are not significantly different.

The average iron content in fresh French bean pods was statistically highest in organic products stores. Iron content in fresh French bean pods ranged from 0.49 to 1.96 mg Fe/100 g. The highest iron content in fresh green beans was found in samples from the 'Garden' organic food store (1.96 mg Fe/100 g), while the lowest was recorded in green beans from the 'Spar' retail chain (0.49 mg Fe/100 g). Statistically, the 'Garden' organic products store displayed the highest iron concentration in fresh French bean pods.

The average zinc content in the dry matter of French bean pods was highest in organic products stores, which also exhibited the statistically significant highest mean zinc concentrations. Conversely, the lowest average zinc content in dry matter was observed in market samples (Table 4). The highest zinc concentration in the dry matter of field beans was recorded in the organic store 'Mrkvica' (43.47 mg Zn/kg dry matter), while the lowest was found in the 'Voćarna' market (23.43 mg Zn/kg dry matter). Statistically significant higher zinc concentrations in the dry matter were identified in the organic products stores 'Mrkvica', 'bio & bio', and 'Garden', as well as in the retail chain 'Kaufland'.

Table 4. Zinc content in dry matter of French bean pods determined in samples collected from, markets, retail chains and organic products stores

Sales channels		mg Zn/kg dry matter			
Markets (M)	M1	32.51	bc	27.3	b
	M2	25.96	de		
	M3	23.43	f		
Retail chains (RC)	RC1	25.42	de	31.86	b
	RC2	39.65	a		
	RC3	30.51	cd		
Organic products stores (OPS)	OPS1	37.67	ab	41.02	a
	OPS2	41.90	a		
	OPS3	43.47	a		

Different letters represent significantly different values according to Tukey's test, p≤0.05. The non-letter values are not significantly different.

The zinc content in fresh French bean pods from retail stores ranged from 0.19 to 0.42 mg Zn/100 g fresh weight (Table 5).

Table 5. Zinc content in fresh matter of French bean pods determined in samples collected from, markets, retail chains and organic products stores

Sales channels		mg Zn/100 g in fresh matter			
Markets (M)	M1	0.41	A	0.29	b
	M2	0.27	C		
	M3	0.19	D		
Retail chains (RC)	RC1	0.27	C	0.25	b
	RC2	0.29	Bc		
	RC3	0.20	D		
Organic products stores (OPS)	OPS1	0.39	A	0.38	a
	OPS2	0.33	B		
	OPS3	0.42	A		

Different letters represent significantly different values according to Tukey's test, p≤0.05. The non-letter values are not significantly different.

The highest zinc concentration in fresh weight was determined in the organic products store 'Mrkvica', while the lowest was observed in the 'Voćarna' market. Statistically, the highest zinc values in fresh weight were found in the organic products stores 'Mrkvica' (0.42 mg Zn/100 g fresh weight) and 'Garden' (0.39 mg Zn/100 g fresh weight), as well as in the 'Dolac' market (0.41 mg Zn/100 g fresh weight).

The average manganese content in the dry matter of French bean pods, by sales channels, was highest in organic product stores, followed by retail chains and markets. Statistically, the highest mean manganese content in dry matter was observed in both retail chains and organic product stores. Manganese concentrations in the dry matter of French bean pods ranged from 2.53 to 42.3 mg Mn/kg dry matter (Table 6). The highest manganese content in dry matter was recorded in the 'Kaufland' retail chain (42.3 mg Mn/kg dry matter), while the lowest was found in the 'Kvatrić' market (2.53 mg Mn/kg dry matter). The statistically highest value was observed in the 'Kaufland' retail chain (42.3 mg Mn/kg dry matter), with additional significant values recorded in the 'bio & bio' (33.48 mg Mn/kg dry matter) and 'Mrkvica' (32.85 mg Mn/kg dry matter) organic products stores, as well as in the 'Spar' retail chain (32.94 mg Mn/kg dry matter).

Table 6. Manganese content in dry matter of French bean pods determined in samples collected from, markets, retail chains and organic products stores

Sales channels		mg Mn/kg dry matter			
Markets (M)	M1	6.29	Cd	8.14	b
	M2	2.53	D		
	M3	15.61	Bc		
Retail chains (RC)	RC1	5.20	Cd	26.81	a
	RC2	42.30	A		
	RC3	32.94	A		
Organic products stores (OPS)	OPS1	18.94	Bc	28.42	a
	OPS2	33.48	a		
	OPS3	32.85	a		

Different letters represent significantly different values according to Tukey's test, p≤0.05. The non-letter values are not significantly different.

In fresh French bean pods, the highest average manganese content across distribution channels was observed in organic products stores, while the lowest was found in markets. Statistically, the highest average manganese content in fresh French bean pods was detected in organic

products stores and retail chains. The manganese content in fresh produce ranged from 0.03 to 0.32 mg Mn/100 g fresh weight (Table 7). The highest manganese concentration in fresh French bean pods was identified in the 'Mrkvica' organic products store, while the lowest was observed in the 'Kvatrić' market. Statistically, the highest manganese values in fresh French bean pods were found in the 'Mrkvica' organic products store (0.32 mg Mn/100 g fresh weight), followed by the 'Kaufland' retail chain (0.31 mg Mn/100 g fresh weight) and the 'bio & bio' organic food store (0.26 mg Mn/100 g fresh weight). The lowest statistically significant value was recorded in the 'Kvatrić' market, with a manganese content of 0.03 mg Mn/100 g fresh weight.

Table 7. Manganese content in fresh matter of French bean pods determined in samples collected from, markets, retail chains and organic products stores

Sales channels		mg Mn/100 g in fresh matter			
Markets (M)	M1	0.08	de	0.08	b
	M2	0.03	e		
	M3	0.13	cd		
Retail chains (RC)	RC1	0.05	de	0.19	a
	RC2	0.31	a		
	RC3	0.22	b		
Organic products stores (OPS)	OPS1	0.20	bc	0.26	a
	OPS2	0.26	ab		
	OPS3	0.32	a		

Different letters represent significantly different values according to Tukey's test, p≤0.05. The non-letter values are not significantly different.

The average copper content in the dry matter of French bean pods is highest in retail chains and lowest in markets. The copper concentrations in the dry matter of field beans ranged from 6.57 to 9.74 mg Cu/kg dry matter (Table 8). The highest copper content was observed in the 'Eurospin' retail chain, while the lowest value was found in the 'Mrkvica' organic products store. Statistically, the highest copper content in the dry matter of French bean pods was recorded in the 'Eurospin' retail chain, with additional significant concentrations found in the 'bio & bio', 'Kaufland', 'Garden', 'Dolac', 'Kvatrić', and 'Spar'.

In contrast, the average copper content in fresh French bean pods was similarly high in organic products stores and markets, but comparatively lower in retail chains. Copper concentrations in the fresh matter of French bean pods ranged from 0.05 to 0.11 mg Cu/100 g fresh weight (Table 9). The highest copper content was detected in the 'Dolac' market, while the lowest was found in the 'Spar' retail chain. Statistically, the highest values were recorded in the 'Dolac' market (0.11 mg Cu/100 g fresh weight), followed by the 'Eurospin' retail chain (0.10 mg Cu/100 g fresh weight) and the 'Garden' organic products store (0.09 mg Cu/100 g fresh weight).

Table 8. Copper content in dry matter of French bean pods determined in samples collected from, markets, retail chains and organic products stores

Sales channels		mg Cu/kg dry matter			
Markets (M)	M1	8.58	abc	7.75	-
	M2	7.82	abc		
	M3	6.85	bc		
Retail chains (RC)	RC1	9.74	a	8.74	-
	RC2	9.12	abc		
	RC3	7.37	abc		
Organic products stores (OPS)	OPS1	8.81	abc	8.19	-
	OPS2	9.20	ab		
	OPS3	6.57	c		

Different letters represent significantly different values according to Tukey's test, p≤0.05. The non-letter values are not significantly different.

Vegetables represent a crucial component of a healthy diet due to their high content of carbohydrates, phytonutrients, fiber, vitamins, and minerals, serving as an essential source of nutritionally valuable elements. The dietary guidelines established by the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) recommend a minimum daily intake of 400 g of fruits and vegetables to prevent nutrient deficiencies.

Table 9. Copper content in fresh matter of French bean pods determined in samples collected from, markets, retail chains and organic products stores

Sales channels		mg Cu/100 g in fresh matter			
Markets (M)	M1	0.11	a	0.08	-
	M2	0.08	bcd		
	M3	0.06	de		
Retail chains (RC)	RC1	0.10	ab	0.07	-
	RC2	0.07	de		
	RC3	0.05	e		
Organic products stores (OPS)	OPS1	0.09	abc	0.08	-
	OPS2	0.07	cde		
	OPS3	0.06	de		

Different letters represent significantly different values according to Tukey's test, p≤0.05. The non-letter values are not significantly different.

Plants absorb minerals from the soil, water, and air, and thus the mineral composition of vegetables is influenced by various factors, including cultivation methods (organic versus conventional, which is closely associated with fertilization practices), environmental conditions, and environmental stresses such as salinity, drought, irrigation water quality, and extreme temperatures. Elevated concentrations of toxic elements in plants can interfere with the uptake of essential nutrients, leading to disruptions in physiological functions such as electron transfer, photosynthesis, respiration, metabolic and enzymatic processes, ultimately affecting plant growth and development (Popović-Djordjević et al., 2021). Same authors conducted a study on various types of organically and conventionally grown vegetables (cabbage, kohlrabi, Brussels sprouts, beetroot, carrots, potatoes, and onions) sourced from urban markets. Their findings revealed that the type of vegetable has a more pronounced effect on the mineral composition than the production method. In a similar research, Hunter et al. (2011) reported significantly higher concentrations of microelements in organically grown fruits, vegetables, French bean pods, and cereals. Furthermore, Czech et al. (2022) observed in their study on various species within the genus *Allium* that organically grown vegetables exhibited higher levels of minerals such as calcium, magnesium, iron, zinc, copper, and manganese, as well as bioactive compounds. This study concluded that the consumption of organically grown *Allium* vegetables confers a more favorable effect on human health. Additionally, Akbaba et al. (2012) found that in their study on *Phaseolus vulgaris* L. (beans), organically grown beans contained higher concentrations of essential elements including calcium, iron, manganese, phosphorus, zinc, chlorine, potassium, sodium, magnesium, and silicon, a finding that aligns with the results of the present study.

CONCLUSIONS

The highest dry matter content in pod samples was observed in the average sample by sales channels at the markets (10.38%). The highest average concentrations of iron, zinc, and manganese in both the dry and fresh mass of French bean pods were found in organic products stores, with values of 115.82 mg Fe/kg dry matter (DM) and 1.12 mg Fe/100 g fresh mass, 41.02 mg Zn/kg DM and 0.38 mg Zn/100 g fresh mass, and 28.42 mg Mn/kg DM and 0.26 mg Mn/100 g fresh mass. The highest average copper content in dry matter was recorded in pods sampled from retail chains (8.74 mg Cu/kg DM). In terms of fresh matter, the highest average copper concentration was measured in samples from both markets and organic products stores, where it was 0.08 mg Cu/100 g fresh mass in each distribution channel.

When comparing these measured values with the recommended daily intake values outlined in the Regulation (EU) on Food Information to Consumers, it can be concluded that the consumption of 100 g of the tested French bean pods contributes to 4.21-8.0% of the daily human requirement for iron, 2.5-3.8% for zinc, 4.0-13.0% for manganese, and 7.0-8.0% for copper.

REFERENCES

Akbaba U., Şahin Y., Türkez H. (2012). Comparison of element contents in haricot beans grown under organic and conventional farming regimes for human hutrition and health. *Acta Sci. Pol., Hortorum Cultus, 11*(2), 117-125.

AOAC (2023). Officinal Method of Analysis of AOAC International, Gaithersburg, Maryland, USA.

Bahat Z., Stepinac D. (2011). Nedostatak željeza kod biljaka s različitim mehanizmima usvajanja željeza, „case study“: kukuruz i uljana repica, Agronomski fakultet Sveučilišta u Zagrebu, Iron deficiency in plants with different iron uptake mechanisms, "case study": corn and oilseed rape, Faculty of Agriculture, University of Zagreb, Zagreb, str. 13.

Carović-Stanko K., Maloić M., Pintar J., Liber Z., Radosavljević I., Bedeković D., Guberac S., Očić V., Lazarević B. (2018). Nutritional quality of phaseolin types and morphotypes of green bean (*Phaseolus vulgaris* L.). *Agrociencia, vol.52*, no.4: 523-537.

Chen G., Li J., Han H., Du R., Wang X. (2022). Physiological and Molecular Mechanisms of Plant Responses to Copper Stress. *Int. J Mol Sci., 23*(21): 12950.

Czech A., Szmigielski M., Sembratowicz I. (2022) Nutritional value and antioxidant capacity of organic and conventional vegetables of the genus *Allium*. *Scientifc Reports*, 1-9

Hafeez B., Khanif Y. M., Saleem M. (2013). Role of Zinc in Plant Nutrition - A Review, *American Journal of Experimental Agriculture, 3*(2): 374-391.

Hunter D., Foster M., McArthur J. O., Ojha R., Petocz P., Samman S. (2011) Evaluation of the Micronutrient Composition of Plant Foods Produced by Organic and

Conventional Agricultural Methods. *Critical Reviews in Food Science and Nutrition*, 51: 571-582.

La Torre A., Iovino V., Caradonia, F. (2018). Copper in plant protection: current situation and prospect. *Phytopathologia Mediterranea, 57*, 2: 201-236.

Lazarević B., Poljak M. (2018.). Fiziologija bilja, Sveučilište u Zagrebu, Agronomski fakultet, Zagreb.

Lešić R., Borošić J., Buturac I., Herak Ćustić M., Poljak M., Romić D., (2016.). Povrćarstvo, Vegetable production, Zrinski, Čakovec.

Miljković I., Rastija D., Dugalić K., Puškar B., Andrišić M., Rašić D. (2018). Mikroelementi u tlu i lišću jabuka u voćnjacima Slavonije i Baranje, Microelements in soil and apple leaves in orchards of Slavonia and Baranja. *Pomologia Croatica*. *Vol. 22*. br. 3-4: 67-86.

Popović-Djordjević J. B., Kostić A. Ž., Rajković M. B., Miljković I., Krstić Đ., Caruso G., Moghaddam S. S., Brčeski I. (2021) Organically vs. Conventionally Grown Vegetables: Multi-elemental Analysis and Nutritional Evaluation. Biological Trace Element Research. file:///C:/Users/Administrator/Downloads/Popovi-Djordjevi2021_Article_OrganicallyVsConventionallyGro.pdf

REGULATION (EU) No 1169/2011 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004

Rudani K., Patel V., Prajapati K. (2018). The importance of zinc in plant growth – a review. *International Research Journal of Natural and Applied Sciences, Volume 5*, Issue 2: 38-48.

Rusjan, D. (2012). Copper in Horticulture. *Fungicides for Plant and Animal Diseases*. 2012, 257-278.

Sadeghzadeh B. (2013). A review of zinc nutrition and plant breeding. *Journal of Soil Science and Plant Nutrition, 13*(4): 905-927.

Vukadinović V., Vukadinović V. (2011.). Ishrana bilja, Sveučilište Josipa Juraja Strossmayera u Osijeku, Poljoprivredni fakultet u Osijeku, Plant Nutrition, Josip Juraj Strossmayer University of Osijek, Faculty of Agriculture in Osijek, Osijek.

Vukadinović, V., Jug I., Đurđević B. (2014.). Ekofiziologija bilja, Plant Ecophysiology, naklada NSS, Osijek.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

THE INFLUENCE OF GENOTYPE AND ENVIRONMENTAL FACTORS ON THE NUTRITIONAL COMPOSITION OF *ROSA CANINA* L. FRUITS

Rodica SOARE[1], Cristina BĂBEANU[2], Maria DINU[3]

[1]University of Craiova, Faculty of Agriculture, 19 Libertatii Street, Craiova, Dolj, Romania
[2]University of Craiova, Faculty of Science, 13 A.I. Cuza Street, Craiova, Dolj, Romania
[3]University of Craiova, Faculty of Horticulture, 13 A.I. Cuza Street, Craiova, Dolj, Romania

Corresponding author email: dinumariana@hotmail.com

Abstract

This study aimed to determine how factors such as genotype and place of cultivation influence the variation of nutritional compounds that constitute the nutraceutical profile of Rosa canina L. fruits. These fruits can be integrated as a functional food in the daily life of the Romanian population. Twelve genotypes of Rosa canina L. were studied, for which the following average values were determined and recorded: total dry matter (46.54%); titratable acidity (40.32 mg/s fw); reducing sugar content (23.32%); total polyphenols (48.07 mg GAE/100 g fw), ascorbic acid content (7.04 mg/100 g fw); ABTS antioxidant activity (182.48 µMTE/g fw) mg/100 g). The results obtained showed that the determining factor is the genotype that induces significant variations in the content of nutritional compounds. The high values of the nutritional compounds determined in this study justify the high nutritional potential of these fruits originating from spontaneous flora and their use as natural medicines.

Key words*: antioxidant activity, Rosa canina L, total polyphenols, vitamin C.*

INTRODUCTION

For more than 2,000 years, the pseudofruits of *Rosa canina*, known as rose hips, have been used as herbal remedies (Mitroi et al., 2023). Studies on the first uses of *Rosa canina* in traditional medicine date back to ancient Greece. Hippocrates used the fruit in various recipes, but its precise use is unknown. The Roman naturalist Plinius the Elder gave the species the name “dog rose” in the belief that the root of the plant could cure the bite of a rabid dog, and during World War II, Britain, unable to import fresh citrus fruits, used rose hips as an important source of vitamin C (Mabellini et al., 2011).

Rosa canina L. is a shrub belonging to the *Rosaceae* Family, which contains over 100 species widely distributed in Europe, Asia, Africa, the Middle East and North America. This shrub has numerous uses: in food, in natural medicine, ornamental plant, rootstock for the reproduction of the *Rosa* genus or in agri-environmental measures to combat wind deflation (Iancu et al., 2020). Rosa fruits are among the important products in terms of global production and trade as organic foods collected from nature (Pećinar et al., 2021; Soare et al., 2015a).

They vary in size and color, depending on the genotype, and are recognized for their nutritional characteristics (Peña et al., 2023). In recent years, there has been a growing interest in edible wild fruits, including rose hips, which possess several properties that are beneficial to human health (Demir et al., 2021). Fruits rose hips, have nutritional, pharmacological and cosmetic value. They are very rich in vitamin C, containing over 2000 mg of this substance per 100 grams of fruit. Therefore, they are used in the food industry in the production of syrups, teas, jams, and jellies.

Fruits are an excellent source of nutrients and bioactive compounds that are effective in protecting the human body against various diseases related to oxidative stress, such as cancer, Parkinson's disease, Alzheimer's disease and atherosclerosis (Mitroi et al., 2023). Rose hips are highly valued for their taste, color and aroma, in line with their recognition as one of

the richest sources of pro-health compounds (Kayahan et al., 2023). The mixed sweet-sour taste of the fruits is caused by citric acid, ellagic acid and malic acid (Gunaydin & Alibas, 2023). Rosa canina fruits reach physiological maturity in August-September and their nutritional value is given by the high content of vitamin C compared to other fruits (Roman et al., 2013; Demir & Özcan, 2001). In addition to vitamin C, rose hips also contain a series of other phytochemicals such as phenolic compounds, organic acids, pectins, fatty acids, but also vitamins B1, B2, K, PP, E, macro and microelements, etc. (Mitroi et al., 2023).

Numerous studies have reported different levels of total sugars organic acids and bioactive compounds (Akagić et al., 2020; Fetni et al., 2020). Higher concentrations of bioactive compounds and higher levels of antioxidant activity are of great interest and could contribute to the development of functional foods or for preventive or therapeutic use in various pathologies (Peña et al., 2023).

The aim of this work was to identify valuable rosehip genotypes existing in the south-western part of Romania. The selected genotypes were studied from the point of view of the nutritional characteristics of the fruits. Knowledge of the nutritional characteristics of the fruits is important for the selection of biotypes that can be promoted and successfully used in breeding programs, but also in the food and pharmaceutical industries.

MATERIALS AND METHODS

For the study, 100 rose hips from 12 genotypes of *Rosa canina* were collected from the spontaneous flora in the area of the Didactic Station of the University of Craiova, South-West Romania (44°19′ north latitude and 23°48′ east longitude and an altitude above sea level ranging from 176-190 m (https://banumaracine.ucv.ro). This species is very resistant to environmental variations and grows well on rocky, degraded and nutrient-poor soils. The climate variability in southwest Romania is temperate-continental plain, where summers are hot and dry and winters are mild and short, the average annual temperature is 11-12°C, which ensures favorable conditions for the growth and development of *Rosa canina* plants.

Meteorological data were recorded in the wild rosehip fruit harvesting area during 2021 and 2022 (Table 1).

The fruits were harvested at the optimal stage of ripeness for processing (end of October). Immediately after harvesting, the samples were transported to the laboratory and analyzed for the following biochemical parameters, namely: total dry matter, reducing carbohydrates, vitamin C and total phenols.

Table 1. The climatic conditions in South-West Romania

Month	Average temperature (°C)		The relative humidity of the air (%)		Rainfall (mm)	
	2021	2022	2021	2022	2021	2022
May	16.6	17.5	65	59	52	45
June	+20.3	22.5	72	62	106	60
July	25.6	25,1	55	52	17	36
August	25.3	24.9	50	59	13	64
September	18.6	17.9	54	70	11	110

*https://rp5.ru/Vremea_în_Craiova

.

Determination of chemical composition

Dry matter content (%) was determined gravimetrically by drying ground fruit of R. canina to a constant weight at 105°C.

Reducing sugars (%) were extracted in distilled water (1:50 w/V), 60 minutes at 60˚C and assayed colorimetric with 3,5-dinitrosalicylic acid reagent using glucose as standard (Soare al., 2017). At 1 mL extract, 2 mL of 3,5-dinitrosalicylic acid reagent (1 g 3,5- dinitrosalicylic acid dissolved in 20 mL of 2M NaOH, 50 mL of distilled water, and 30 g of Na-K- tartrate and the final volume was made up to 100 mL) was added. The mixture was incubated for 10 min at 100˚C, cooled and the absorbance was measured at 540 nm after color development. D-Glucose was used as standard and the results were expressed in % fresh mass basis.

The titratable acidity was determined by titration with 0.1N sodium hydroxide (NaOH), using phenolphthalein as indicator and expressed as mg citric acid/g fw.

Ascorbic acid was extracted in 2% hydrochloric acid, HCl; 5:50 w/v (Soare et al., 2017). The determination of ascorbic acid is performed from the supernatant with iodometric redox titration in which iodine reacts with ascorbic acid, oxidizing it to dehydroascorbic acid. The ascorbic acid content was expressed as mg/g fresh weight.

Methanolic extract: For the determination of phenols and antioxidant activity the samples

were extracted with 80% aqueous methanol (1:10 w:v) by sonicating for 60 min in a sonicate bath Fungilab (Madrid, Spain) equipped with a digital timer and a temperature controller at 24°C. The resulting slurries were centrifuged at 10000 g for 10 minutes and the supernatants were analyzed.

The total phenolics content was determined colorimetric at 765 nm by using the Folin-Ciocalteu reagent (Soare et al., 2017). 0.5 mL methanolic extract of the sample is mixed with 0.2 mL Folin Ciocalteu reagent and 1.8 mL distilled water and after 5 minutes 1.5 mL 7.5% Na_2CO_3 is added. The mixture is left to stand in the dark and the absorbance at 765 nm is read after 60 min. The total phenolic content (TPC) was calculated using a standard curve prepared using gallic acid and expressed as mg GAE/g fw.

ABTS (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) radical cation scavenging activity was measured colorimetric at 734 nm using Trolox as standard (Soare et al., 2017). ABTS was dissolved in water to a 7 mmol/L concentration. ABTS radical cation was produced by reacting ABTS stock solution with 2.45 mmol/L potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12-16 h before use. The ABTS radical cation solution was diluted with ethanol to an absorbance of 0.70 at 734 nm. 0.1 mL of the sample extract was mixed with 2.9 mL of diluted ABTS radical cation solution. After reaction at room temperature for 6 min, the absorbance at 734 nm was measured. The Trolox calibration curve was plotted as a function of the percentage of ABTS radical cation scavenging activity. The final results were expressed as μM TE/g fw.

All spectrophotometric measurements were performed with a Thermo Scientific Evolution 600 UV-Vis spectrophotometer with VISION PRO software. All determinations were performed in triplicate.

Statistical Analysis

The data obtained were analyzed, and all results were expressed as means. The statistical significance of differences between variants was determined with the analysis of variance (ANOVA: single factor), and the means were compared using Duncan's multiple range test ($p \leq 0.05$).

RESULTS AND DISCUSSIONS

The degree of maturity and the harvest period of the rose hips significantly determine their biochemical composition profile. Within these genotypes, the total dry matter recorded a variation amplitude between 31.54 and 46.45%. The highest content was recorded in genotype 10 and was followed by genotypes G4, G5, G6, and G8, while genotypes G1, G7, and G12 recorded lower values. In this case, it can be stated that the dry matter in rose hips from spontaneous flora varies depending on location and genotype. Paunović et al., (2019), reported dry matter content values of 52.28% in fresh fruits and up to 90.33% in dried fruits.

Table 2. Biochemical content of Rosa canina genotypes from the spontaneous flora

Rosehip genotypes	Dry matter content (%)	The titratable acidity (mg citric acid/g fw)	Reducing sugars (%)
G1	31.98f	42.1a	19.2c
G2	35.92^{e}	24.0^{e}	18.0^{cd}
G3	38.30^{d}	31.68^{b}	18.58^{c}
G4	41.12^{c}	25.6^{d}	19.82^{b}
G5	42.28b^{c}	26.24^{d}	20.75^{b}
G6	42.26b^{c}	30.72^{bc}	23.06^{a}
G7	31.54^{f}	29.44^{c}	20.53^{b}
G8	42.88^{b}	28.16^{cd}	17.19^{d}
G9	39.12^{d}	25.6^{d}	18.29^{cd}
G10	46.45^{a}	40.32^{a}	23.32^{a}
G11	35.44^{e}	29.44^{c}	16.69^{d}
G12	31.90^{f}	21.76^{f}	14.09^{e}
$p \leq 0.05$	1.64	2.05	1.31

Mean values with different superscript letters significantly differ, and statistical significance was set at $p < 0.05$.

Sugars and organic acids are the most common soluble constituents of fruits, with important influence on taste and nutritional properties (Agagik et al., 2020).

Reducing carbohydrates in *Rosa canina* fruits ranged between 14.06 and 23.32% (Table 2). In the specialized literature, a wide variability of carbohydrate content has been reported, which is influenced by genotype, altitude or degree of fruit ripening. Thus, Rosu et al. (2011), reported the total sugars content in the fruits of rose species of 17.63 g/100 g fw to 12.05 g/100 g fw, and Gunes & Dolek (2024) reported values from 9.28-13.90%.

Also, the acidity of fresh *Rosa canina* fruits recorded values from 21.76 to 42.1 mg citric

acid/g fw. These results are in agreement with the studies of Vasilj et al. (2024), which reported values up to 45.1% total acids, and higher than those reported by Güneş et al. (2016), which ranged from 11.1 to 36.7 g kg^{-1} citric acid, influenced by the harvest period.

Antioxidants play an important role in the body in defense mechanisms against pathologies related to free radical attack.

The ascorbic acid (vitamin C) content identified in the rose hip genotypes ranged from 2.64 to 7.04 mg/g f.w. (Figure 1). The influence of genotype on the ascorbic acid content of rose hips recorded in the present study is also confirmed by other authors. Similar results to our study were reported by Peña et al. (2023) who reported values of vitamin C content (the ascorbic acid concentration ranged from 6.0 to 8.2 mg g^{-1} FW).

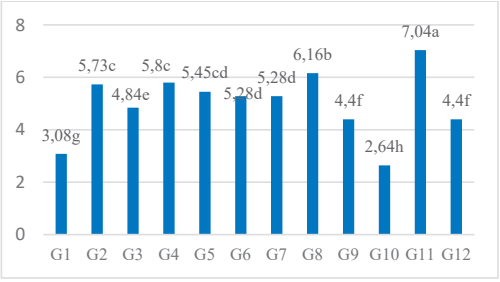


Figure 1. The vitamin C content (mg/g fw) of *Rosa canina* genotypes from the spontaneous flora ($p \leq 0.05 = 0.35$)

Some authors have stated that the vitamin C content of rose hips can also be influenced by the species. Thus, the highest vitamin C content was found in *R. rugosa* and *R. rubiginosa* of 20.9 and 13.9 mg/g dry matter, respectively, while the lower content of ascorbic acid was found in *R. micrantha* (6.1 mg/g dry matter) (Guantaroi et al., 2023). Also, Bakhtiar et al. (2023) reported a vitamin C content of 103.51 and 419.70.12 mg/100 g DW in the fruits of *Rosa canina* ecotypes, collected from eight provinces of Iran, and Bozhuyuk et al. (2021) reported a content of rose hip ecotype located in the East Anatolia of Turkey, ranging from 430-690 mg per 100 g FW (fresh weight).

In a comparative study conducted by Kerasioti, et al. (2019) on species of the Rosaceae family collected from Greece, the variation in antioxidant activity determined by the DPPH test on rosehip fruits was as follows: *Rosa canina* (100 µg/mL) > *Rosa sempervivens* (130 µg/mL) > *Pyracantha coccinea* (500 µg/mL).

In Romania, in the north-eastern part (400-1060 m altitude) Rosu et al. (2011) found vitamin C between 616 and 866 mg/100 g FW in fruits of *Rosa caesia* and *R. rubiginosa* in Romania, and Roman et al. (2013), between 112 and 360 mg per 100 g of fresh rose hips.

Ascorbic acid, phenolic compounds and flavonoids contribute to the antioxidant activities of fruits and, with higher content, fruits exhibit stronger antioxidant activities (Miljković et al., 2024).

The significant differences recorded in the content of phenolic total in fresh rosehip fruits (Figure 2) can be explained by genetic variation between genotypes and the influence of environmental factors in the growing areas.

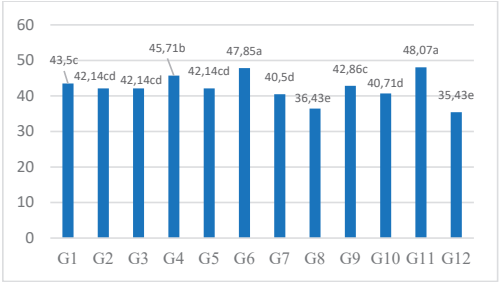


Figure 2. The total phenolic content (mg GAE/g fw) of *Rosa canina* fruits ($p \leq 0.05 = 1.81$)

Munteanu et al. (2023) argue that phenolic compounds are often produced in response to the stress of environmental factors, such as exposure to UV radiation, temperature, humidity and soil nutrient availability. Geographic location also plays an important role, contributing through specific microclimate and soil characteristics. For example, research conducted prior to this study shows that regional variations influence phenolic content in similar plants due to adaptation to local stress (Giosanu et al., 2018; Giura et al., 2019). Therefore, genotypes from the wild flora can accumulate varying amounts of phenolic compounds to better adapt to local conditions. This complex interaction between genetics and environment is essential for the variability observed in the phenolic composition of fruits. In the present study, the content of total phenolic compounds ranged from 35.43 to 48.07 mg GAE/g fw

(Figure 2). The values obtained in this study are comparable to those reported by other authors, where the highest and lowest levels of total phenolics in rose hips were 52.87 and 104.52 mg GAE/g DW (Bakhtiar et al., 2023) or between 390 and 532 mg gallic acid equivalent per 100 g FW (Bozhuyuk et al., 2021), and Soare et al. (2015) reported values of up to 48.07 mg GAE/kg^{-1} in Romania (Oltenia).

The antioxidant properties of rose hips can be influenced by numerous factors, such as its geographical location, ripening stage (Skrypnik et al., 2019), meteorological conditions (Mitroi et al., 2023). The ABTS values of the analyzed genotypes are presented in Figure 3, and the results indicate significant differences between them, in terms of antioxidant capacities. Thus, the highest results were recorded at G1 (182.48 mg/g), followed by G3 (180.33 µM TE/g fw.) and G11 (179.69 µM TE/g fw.).

Bozhuyuk et al. (2021) reported antioxidant capacity values from *R. canina* fruits ranging from 19.7 mg ascorbic acid equivalent per g FW to 34.7 mg ascorbic acid equivalent per g FW, depending on the ecotype. Bünyamin et al. (2021) reported values between 39,510 and 72,673 mmol kg^{-1} for *R. canina* fruits collected from the natural flora of Mesudiye (Ordu) and Talas (Kayseri) districts, Turkey, and Demir et al. (2014) reported values of 35.51 mmol TE g^{-1} by the ABTS method. Differences between studies may be attributed to different species, ecotypes or extraction methods.

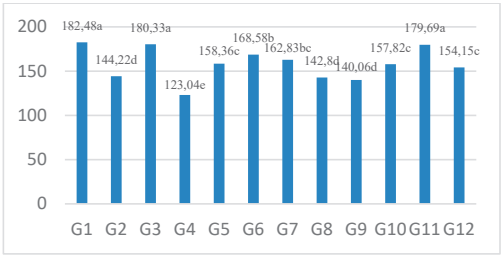


Figure 3. The antioxidant activity (µM TE/g fw) of *Rosa canina* ($p \leq 0.05 = 6.76$)

According to data from scientific literature, the concentration of nutrients in rose hips can be influenced by several parameters, namely: average temperature in the researched area, amount of precipitation during the growing season, species, genotype and altitude, but also by the time of harvest.

CONCLUSIONS

Evaluating the recorded results, it is found that the genotype has the greatest impact on the concentration of nutraceutical compounds. Within the 12 genotypes, a large variation in nutritional values can be observed regardless of the type of compound analyzed. The rosehip genotypes collected from the spontaneous forest in south-western Romania have proven to be rich in bioactive compounds and have the potential to be introduced into germplasm collections. Their analysis indicates that the genotypes presented large variations regarding the content of reducing carbohydrates, vitamin C, antioxidant activity and total phenols. The nutraceutical value of the *Rosa canina* species is undeniable and represents an important source of vitamins and other nutrients very necessary for the human body, which is why the consumption of these fruits can only help in the prophylaxis and prevention of various pathologies.

REFERENCES

Akagić, A.; Oras, A.V.; Oručević Žuljević, S.; Spaho, N.; Drkenda, P.; Bijedić, A.; Memić, S.; Hudina, M. (2020). Geographic Variability of Sugars and Organic Acids in Selected Wild Fruit Species. *Foods*, *9*, 462

Bakhtiar, Z., Eghlima, G., Hatami, M. et al. (2023). Quantification of fatty acids in seed oil and important bioactive compounds in Iranian *Rosa canina* L. ecotypes for potential cosmetic and medicinal uses. *Scientific Reports, 13*, 22721

Bozhuyuk, M.R.; Ercisli, S.; Karatas, N.; Ekiert, H.; Elansary, H.O.; Szopa, A. (2021). Morphological and biochemical diversity in fruits of unsprayed *Rosa canina* and *Rosa dumalis* ecotypes found in different Agroecological Conditions. *Sustainability 13*, 8060.

Bünyamin, D., Bahadır, S., Mehmet, Y., Ahmet, S., Ercan Y., Orhan, K., Günseli, B. A., Szai E. (2021). Biochemical composition and shape-dimensional traits of rosehip genotypes. *Folia Horiculturae, 33*(2), 1-16.

Demir, T. (2021). Effects of green tea powder, pomegranate peel powder, epicatechin and punicalagin additives on antimicrobial, antioxidant potential and quality properties of raw meatballs. Molecules, *26*(13), 4052.

Demir F, Özcan M (2001). Chemical and technological properties of rose (*Rosa canina* L.) fruits grown wild in Turkey. *Journal of Food Engineering 47*(4), 333-336.

Fetni, S.; Bertella, N.; Ouahab, A. (2020). LC–DAD/ESI–MS/MS Characterization of Phenolic Constituents in *Rosa canina* L. and Its Protective Effect in Cells. *Biomedical Chromatography*, 34, e4961.

Giosanu, D.; Bărbuceanu, M.; Anghel, M.; Vîjan, L. (2018). The determination of the content of phenolic compounds from different Romanian wines using Folin-Ciocalteu method. *Current Trends Natural Scences 7*, 155-159.

Giura, S.; Botu, M.; Vulpe, M.; Vîjan, L.E.; Mitrea, R. (2019). Evolution of the polyphenols, flavonoids, and tannins content in walnut leaves and green walnut husk during growing season. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 47,* 1264-1271.

Guantario B, Nardo N, Fascella G, Ranaldi G, Zinno P, Finamore A, Pastore G, Mammano MM, Baiamonte I, Roselli M. (2023). Comparative Study of Bioactive Compounds and Biological Activities of Five Rose Hip Species Grown in Sicily. *Plants*, 23; 13(1), 53.

Gunaydin, S., Alibas, I. (2023). The influence of short, medium, and long duration common dehydration methods on total protein, nutrients, vitamins, and carotenoids of rosehip fruit (*Rosa canina* L.), *Journal of Food Composition and Analysis, 124.*

Güneş, M.; Dölek, Ü.; Elmastaş, M. (2016). Pomological changes in some rosehip species during ripening. *JAFAG*, 33, 214-222.

Günes, M. and Dölek U. (2024). Changes in Total Sugar, Ascorbic Acid, Alpha-Tocopherol and Beta-Carotene Contents of Rosehip Fruits Based on Harvest Times. *Journal of Agricultural Science and Technology, 26*(4), 833-846.

Iancu, P., Soare, R., Dinu, M, Soare, M., Bonea, D., Popescu, M. (2020). Analysis of the existing research regarding the use of the species *Rosa canina*. *Scientific Papers. Series B, Horticulture*, *LXIV, 2*, 325-331.

Kayahan, S., Ozdemir, Y. & Gulbag, F. (2023). Functional Compounds and Antioxi dant Activity of Rosa Species Grown In Turkey. *Erwerbs-Obstbau 65*, 1079-1086.

Kerasioti, E.; Apostolou, A.; Kafantaris, I.; Chronis, K.; Kokka, E.; Dimitriadou, C.; Tzanetou, E.N.; Priftis, A.; Koulocheri, S.D.; Haroutounian, S.A.; et al. (2019). Polyphenolic composition of *Rosa canina*, *Rosa sempervivens* and *Pyrocantha coccinea* extracts and assessment of their antioxidant activity in human endothelial cells. *Antioxidants*, 8, 92.

Mabellini, A., Ohaco, E., Ochoa, M.R., Kesseler, A.G, Marquez, C.A, Michelis, A.D. (2011). Chemical and physical characteristics of several wild rose species used as food or food ingredient. *International Journal of Industrial Chemistry 2*, 158-171.

Miljković VM, Nikolić L, Mrmošanin J, Gajić I, Mihajilov-Krstev T, Zvezdanović J, Miljković M. (2024). Chemical Profile and Antioxidant and Antimicrobial Activity of *Rosa canina* L. Dried Fruit Commercially Available in Serbia. *International Journal of Molecular Sciences, 25*(5), 2518.

Mitroi, C.L., Lucan (Banciu), C.A., Guran (Radu), A., Şibu (Ciobanu), A., Poja, A. (2023). The antioxidant activity of rosehip (*Rosa canina* L.) - A review. *Journal of Agroalimentary Processes and Technologies, 29*(2), 55-58.

Munteanu, A.L.; Vijan, L.E.; Topală, C.M.; Mitrea, R. (2023). Influence of the phytosanitary status, cultivar, and harvest time on the phenolic, chlorophyll, and alkaloid content of Rosa sp. leaves. *Horticulturae, 9,* 1169.

Paunović, D., Kalusevıc, A., Petrović, T., Urosević, T., Djınović, D., Nedović, V., Popović-Djordjević, J. (2019). Assessment of chemical and antioxidant properties of fresh and dried rosehip (*Rosa canina* L.). *Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 47*(1), 108-113.

Pećinar I, Krstić D, Caruso G, Popović-Djordjević JB (2021). Rapid characterization of hypanthium and seed in wild and cultivated rosehip: application of Raman microscopy combined with multivariate analysis. *Royal Society Open Science, 8*(3): 202064.

Peña, F.; Valencia, S.; Tereucán, G.; Nahuelcura, J.; Jiménez-Aspee, F.; Cornejo, P.; Ruiz, A. (2023). Bioactive Compounds and Antioxidant Activity in the Fruit of Rosehip (*Rosa canina* L. and *Rosa rubiginosa* L.). *Molecules*, 28, 3544.

Rosu, C.M.; Manzu, C.; Olteanu, Z.; Oprica, L.; Oprea, A.; Ciornea, E.; Zamfirache, M.M. (2011). Several fruit characteristics of *Rosa* sp. genotypes from the Northeastern region of Romania. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 39*, 203-208.

Roman, I.; Stanila, A.; Stanila, S. (2013). Bioactive compounds and antioxidant activity of *Rosa canina* L. biotypes from spontaneous flora of Transilvania. *Chemistry Central Journal. 7*, 2–10.

Skrypnik, L., Chupakhina, G., Feduraev, P., Chupakhina, N., Maslennikov, P. (2019). Evaluation of the rose hips of Rosa canina and *Rosa rugosa* Thunb. As a valuable source of biological active compounds and antioxidants on the Baltic Sea coast. *Polish Journal of Natural Science, 34*(3), 395-413.

Soare, R., Dinu, M., Babeanu, C., Popescu, M., Popescu, A. (2017). Nutritional value and antioxidant activies in fruit of some cultivars of pepper (*Capsicum annuum* L.), *Journal of Agroalimentary Processes and Technologies*, *23*(4), 217-222.

Soare, R., Bonea, D., Iancu, P., Niculescu, M. (2015a). Biochemical and Technological Properties of Rosa Canina L. Fruits from Spontaneous Flora of Oltenia, Romania. *Bulletin UASVM Horticulture*, *72*(1).182-186

Soare, R., Băbeanu, C., Bonea, D., Păniță, O. (2015b). The content of total phenols, flavonoids and antioxidant activity in Rosehip from the spontaneous flora from south Romania. *Scientific Papers-Series A, Agronomy, 58*, 307-314.

Vasilj, V., Brekalo, H., Petrović, D., Šaravanja, P., Batinić, K. (2024). Chemical composition and mineral content of fresh and dried fruits of the wild rosehip (*Rosa canina* L.) population. *Journal of Central European Agriculture*, *25*(1), 179-193.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

APPLICATION OF WEB-BASED TOOLS FOR DOCUMENTATION OF HORTICULTURE LOCAL VARIETIES - BULGARIAN AND SLOVAKIAN EXAMPLE

Nikolaya VELCHEVA[1], Asya STOYANOVA-DOYCHEVA[2], Pencho MALINOV[2], Sebiha MADANSKA[2], Ľubomír MENDEL[3], René HAUPTVOGEL[3]

[1]Agricultural Academy, Institute of Plant Genetic Resources, 2 Druzhba Str., Sadovo, Bulgaria
[2]Plovdiv University, Department of Computer Systems, 24 Tzar Asen Str., Plovdiv, Bulgaria
[3]National Agricultural and Food Centre, Research Institute of Plant Production, 122 Bratislavská cesta Str., Piešťany, Slovak Republic

Corresponding author email: nikolaya_velcheva@abv.bg

Abstract

Plant genetic resources play an important role in maintaining global food security, conserving biodiversity and promoting sustainable agriculture. The paper aims to explore the data management, digitization and cataloguing the horticulture traditional varieties conserved ex situ/on farm. The approaches include development of optimised databases and web-based tools in accordance with FAO/Bioversity descriptors. Intelligent technologies are used for creating public access under the Nagoya Protocol and equitable sharing of benefits from the use of gene pool under the International Treaty on Plant Genetic Resources for Food and Agriculture. The interdisciplinary approach for promoting the local varieties is a part from their valorisation as biocultural heritage. The results have an impact to the modernisation of genebank documentation systems and for reintroduction of plant diversity to the home gardens and farms. The study contributes to environmental protection and sustainable preservation of gene pool, according to the national conservation strategies of Bulgaria and Slovakia.

Key words*: plant diversity, horticulture crops, genebanks, information systems, European networks.*

INTRODUCTION

Plant diversity is essential for food security, sustainable agriculture and environmental protection (Rocchi et al., 2016). The European Strategy for Genetic Resources highlights the need to preserve the gene pool for the present and future of humanity and emphasizes the obligation of gene banks to provide free access to information on the biological potential of the stored gene pool (EC, 2020). In recent decades, more and more old varieties have been replaced by new ones, and although the demand for traditional products is increasing, they are threatened with extinction, due to both climatic and anthropological factors (Britwum and Demont, 2022). In these circumstances, combining *ex situ* and *on farm* conservation approaches to protect biodiversity is a research priority (Arndorfer et al., 2009). International cooperation between genebanks is crucial to ensure the global conservation and sustainable use of genetic resources within the framework of interoperability in the European Research Area. The Green Deal promotes environmental practices, some of which are the inclusion of cereals and legumes in crop rotation, the search for solutions for natural enrichment of soils with nitrogen, limiting mineral nutrition, green manure, conservation agriculture, which have a positive impact on the protection of natural resources. Genetic resources are a tool that, if used sustainably, has the potential to create innovations and support the green transition (Reidsma et al., 2010; Halewood et al., 2018). The European Strategy for the Promotion of Protein Crops aims to support the production of legumes in the European agricultural sector (Brezeanu et al., 2021). There is a wide diversity of old and traditional horticulture varieties contributing to the households and accessible at the local markets in the rural areas in some European countries as Bulgaria, Romania, Slovakia (Benková et al., 2017; Ivanova et al., 2023; Stan et al., 2024).

According to the authors Smyth et al. (2020) the digitization of biological information for genetic resources with proven and potential

value means online publicly available information resources, described by a standardized descriptor, allowing data processing through the application of statistical models for automated processing and intelligent technologies. These data are characterized by high public value, having the potential to discover valuable traits and qualities for breeders in the stored samples that can help create genetic diversity and new varieties that respond to modern climatic and social challenges (Vinatoru et al., 2024).

The European electronic catalogue EURISCO aims to expand the possibilities of users by providing open access not only to passport description, but also to characterization and evaluation data of accessions, stored in the European genebanks (Weise, 2021; Kotni et al., 2023). The process of digitizing the plant gene pool is part of the overall transformative technological change that is being observed in all spheres of the global economy (Darnala et al., 2022; Shaw et al., 2023; Shelef et al., 2017; Tienhaara et al., 2019; Weise et al., 2025).

Digitization in Bulgarian and Slovakian agriculture and, in particular, in the management of plant genetic resources significantly improves the efficiency of the process of storing and using the gene pool (AEGIS, 2022a; AEGIS, 2022b; Mendel et al., 2019). The initial selection of accessions from genebanks for conducting scientific experiments is carried out on the basis of available passport information, such as taxonomic description and ecological-geographical characteristics, especially important for local populations (Thormann et al., 2012).

The aim of the study is to evaluate the development and use of web-based tools for inventory and digitization of local diversity of horticulture traditional varieties as an innovative solution to climatic and social challenges.

MATERIALS AND METHODS

The explored data was generated using information techniques for sortation of the national registers of Bulgarian and Slovakian genebanks, curators` data and interviews of local farmers via questionnaire.

Slovakian (https://griss.vurv.sk/) and Bulgarian (https://genbank.uni-plovdiv.net/) plant germplasm information systems are fully compatible with the passport descriptors standard of FAO/Bioversity (2017).

Information systems are conceptually, methodically and methodologically compatible with the *online* search systems in Europe – EURISCO (https://eurisco.ipk-gatersleben.de/) and GRIN (https://www.ars-grin.gov/) of USA.

For the survey local varieties were collected from rural areas and accessions from the genebank collections of both countries were exchanged by Standard Material Transfer Agreement.

RESULTS AND DISCUSSIONS

During 2024-2025 a qualitatively new cooperation between the Bulgarian and Slovakian genebanks by sharing experience and good practices in the field of conservation, characterization, evaluation and documentation of plant genetic resources is established. The information services are improved focused on the quality, management and sharing the data through digitization of local diversity according to descriptors of FAO/Bioversity. The interdisciplinary approach combines agricultural sciences and information technologies to modernize the documentation systems of the genebanks in both countries. As a result, through the exchange of new knowledge and experience, the quality of the digitization and computerization of the national information systems of the plant gene pool for food and agriculture in Slovakia and in Bulgaria is increased and improves the standardization of information flows in the framework of maintaining compatibility and interoperability in the European area.

The inventory of the *ex situ* collections stored in the genebanks via EURISCO catalogue shows that the Bulgarian collection in the National genebank in Sadovo consists 66,798 accessions with diverse geographical origin and biological status. The *ex situ* collection of Slovakian genebank includes 21,240 accessions in active collection and 5,529 accessions in basic collection. In the EURISCO catalogue, the National Agricultural and Food Centre in Piešťany presents data on 17,614 accessions.

European and international policies, standards and good practices for documentation of the plant gene pool are followed. The partners are working on enrichment the diversity of *ex situ* collections in Bulgaria and Slovakia.

As a result of germplasm exchange the Bulgarian collection is enriched with 66 new accessions with Slovakian origin, including 12 acc. cereals, 18 acc. beans, 10 acc. pea, 13 acc. pepper, 4 acc. pumpkin, 3 acc. tomato, leafy vegetables and spices (Figure 1).

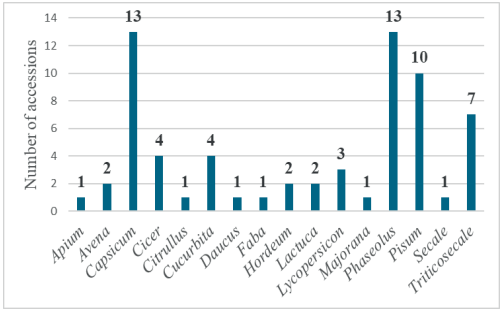


Figure 1. Enrichment of Bulgarian genebank through DIGIVALPGR project *with* new germplasm accessions

The Slovakian genebank has been enriched with seed materials from 40 local accessions from the curators` collections in Sadovo and with 17 traditional Bulgarian varieties (Figure 2).

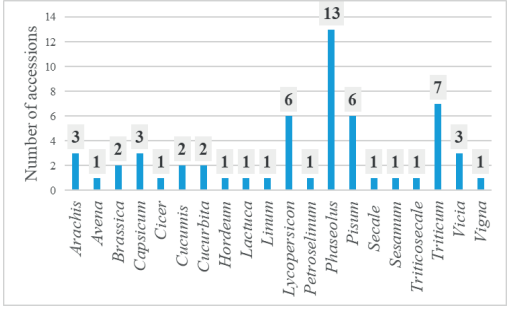


Figure 2. Enrichment of Slovakian genebank through DIGIVALPGR project with new germplasm accessions

Although the majority of horticultural production in Europe currently depends on formally registered, genetically uniform cultivars, local varieties are still cultivated *on farm*. In 2024 seven local varieties from traditional crops, including four common bean accessions, two tomato accessions and a pumpkin from home gardens in two different localities in the Rhodope Mountains (Bratsigovo, Pazardzhik region and Sarnitsa near Velingrad) have been collected and conserved in the Bulgarian genebank (Table 1). The climate conditions in Sadovo region are suitable for regeneration and multiplication of vegetable crops. The temperature and precipitation values are within the limits of their biological requirements or deviate insignificantly from them.

Table 1. Collected local horticulture varieties from mountain and semi-mountain regions in South Bulgaria

COLLNUMB	GENUS	SPECIES	ACCENAME	LOCALITY	ELEVATION
C4E0223	*Phaseolus*	*vulgaris*	Prevren	Bratzigovo	450 m
C4E0224	*Lycopersicon*	*esculentum*	Local pink	Bratzigovo	450 m
C4E0225	*Lycopersicon*	*esculentum*	Local red	Bratzigovo	450 m
C4E0226	*Cucurbita*	*moschata*	Barunche	Sarnitsa	1250 m
C4E0227	*Phaseolus*	*vulgaris*	Rancha	Sarnitsa,	1250 m
C4E0228	*Phaseolus*	*coccineus*	Pehlivan	Sarnitsa	1267 m
C4E0229	*Phaseolus*	*vulgaris*	Bashev	Sarnitsa	1259 m

A questionnaire for collecting passport and ecological-geographic characteristics has been developed and is being filled out (Figure 3). Partners agreed on the use of appropriate templates to collect occurrences data.

	QUESTIONNAIRE for collecting data during the collecting missions for farmer's variety/landrace by DIGIVALPGR project
1	Accession number
2	Collection date
3	Researcher who collected the accessions
4	Collection site/place 1) Country/city/village/locality 2) Latitude 3) Longitude 4) Altitude 5) Characteristics of the area - flat, mountainous, semi-mountainous, etc. 6) Climatic features - temperatures, precipitation during the growing season
5	Farmer information 1) Name and surname 2) Age 3) Main activity
6	Crop/taxonomic description
7	Local accession name
8	Biological status 1) Wild / Crop wild relatives 2) Local / traditional variety, population 3) Old variety (with reduced use)
9	Characteristics of the accession 1) Agronomic - cultivation technology, early maturity, yield, etc. 2) Product quality – shape, color, taste, etc. 3) Resistance to diseases, enemies, drought, etc. 4) Irrigated / non-irrigated conditions 5) Specific features – distinguishing qualities and signs 6) Where the accession was obtained from and how long it has been grown on the farm
10	Farm information 1) Total area of used agricultural land in the farm 2) Area on the farm occupied by the accession 3) Reasons for growing the accession – traditional, cultural/religious, market, palatability, early maturity, disease resistance, etc. 4) Direction of production – for domestic consumption, local market, ecotourism, for the country/abroad, direct market/through an intermediary, etc. 5) How do they get seeds - do they collect themselves, from neighbors or from the local market
11	Traditional recipes for using the accession and other additional information
12	Photo of accession

Figure 3. Questionnaire for collecting data during collecting missions via interviewing of local farmers

The upgrade of the genebank information system in the Institute of Plant Genetic Resources in Sadovo was aimed at achieving two main objectives: (1) Expanding, increasing the quality and updating the database with information on expeditions for the *ex situ* collections and (2) Ensuring *open access* to these resources for all users.

The created register with *open access* to the plant genetic resources is focused on the local gene pool as an effective approach for its valorization. The use of smart technologies in support of the cause of public responsibility for the preservation of plant biodiversity for current and future generations attracts many followers and young people with ecological civic position. A convenient application for *open access* to the genebank has been developed (Figure 4).

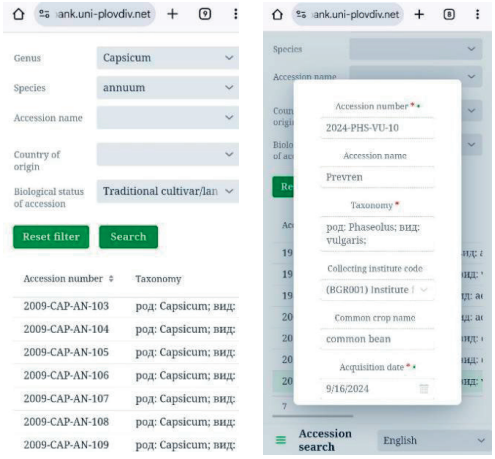


Figure 4. Mobile application of the *open access information* system of Bulgarian genebank

The main search criteria are based on the passport data of the plant genetic resources. After performing a search, users can view detailed information about the selected accession (Figure 5).



Figure 5. Detailed information for accession in *open access* in system of Bulgarian genebank

This information includes the complete set of its passport data. In the future, the *open access* search will be expanded to include more commonly known plant names, which will make it easier for farmers and other non-expert users to navigate the system. To support the implementation of this feature, a study was conducted on the plant genetic resources information system in Slovakia.

Inventory, collection, conservation and documentation of localities are of considerable importance for promoting actions aimed at enhancing the preservation and utilization of horticulture diversity. The Expeditions Module has been developed and includes key information about the conducted collecting missions, the participants involved, the funding organizations, as well as data on the new accessions (Figure 6).

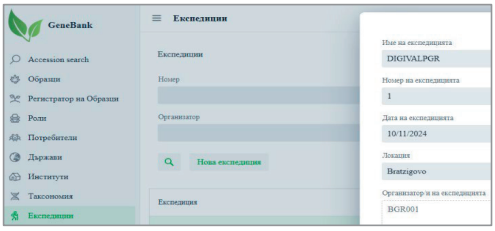


Figure 6. Expedition module in the information system of *Bulgarian* genebank

The necessity of keeping information for distribution of local varieties gives advantages for authorities to effectively plan and implement systematic conservation and utilization strategies for biodiversity (Raggi et al., 2024).

To facilitate user access in the Expedition module we developed search filters by various criteria - such as sample, participant, or funding project. The expedition data has been imported into the system's database, and relevant information has also been integrated into the GenBank Ontology, which was developed under the BGPLANTNET project (Stoyanova-Doycheva et al., 2020). As a part of the development process, an analysis of the genebank information system in Slovakia was carried out to adopt best practices and encourage information exchange between the two systems.

Slovakian information system GRISS is designed for complex information management

of the National genebank and supports all conservation processes. An information system is accessible through the web interface as searchable catalogue used by the curators of collections of plant genetic resources and from breeder's institutions, universities, public institutions and others. GRISS offers an online web portal solution for ordering germplasm materials in accordance with the international principles and the National Programme.

CONCLUSIONS

Digitization and the developed web-based applications increase and optimize the use of the gene pool in breeding programs for the adaptability of genetic materials to changing climatic factors and satisfy the needs of farmers to return the traditional varieties to practice.
The study is in line with the EU 2030 strategy for the conservation of biodiversity, is addressed to the implementation of national programs on Plant Genetic Resources for Food and Agriculture in Bulgaria and Slovakia.
The results of the study find their application in the overall process of protecting biodiversity and nature, increasing the resilience of crops to dynamic environmental conditions in order to guarantee the production of healthy and high-quality food.

ACKNOWLEDGEMENTS

This research work was carried out under the SVK-BGR cooperation project DIGIVALPGR "Digitalization and valorisation of local plant genetic resources in Bulgaria and Slovakia in the framework of maintaining compatibility and interoperability in the European area" with the support of Bulgarian National Science Fund, Contract number KP-06-Slovakia/9/13.08.2024 and Slovak Research and Development Agency, Contract number SK-BG-23-0005/17.09.2024.

REFERENCES

AEGIS (2022a). Genebank Review. *IPGR-Sadovo.* Bulgaria, October 27th, 2022. (https://www.ecpgr.cgiar.org/fileadmin/templates/ecpgr.org/upload/AEGIS/PEER_REVIEWS/GBank_Review_IPGR-Sadovo.pdf)

AEGIS (2022b). Genebank Review. *NPPC-RIPP-Piešťany,* Slovak Republic, September 18th, 2022. (https://www.ecpgr.cgiar.org/fileadmin/templates/ecpgr.org/upload/AEGIS/PEER_REVIEWS/Peer_Review_report_AGENT_NPPC.pdf)

Arndorfer, M., Kajtna, B., Vorderwülbecke, B. (2009). Integrating *ex situ* and *on farm* conservation approaches in the management of local vegetable diversity in Austria. *Acta Hortic.*, 817, 333-340.

Bachev, H. (2020). Digitalisation of Bulgarian agriculture and rural areas. *Bulgarian Journal of Agricultural Economics and Management*, *65*(2), 3-24.

Benková, M., Čičová, I., Benediková, D., Mendel, Ľ., Glasa, M. (2017). Variability of old sweet cherries found in Slovak regions and their preservation. *Proc. of the Latvian Academy of Sciences: Section B: Natural, Exact, and Applied Sciences*, *71*(3), 184-189. (https://www.degruyter.com/downloadpdf/j/prolas.2017.71.issue-3/prolas-2017-0031/prolas-2017-0031.pdf).

Brezeanu, C., Brezeanu, P.M., Calara, M., Ambarus, S. and Cristea, T.O. (2021). The role of food legume species in the context of sustainable agriculture. Food Security, Agrobiodiversity, Conservation and Human Health. *Scientific Papers. Series B, Horticulture*, *LXV* (1), 382-391.

Britwum, K., Demont, M. (2022). Food security and the cultural heritage missing link. *Global Food Security*, 35, 100660.

Darnala, B., Amardeilh, F., Roussey, C., Todorov, K., Jonquet, C. (2022). Ontological representation of cultivated plants: linking botanical and agricultural usages. In: *Modular Knowledge 2022*.

EC (2020). EU Biodiversity Strategy for 2030. Bringing nature back into our lives. *Communication for the Commission to the European Parliament, the Council, the European Economic and Social Committee and the Committee of the regions*, p. 25.

FAO/Bioversity (2017). Multi-Crop Passport Descriptors, *Alliance of Bioversity*, Rome, Italy. (https://www.ecpgr.cgiar.org/fileadmin/templates/ecpgr.org/upload/EURISCO/EURISCO_MCPD2_descriptors_updated_November_2017.pdf)

Halewood, M., Chiurugwi, T., Sackville Hamilton, R., Kurtz, B., Marden, E., Welch, E., Powell, W. (2018). Plant genetic resources for food and agriculture: opportunities and challenges emerging from the science and information technology revolution. *New Phytologist*, *217*(4), 1407-1419.

Ivanova, T., Marchev, A., Chervenkov, M., Bosseva, Y., Georgiev, M., Kozuharova, E., Dimitrova, D. (2023). Catching the Green – Diversity of Ruderal Spring Plants Traditionally Consumed in Bulgaria and Their Potential Benefit for Human Health. *Diversity*, *15*(3), 435.

Kotni, P., Hintum, T. van, Maggioni, L., Oppermann, M., Weise, S. (2023). EURISCO update 2023: the European Search Catalogue for Plant Genetic Resources, a pillar for documentation of genebank material, *Nucleic Acids Research*, 51, D1, D1465–D1469.

Mendel, L., Hauptvogel, P., Čičová, I. (2019). Plant Genetic Resources Information System of Slovakia as the Primary Source of the Information. *Agrobiodiversity for Improving Nutrition, Health and*

Life Quality, Nitra, 3, 178-185. (https://agrobiodiversity.uniag.sk/scientificpapers/article/view/262).

Raggi, L., Spataro, G., Negri, V. (2024). Landrace *in situ (on-farm)* conservation: European Union achievements. *Biodiversity and Conservation*. https://doi.org/10.1007/s10531-024-02893-y.

Reidsma, P., Ewert, F., Oude Lansink, A., Leemans, R. (2010). Adaptation to climate change and climate variability in European agriculture: The importance of farm level responses. *European Journal of Agronomy*. *32*(1), 91-102.

Rocchi, L., Paolotti, L., Cortina, C., Boggia, A. (2016). Conservation of landrace: The key role of the value for agrobiodiversity conservation. An application on ancient tomatoes varieties. *Agriculture and Agricultural Science Procedia*, 8, 307-316.

Shaw, P.D., Weise, S., Obreza, M., Raubach, S., McCouch, S., Kilian, B., Werner, P. (2023). Database solutions for genebanks and germplasm collections. *Plant genetic resources for the 21st century*, 285-309.

Shelef, O., Weisberg, P.J., Provenza, F.D. (2017). The value of native plants and local production in an era of global agriculture. *Frontiers in plant science*, 8, 2069.

Smyth, S.J., Macall, D.M., Phillips, P.W., de Beer, J. (2020). Implications of biological information digitization: Access and benefit sharing of plant genetic resources. Journal of World Intellectual Property, 23(3-4), 267-287. https://doi.org/10.1111/jwip.12151.

Stan, E., G., Iliescu, L., M., Stanica, F. (2024). Valorization of Sweet Potato (*Ipomoea batatas (L.) Lam*) and Customers' Perception on Some Innovative Products. *Scientific Papers. Series B, Horticulture*, Vol. LXVIII, Issue 1, Print ISSN 2285-5653, 893-898.

Stoyanova-Doycheva, A., Ivanova, V., Doychev, E., Spassova, K. (2020). Development of an Ontology in Plant Genetic Resources, *IEEE 10th International Conference on Intelligent Systems*, 246-251, DOI: 10.1109/IS48319.2020.9199935.

Thormann, I., Gaisberger, H., Mattei, F., Snook, L., Arnaud, E. (2012). Digitization and online availability of original collecting mission data to improve data quality and enhance the conservation and use of plant genetic resources. *Genetic Resources and Crop Evolution*, 59(5), 635-644.

Tienhaara, A., Ahtiainen, H., Pouta, E., Czajkowski, M. (2019). Role of information in the valuation of unfamiliar goods – the case of genetic resources in agriculture. *Working Paper – Faculty of Economic Sciences*, University of Warsaw, WP7/292.

Vinatoru, C., Peticila, A., Musat, B., Bratu, C., Negosanu, G., Popescu, M. (2024). New Cherry Tomatoes Varieties Obtained at BRGV Buzău. *Scientific Papers. Series B, Horticulture*, *Vol. LXVIII*, Issue 2, Print ISSN 2285-5653, 620-628.

Weise, S. (2021). Data management for pre-serving genetic diversity: Experiences and challenges. *34th Meeting EUCARPIA Fodder Crops*, 12-16. https://doi.org/10.5507/vup.21.24459677.

Weise, S., Blattner, F. R., Börner, A., Dehmer, K. J., Grübe, M., Harpke, D., Lohwasser, U., Oppermann, M., Stein, N., Willner, E. & Nagel, M. (2025). The German Federal *Ex Situ* Genebank for Agricultural and Horticultural Crops–Conservation, exploitation and steps towards a bio-digital resource centre. *Genetic Resources*, S2, 91-105. https://doi.org/10.46265/genresj.GYDY5145.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

INNOVATIVE TECHNIQUES FOR MONITORING AND EFFICIENT MANAGEMENT OF THE BULGARIAN GENEBANK

Nikolaya VELCHEVA[1], Gergana DESHEVA[1], Katya UZUNDZHALIEVA[1], Asya STOYANOVA-DOYCHEVA[2], Lyubka DOUKOVSKA[3], Valko NESHEV[1]

[1]Agricultural Academy, Institute of Plant Genetic Resources, 2 Druzhba Str., Sadovo, Bulgaria
[2]Plovdiv University, Department of Computer Systems, 24 Tzar Asen Str., Plovdiv, Bulgaria
[3]Bulg. Academy of Sciences, Institute of Information and Communication Technologies, Acad. Georgy Bonchev Str., Building 2, Sofia, Bulgaria

Corresponding author email: nikolaya_velcheva@abv.bg

Abstract

Every country is responsible for preserving its gene pool as a national capital. The genebank, located in Sadovo, is the main center for the long-term conservation of the plant diversity of Bulgaria. The research focuses on the development and implementation of innovative technologies for the management and storage of plant genetic resources. The main goal is to optimize the quality and efficiency of the processes in the National Genebank by intelligent systems and sensor networks to facilitate the monitoring of the accessions. The component for notification of control tests of the seeds and an interface for monitoring the chambers, as well as algorithms for intelligent search in ontologies are implementing. The importation of evaluation seed data from external devices is answering the need of digitalization of the genebank. The results have a significant scientific and public contribution leading the increase of the functionality and security of the genebank fund. The IS-PGR-SADOVO project supports the establishment of the quality standard for ex situ storage, in accordance with the Operational Guide for the Bulgarian Genebank, approved by the ECPGR Management Committee (2023).

Key words*: seed accession, genebank, long-term conservation, innovation, digitalization.*

INTRODUCTION

Plant genetic resources for food and agriculture are critically important for the sustainable food production in conditions of an ever-growing population and climate change (ITPGRFA, 2009). An important step in this direction is increasing the adaptability and productivity of crops in conditions of stress and reduced inputs, while ensuring sustainable development and protection of natural resources (Pilling et al., 2020). Each country is responsible for preserving its gene pool as a national treasure – a resource with biological and economical value, initial material for creation of new varieties that meet the requirements of current and future generations (Mattana et al., 2021). Conservation and documentation of genetic diversity goes beyond the mere storage of species and accessions, as it preserves traditional practices and cultures, including the varieties of local communities. These people are the stewards of the world's crop biodiversity (Arnaudova et al., 2015).

One of the main priorities until 2030 at the European level is the guarantee of *open access* to the genebank funds for all users (ECPGR, 2021). *Ex situ* collections are the basis of bioeconomy and agri-food chain in conditions of climate change. The report of FAO (2025), based on information provided by 128 countries and several international research centres, highlights that losses in plant diversity continue, but there is progress in its conservation, unfortunately the deep gaps in digitalization of the world's *ex situ* collections are alarming. To increase efficiency of preservation of *ex situ* collections it is recommended to implement innovative technologies for documentation of tests for viability and quality of seeds in the process of storage, intelligent monitoring and control of

risk in chambers for guaranteed conservation that improve the genebank operations and the management system (Ay et al., 2024; Hintum and Wijker, 2024).
Many authors published results that reveal the broad potential of leverage new technologies in genomics and phenotyping to improve the utilization of conserved diversity (Arend et al., 2022; Anglin et al., 2025). Establishing of a "digital genebank" is an innovative approach that aims to decipher the genetic composition of stored collections, which is a prerequisite for solving a huge number of problems related to increasing the resilience to pests and diseases and adaptability to environmental changes of the new varieties (Kim et al., 2023).
Appropriate example is HortDB V1.0 – a genomic database of horticultural plants, providing bioinformatic information for researchers and crop breeders. All data and tools are with open access (Li et al., 2024).
A common priority for each of the National Genebanks is the expanding the scope of intelligent systems in the process of management, preservation and use of plant diversity (Mendel et al., 2019).
At IPGR - Sadovo is situated the National Seed Genebank of Bulgaria and the Institute coordinates all activities and represents the country in the European program on plant genetic resources (ECPGR).
The aim of the study is to analyse the available passport information of the Bulgarian Genebank collection and to present the methodological process for integration of innovative techniques for monitoring and efficient management of the conservation process.

MATERIALS AND METHODS

The Information Centre for Plant Genetic Resources at IPGR - Sadovo was created in 1982. It was completely renovated in the period 2019-2023 when also the National GeneBank Information System for plant genetic resources was established (Doychev et al., 2020; Velcheva et al., 2022). The Information Centre works according to the international standard for documentation of FAO/Bioversity (2017) for *ex situ/on farm* conservation of plant genetic resources and FAO/Bioversity (2022) for *in situ* preservation of crop wild relatives. It is following the priorities for *open access* to the genebank collections in Europe, for available food plants databases as a bioresource for crop breeding and agriculture, and for maintaining a global system for free germplasm exchange between all users (French, 2018; Brink and Hintum, 2020; ECPGR, 2021).
The electronic database of Bulgarian Genebank contains the following passport information: taxonomic description, catalogue number, date of acquisition, country of origin, donor of the sample, collection site, ecology-geographical characterization, biological status, type of storage: long-term (base collection), medium-term (exchange collection), short-term (working collection), *in vitro*, cryo-conservation, field collection, botanical garden and agroecological data for *in situ* preservation of crop wild relatives. The National Seed Genebank in Sadovo represents Bulgaria in the European electronic catalogue on plant genetic resources EURISCO (http://eurisco.ecpgr.org/).
The enrichment, storage, characterization and evaluation, reproduction and multiplication of the genebank collections are a result of hard work of the crop curators, conservation and documentation specialists from the Department "Plant Genetic Resources".
The medium-term collection is stored at temperature from 0 to +6°C for a period of up to 10 years. 45,000 seed accessions are maintained in the base collection under long-term storage conditions with low moisture contents (5±2%) in three laminated aluminium foil packets at –18°C. Seed viability is detected on the basis of germination rate. The germination tests are conducted at regular intervals: before the storage in the genebank, and periodically every 10 years (for specific crops – every 5 years). The moisture content of seed accessions, both before and after the time of storage, is determined. Genebank Standards for Plant Genetic Resources for Food and Agriculture is followed (FAO, 2013).
During the period 2024-2027, according to the IS-PGR-SADOVO project, financed by the Bulgarian National Science Fund of the Ministry of Education and Science, intelligent techniques and sensor network to support and automatizing all the processes related to the management and monitoring of the national

plant gene pool conserved at IPGR - Sadovo started to be developed. The component for notification of control seed germination tests and an interface for monitoring the chambers, as well as algorithms for intelligent search in ontologies are implementing. The importation of biometrics data of evaluated seed samples from external devices is planned to be digitalized.

The IS-PGR-SADOVO project supports the establishment of quality standard for *ex situ* storage, in accordance with the Operational Guide for the Bulgarian Genebank, approved by the ECPGR Management Committee (ECPGR, 2023) and the European research cooperation on plant genetic resources conservation and use (Goritschnig et al., 2025).

RESULTS AND DISCUSSIONS

Data management practices in Bulgarian genebank vary widely, reflecting the different methodologies, and progress in the technological infrastructures. The study starts with analysis the genebank information management by historical periods as introduction to the project initiative (Figure 1). The results provide insights into existing workflows and highlighted the roles of key responsible actors.

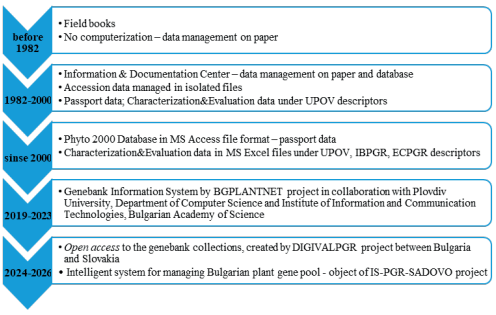


Figure 1. Historical periods of genebank documentation

The current digitalization activities in Bulgarian genebank follow the modern approaches aimed at compliance with the European policy for standardization of information in relation to sustainable conservation, *open access* to gene pool and equitable sharing of the benefits of these valuable resources among all participants in the agro-food chain. The GeneBank Information System is designed for a comprehensive description of accessions by passport, evaluation and characterization data, as well as managing of all storage and monitoring processes. The automatic output in excel file format according to standard descriptors makes easy and effective transfer of data for Bulgarian accessions to EURISCO intranet system.

According to EURISCO there are 66,555 preserved seed samples at IPGR - Sadovo from 70,990 totally registered as Bulgarian gene pool (Table 1). 25.65% from the *ex situ* collection in the National Seed Genebank is with local origin. Through the National Focal Point, accessions from the breeding collections of other institutes of the Agricultural Academy have been included in the European search catalogue EURISCO.

Table 1. Status of the Bulgarian *ex situ* National Inventory in EURISCO

Institute FAO code	Holding institute name	Number of accessions	Acc. with Bulgarian origin
BGR001	Institute of Plant Genetic Resources, Sadovo	66,555	17,073
BGR005	Institute of Rose and Essential-oil Plants, Kazanlak	563	4
BGR007	Institute of Maize, Knezha	13	13
BGR015	Institute of Forage Crops, Pleven	2	2
BGR029	Dobrudzha Agricultural Institute, General Toshevo	3,857	1,829
Total number of accessions		70,990	18,921

The data for monitoring of crop wild relatives in Bulgaria covers 243 populations, available in EURISCO website for *in situ* preservation with prioritized taxa: *Achillea, Aegilops, Astragalus, Bromus, Calamagrostis, Clinopodium, Dactylis, Daucus, Glycirrhiza, Hedysarum, Heracleum, Lepidium, Limonium, Lotus, Mentha, Paeonia, Serratula, Silene, Stachys, Trifolium*. The neighbour country Romania is also characterized by a high number of crop wild relatives, the Şandru (2021) presents a checklist of 525 native species. The stated

examples confirm the presence of rich biodiversity in the Balkans.

Development and implementation of innovative technologies for the management of plant genetic resources in Bulgarian genebank include the following steps:

(1) Improve the open access to the plant gene pool by all users, regardless of their knowledge through ontologies;

(2) Managing the germination tests and alert warning system for needed control checks;

(3) Implementation of a control system using QR codes facilitate and optimize the genebank activities;

(4) Digitalization of results from evaluation of accessions with external devices – seed analyser, which will increase the quality of the decisions taken for the storage process;

(5) Monitoring the conditions in the medium- and long-term storage chambers, in the laboratories and in the seed drying room with sensor network.

The genebank has refrigeration systems set up to maintain specific seed storage conditions depending on whether the storage is medium- or long-term. The technical staff inspects these installations daily and monitors the status of the storage conditions. The proposed for construction sensor network provides constant monitoring of the conditions in the cold storage chambers, providing the genebank employees with continuous information about the state of the seeds. The software component allows the monitoring of sensor data in real time, which facilitates the detection of potential problems with the refrigeration installations and enable a quick response if a change in storage conditions or additional test is required. A sensor network specification to alert the genebank staff when there are problems with the refrigeration installations, in the dryer room and laboratories is created.

The smart technology in germplasm conservation reduces the level of risk for loss of accessions and allow wider access of users to the resources, stored in the genebank.

Digitalization and open access to the gene pool develop the social interest in plant genetic resources. The available data increases the possibilities for partnership of the Bulgarian genebank with agricultural and horticultural crops breeders who are searching for valuable donors of important traits in the direction of sustainable agriculture in climate change conditions.

To effectively manage the implementation of the IS-PGR-SADOVO project and ensure the achievement of the goals, the main activities are grouped into six work packages (Figure 2).

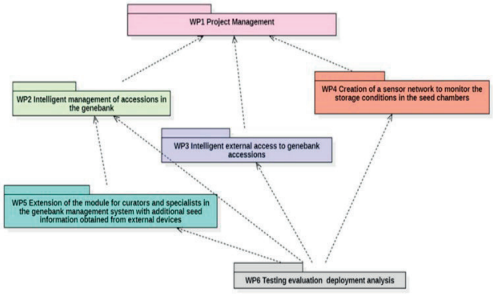


Figure 2. Strategically work packages and interaction between them

Curators conduct experiments with accessions, listed in their collections, and record and analyse their results. When a new accession is registered in the genebank documentation system with its passport data, it refers to a designated collection for storage, study, reproduction and multiplication for germplasm free exchange. The experiments are currently related to the measurement of evaluative characteristics by unified international descriptors.

The measuring system for analysis of accessions scans the seeds and evaluate the total number of seeds, 1,000 grain weight, grain size (length, width), determination of dark or light grains, fractions by size (length, width), calculation of hectolitre mass, determination of purity of the accession, etc. A relational database with biometric assessment of different accessions and plant species maintained in the genebank is created. The system provides capabilities to detect colour abnormalities in seed accessions and to record colour images for documentation purposes. The data obtained from the measurement system is incorporated into the Intelligent Management System of the Bulgarian plant gene pool, stored in the genebank of IPGR-Sadovo, and access to a wide range of users will be provided.

All data in the GeneBank Information System is provided by curators, conservation and

documentation specialists online via the created interface. There are two functional and interdependent parts: (1) Non-public part, accessible for authorized users – curators, conservation and documentation specialists through a user name and password; and (2) Public part – a web application, designed with open access for all users. The Bulgarian genebank is freely accessible at the web portal. Internally it is composed for three parts: passport data, characterization/evaluation data and storage data.

Passport data present basic information on crops – a unique identifier of accession (ACCNUMB), genus, species, accession name, as well as information about holding institute, country of origin, status of accessions, source of the material, date of collection, type of storage, whether the genotype has safety duplication in another genebank, etc. The accession number consists of year, genus code, species code and the serial number of the accession in the crop collection. Passport database also contains information about the crop wild relatives, received from monitoring and *in situ* preservation such as taxonomy, geographical data and description of the collection site. All passport data is compliant with the FAO/Bioversity descriptors (2017; 2022). Figure 3 presents the main screen of GeneBank Information System with passport data of the crops.

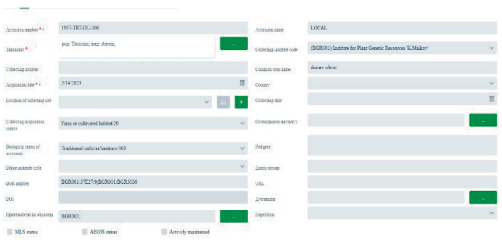


Figure 3. Passport data of the crops in GeneBank Information System

Characterization and evaluation data module constitute the basis for standardized describing system that provides information according to international descriptors by crop or crop group. The results are based on 2-3 years field experiments of crops. Figure 4 represents the part of the system where curators insert the evaluation characteristics about accessions.

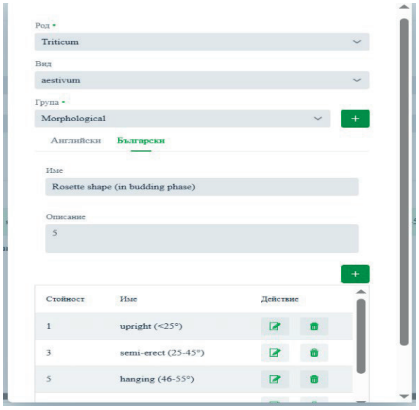


Figure 4. Evaluation characteristics in GeneBank Information System

In this case, this is an evaluation characteristic "Rosette shape (in budding phase)" for genus: *Triticum*, species: *aestivum*. Then the values for the different states of the measurement are seen. For example, in the case of rosette shape, a score of 1 is given if it is upright (<25°)

The module for storage documentation is contained in the third part of the system: germination ability, moisture content, number of seeds in the accession, storage date, etc. Figure 5 presents the main screen with storage documentation in the GeneBank Information System. This part of the system is non-public, and the interface is in Bulgarian language.

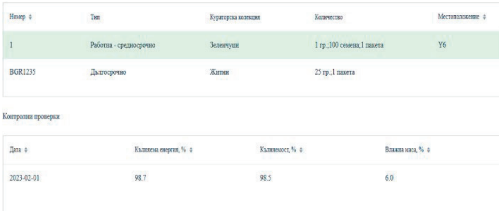


Figure 5. Storage documentation in GeneBank Information System

For each sample, the system allows to see the reproductions that are associated with it, in what storage they are and what control checks have been made for each of them.

Work is currently underway to import historical data on the reproductions and the control checks carried out for them into the system database.

Accessions are distributed free of charge based on signed SMTA (Standard Material Transfer Agreement).

CONCLUSIONS

The study prioritizes GeneBank Information System innovation efforts and strategically plan improvements over short-, medium-, and long-term horizons. This approach supports a strategic focus on using smart technologies for monitoring and management of genebank activities.

The digitalization of gene pool management processes is a prerequisite for increasing the quality of data, better control and sustainable preservation of plant genetic resources.

Access to information on the gene pool qualitatively increases the cooperation of the National Genebank with the Bulgarian breeding programs in connection to the creation of sustainable new varieties that meet the challenges of the present and future environment conditions.

Interdisciplinary collaboration between agriculture and software engineering scientists is a successful approach to continue providing professional support to maintain a continuous improvement process of genebank information management system.

ACKNOWLEDGEMENTS

This research work was carried out under the project IS-PGR-SADOVO “Intelligent system for managing Bulgarian plant gene pool, conserved in the genebank of IPGR - Sadovo” with the financial support of the Ministry of Education and Science, Contract number KP-06-H86/9/09.12.2024 of Bulgarian National Science Fund.

REFERENCES

Anglin, N. L., Wenzl, P., Azevedo, V., Lusty, C., Ellis, D., Gao, D. (2025). Genotyping Genebank Collections: Strategic Approaches and Considerations for Optimal Collection Management. *Plants*, 14(2), 252. DOI: 10.3390/plants14020252.

Arend, D., Psaroudakis, D., Memon, J. A., Rey-Mazón, E., Schüler, D., Szymanski, J. J., Scholz, U., Junker, A., Lange, M. (2022). From data to knowledge – big data needs stewardship, a plant phenomics perspective. *The Plant Journal*, 111(2), 335-347. DOI: 10.1111/tpj.15804.

Arnaudova, Z., Stefanova, V., Haytova, D. (2015). Creating land assessment database for vegetable crops in Perushtitza Village, Bulgaria. *Scientific Papers. Series B, Horticulture*, LIX, 177-184.

Ay, Z., Simon, A., Gyurkó, A., Fekete, E., Horváth, B., Baktay, B. (2024). History and current status of plant genetic resources conserved and maintained by the Hungarian central genebank. *Genetic Resources*, S2, 13-28. DOI: 10.46265/genresj.FCUW9498.

Brink, M., Hintum, T. van. (2020). Genebank operation in the arena of access and benefit-sharing policies. *Frontiers in Plant Science*, 10, 1712. DOI: 10.3389/fpls.2019.01712.

Doychev, E., Malinov, P., Velcheva, N., Duchev, Z. (2020). A Genebank Architecture: A Distributed System for Management of Plant Genetic Resources, 2020 IEEE, *10th International Conference on Intelligent Systems*, 580-583, DOI: 10.1109/IS48319.2020.9199972.

ECPGR. (2021). Plant Genetic Resources Strategy for Europe. Rome, Italy. https://www.ecpgr.cgiar.org/fileadmin/bioversity/publications/pdfs/PGR_STRATEGY_LP_22_Nov_revised.pdf.

ECPGR. (2023). Operational Guide for the Bulgarian Genebank.https://www.ecpgr.org/fileadmin/templates/ecpgr.org/upload/AEGIS/Documents/Procedures_examples/Genebank_manuals/Genebank_Manual_Bulgaria_IPGR-Sadovo_February_2023.pdf.

FAO. (2013). Genebank Standards for Plant Genetic Resources for Food and Agriculture. Rome, Italy, ISBN: 9789251082621, p. 181.

FAO. (2025). The Third Report on The State of the World's Plant Genetic Resources for Food and Agriculture. FAO Commission on Genetic Resources for Food and Agriculture Assessments, Rome. DOI: 10.4060/cd4711en.

FAO/Bioversity. (2017). Multi-Crop Passport Descriptors, Rome, Italy. https://www.ecpgr.cgiar.org/fileadmin/templates/ecpgr.org/upload/EURISCO/EURISCO_MCPD2_descriptors_updated_November_2017.pdf.

FAO/Bioversity. (2022). Descriptors for uploading in situ CWR passport data to EURISCO, Rome, Italy. https://www.ecpgr.org/resources/ecpgr-publications/publication/descriptors-for-uploading-in-situ-cwr-passport-data-to-eurisco-2022.

French, B.R. (2018). The Food Plants International database as a global resource, freely available. *ISHS Acta Horticulturae*, 1205: International Symposia on Tropical and Temperate Horticulture – ISTTH2016, 267-272. DOI: 10.17660/ActaHortic.2018.1205.30.

Goritschnig, S., Weise, S., Guzzon, F., Maggioni, L., Hintum, T. van, Steffensen, L. L., Stein, N., Giuliano, G. (2025). Strengthening European research cooperation on plant genetic resources conservation and use, *Genetic Resources*, S2, 119-134. DOI: 10.46265/genresj.LUZJ7324.

Hintum, T. van, Wijker, E. (2024). Quality management in a genebank environment: Principles and experiences at the Centre for Genetic Resources, The Netherlands (CGN), *Genetic Resources*, S2, 6-12. DOI: 10.46265/genresj.RFXB3570.

ITPGRFA. (2009). International Treaty on Plant Genetic Resources for Food and Agriculture. Rome, Italy. https://www.fao.org/plant-treaty/en/.

Kim, S. H., Subramanian, P., Na, Y. W., Hahn, B. S., Kim, Y. (2023). RDA-Genebank and Digital Phenotyping for Next-Generation Research on Plant Genetic Resources. *Plants*, 12(15), 2825. DOI: 10.3390/plants12152825

Li, Z., Wang, C., Wang, S., Wang, W., Chen, F. (2024). HortDB V1. 0: a genomic database of horticultural plants. *Horticulture Research*, 11(10), uhae224.https://doi.org/10.1093/hr/uhae224.

Mattana, E., Ulian T., Pritchard H. (2021) Seeds as natural capital. Trends in Plant Science, 27(2), 139-146, doi: 10.1016/j.tplants.2021.08.008.

Mendel, Ľ., Hauptvogel, P., Čičová, I. (2019). Plant genetic resources information system of Slovakia as the primary source of the information. *Agrobiodiversity for improving of nutrition, health and quality of life*, 3, 178-185. DOI: 10.15414/agrobiodiversity.

Pilling, D., Bélanger, J., Diulgheroff, S., Koskela, J., Leroy, G., Mair, G., Hoffmann, I. (2020). Global status of genetic resources for food and agriculture: challenges and research needs, *Genetic Resources*, 1 (1), 4-16, DOI: 10.46265/genresj.2020.1.4-16.

Şandru, M.D. (2021). National inventory and prioritization of crop wild relatives from Romania. *Scientific Papers*. *Series A. Agronomy*, 64(2),424-434.

Velcheva, N., Uzundzhalieva, K., Stoyanova-Doycheva, A., Malinov, P. (2022). Establishment of national information system of plant genetic resources in Bulgaria. *Scientific Papers. Series B. Horticulture*, 66(1), 938-943.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

EVALUATION OF THE COMBINING ABILITY AND HETEROSIS IN EGGPLANT PARENTS FROM BRGV BUZĂU FOR OBTAINING VALUABLE F1 HYBRIDS

Costel VÎNĂTORU[1], Adrian PETICILĂ[2], Bianca MUȘAT[1], Camelia BRATU[1], Geanina NEGOȘANU[1], Matilda POPESCU[1]

[1]Plant Genetic Resources Bank - for Vegetables, Floricultural, Aromatic and Medicinal Plants Buzău, 56 Nicolae Bălcescu Blvd, Buzău, Romania
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăşti Blvd, District 1, Bucharest, Romania

Corresponding author email: musat_b@brgv.ro

Abstract

This study evaluates the combining ability and heterosis phenomenon in eggplant (Solanum melongena L.) parents to obtain valuable F1 hybrids. The research was conducted at the Plant Genetic Resources Bank (BRGV) Buzău and included 70 parental lines selected for genetic stability and distinct phenotypic expression. Testing the general (GCA) and specific (SCA) combining ability led to the identification of 31 hybrid combinations with high potential. The results indicated a strong manifestation of heterosis in most combinations, with 23 hybrids showing a 30% yield increase compared to their parents. Additionally, 35 hybrid combinations demonstrated high offspring uniformity, crucial in selecting new genotypes. In conclusion, the study highlights the importance of using local genetic material in eggplant breeding, contributing to reducing dependence on imported hybrid seeds. These findings offer promising perspectives for developing competitive hybrids adapted to local climatic conditions, thereby supporting the progress of the Romanian vegetable sector.

Keywords*: breeding, genetic diversity, hybridization, phenotype, resistance.*

INTRODUCTION

The interest in using hybrid seeds of eggplant (*Solanum melongena* L.) has significantly increased in Romania, especially among growers practicing intensive vegetable farming, both in protected spaces and in open fields. These hybrids stand out due to their higher productivity, superior fruit quality, earliness, and greater resistance to specific pathogens (Kalloo, 1993; Kumar et al., 2020). Although eggplant is an important crop worldwide, modern varieties have a narrow genetic base, making it necessary to expand the germplasm pool used in breeding (Muñoz-Falcón et al., 2009).

The phenomenon of heterosis, which determines the superiority of hybrids over their parents, is well-documented in the specialized literature. Birchler et al. (2010) define heterosis as "a phenomenon in which the offspring resulting from the crossing of distinct individuals exhibit higher or lower trait values compared to the average of the initial parents". This manifestation of hybrid vigor translates into rapid growth, higher productivity, enhanced vitality, and superior plant uniformity (Kumar et al., 2020). Moreover, it has been demonstrated that F_1 hybrids can exhibit greater tolerance to abiotic stress, such as extreme temperatures and water deficiencies, making them more adaptable to changing climatic conditions (Kaushik et al., 2016).

Despite these evident advantages, Romania remains dependent on imported hybrid seeds, as domestic research in this field has been limited. This situation generates economic and agronomic challenges, as imported seeds are often expensive and not always well-adapted to local climatic conditions. Furthermore, in some cases, the import of seed material has facilitated the introduction of new pathogens, leading to significant production losses. According to recent studies, using local genetic material and wild species in breeding programs can contribute to increasing genetic diversity and developing more resistant hybrids (Knapp et al., 2013; Prohens et al., 2012).

To reduce this dependency and support national hybrid seed production, current research focuses

on evaluating the combining ability of local genotypes and determining the heterosis potential of the obtained hybrids. The Plant Genetic Resources Bank (BRGV) Buzău plays a crucial role in this endeavour by collecting, conserving, and improving the local germplasm pool. Identifying the most valuable hybrid combinations and selecting the most performant parent lines are fundamental steps in obtaining competitive F_1 hybrids at both national and international levels (Prohens et al., 2012; Kaushik et al., 2016). Additionally, phenotypic and molecular descriptors, such as those proposed by UPOV and IBPGR, have been successfully used for evaluating segregating generations of interspecific crosses, providing valuable information for selecting the genotypes with the highest potential (Prohens et al., 2013).

MATERIALS AND METHODS

BRGV Buzău has genebanked over 1,000 eggplants (*Solanum melongena* L.) lines at various stages of breeding, focusing on both genetic stability and variety authenticity improvement.
Among these, 443 genotypes are in an advanced breeding stage. Special attention has been given to old local varieties and populations. Notably, the bank's collection includes well-established traditional varieties that were once widely cultivated, such as 'Danubiana', 'Bucureștene', and 'Pana Corbului'. From these, 70 parent lines were selected for their genetic stability and distinct phenotypic expression: 1C, 2C, 3C, 8B, 8C, 12A, 13B, 20, 22A, 23A, 23A2, 23A3, 23F, 23F2, 23N, 24, 25A, 27B, 27B1, 27P, 27P1, 51A, 51A1, 51A2, 51A3, 58, 58A, 58B, 58C, 28, 29A, 29B, 29D, 30A, 30D, 30D1, 30D2, 30E, 31A, 32A, 33D, 33D1, 34A, 40C, 59, 59A, 60, 60A, 61A, 61A1, 61B, 61B1, 61B2, 62, 62A, 62B, 62C, 63, 63A, 63B, 64, 64A, 70, 74, 76, 77, 78, 79, 10, 10A1.
For testing general combining ability (GCA), the well-established traditional varieties 'Bucureștene' and 'Danubiana' were used, as they are known for their genetic recombination potential and have been previously used in hybrid development.
Subsequently, specific combining ability (SCA) testing was conducted using only the parental lines that exhibited both heterosis and uniformity in offspring during the GCA phase. These were subjected to diallel hybridization (each parental line being used as both a maternal and a paternal parent in different crosses), resulting in 31 hybrid combinations.
Parent lines that showed compatibility in hybrid combinations with the tester parents 'Danubiana' and 'Bucureștene' were further promoted to the intensive breeding field for specific combining ability (SCA) testing. Out of the 70 parent lines evaluated in the GCA phase, 31 demonstrated a pronounced heterosis effect, reflected in $\mathbf{F_1}$ hybrids through improved productivity, uniformity, and quality.

Formulas used to determine heterosis intensity
The formula used to calculate the production difference relative to the parental average is called "mid-parent heterosis" (or heterosis relative to the parental mean):

$$(\%) = F1 - \bar{P}/\bar{P} \times 100$$

Where:
F_1 = hybrid performance (e.g., hybrid yield);
$\bar{P}$ = average yield of the two parents (P_1 and P_2).

The formula used to calculate the production difference relative to the best parent is called "high-parent heterosis" (or heterosis relative to the best parent):

$$(\%) = F1 - Pmax/Pmax \times 100$$

Where:
F_1 = hybrid performance (e.g., hybrid yield);
P_{max} = performance of the best parent.

For qualitative phenotypic description, international descriptors from UPOV and IPGRI were used. Statistical analysis was performed using ANOVA in SPSS software.

Description of the 'Bucureștene' variety
The 'Bucureștene' variety, used as a parent, has a globular and slender bush shape. The stem and shoots are light green, while the stem base is woody with a green-brown coloration. The stem height up to the first branching varies between 10 and 15 cm. The fruits are cylindrical, slightly tapered at the tip, with some showing a subtle curvature. At maturity, the fruit develops an intense purple-to-black hue with a strong gloss. The sepals are green and have white spines, which turn brown as the fruit matures. The fruit pulp is dense and pure white.
The main quantitative characteristics of the plant, recorded in two cultivation environments (greenhouse and open field), are presented in Table 1.

Table 1. 'Bucureştene' variety, main characteristics - cultivated in field/greenhouse

Variety/growing medium	Plant height (cm)	Bush diameter (cm)	No. of fruits/plant	Fruit length (cm)	Median diameter of the fruit (cm)	Fruit weight (g)	Total fruit weight per plant (g)
'Bucureştene' - field	62.16±4.4^{a1}	55.4±4.0^{a}	9.66±0.8^{a}	16.42±1.2^{a}	5.56±0.4^{a}	293.2±20.9	2841.84±208.4^{a}
CV	7%	7%	8%	7%	8%	7%	7%
'Bucureştene' - greenhouse	128.2±10.2^{b}	78.4±5.9^{b}	14.52±1.0^{b}	20.42±1.5^{b}	8.1±1.7^{b}	417±28.2^{b}	6076.6±449.0^{b}
CV	8%	8%	7%	7%	21%	7%	7%

[1]Letters represent Duncan test results with confidence interval of 95% and p<0.05

The 'Danubiana' variety, used as a parent, is characterized by a moderate green stem and leaves, with a globular bush shape. The stem height, from ground level to the first branching, varies between 8 and 12 cm. The fruit has an intense black color with a glossy appearance, being elongated-spherical and slightly more voluminous towards the rounded tip. The sepals are green with sparse brown spines. The pulp is slightly dense, with a characteristic white shade. The main quantitative characteristics of the plant, recorded in two cultivation environments (greenhouse and open field), are presented in Table 2.

Table 2. 'Danubiana' variety, main characteristics - cultivated in field/greenhouse

Variety/growing medium	Plant height (cm)	Bush diameter (cm)	No. of fruits/plant	Fruit length (cm)	Median diameter of the fruit (cm)	Fruit weight (g)	Total fruit weight per plant (g)
'Danubiana' - field	59±4.74^{a1}	48±3.84^{a}	6.2±0.49^{a}	15±1.27^{a}	7.4±0.74^{a}	328±29.47^{a}	2033.6±169.24^{a}
CV	8.04%	8.00%	7.93%	8.71%	9.98%	8.98%	8.32%
'Danubiana' - greenhouse	109±8.52^{b}	76±6.36^{b}	12.7±0.98^{b}	15.2±1.38^{b}	9.5±0.79^{b}	458±39.72^{b}	5816.6±481.77^{b}
CV	7.81%	8.37%	7.68%	9.06%	8.32%	8.67%	8.28%

[1]Letters represent Duncan test results with confidence interval of 95% and p<0.05.

RESULTS AND DISCUSSIONS

Table 3. Testing of general combining ability with 'Bucureştene'

Genitor 1	Genitor tester G2	Compatibility	F1 heterosis	F1 uniformity
1C	Bucureştene	NO	-	-
2C	Bucureştene	NO	-	-
3C	Bucureştene	YES	- X	non-uniform
8B	Bucureştene	YES	- X X X	non-uniform
8C	Bucureştene	YES	- X	Uniform
12A	Bucureştene	YES	- X X	Uniform
13B	Bucureştene	YES	+ X X X	Uniform
20	Bucureştene	YES	+ X X X	Uniform
22A	Bucureştene	YES	+ X X	Average
23A	Bucureştene	YES	+ X X	Uniform
23A2	Bucureştene	YES	+ X X X	Average
23A3	Bucureştene	YES	+ X X X	Uniform
23F	Bucureştene	YES	+ X X X	Uniform
23F2	Bucureştene	YES	+ X X	Average
23N	Bucureştene	YES	- X	non-uniform
24	Bucureştene	NO	-	-
25A	Bucureştene	YES	- X X	Average
27B	Bucureştene	YES	+ X	Average
27B1	Bucureştene	YES	+ X X	Uniform
27P	Bucureştene	YES	+ X X X	Uniform
27P1	Bucureştene	YES	+ X X	Uniform
51A	Bucureştene	YES	+ X X X	Average
51A1	Bucureştene	YES	+ X	Uniform
51A2	Bucureştene	YES	+ X X	Uniform
51A3	Bucureştene	YES	+ X X	Uniform
58	Bucureştene	YES	+ X X X	non-uniform
58A	Bucureştene	YES	+ X X X	Uniform
58B	Bucureştene	YES	+ X X	Average
58C	Bucureştene	YES	+ X X X	Uniform
28	Bucureştene	YES	+ X X X	Uniform
29A	Bucureştene	NO	-	-
29B	Bucureştene	NO	-	-
29D	Bucureştene	NO	-	-
30A	Bucureştene	YES	+ X	Uniform
30D	Bucureştene	YES	+ X X	Average
30D1	Bucureştene	YES	- X X X	non-uniform
30D2	Bucureştene	YES	+ X	Average
30E	Bucureştene	YES	- X	Uniform
31A	Bucureştene	YES	+ X X	Uniform
32A	Bucureştene	YES	+ X X X	Uniform
33D	Bucureştene	YES	+ X X	Uniform
33D1	Bucureştene	YES	- X X	Average
34A	Bucureştene	YES	+ X X	Uniform
40C	Bucureştene	NO	-	-
59	Bucureştene	YES	+ X X X	non-uniform
59A	Bucureştene	YES	+ X X	Average
60	Bucureştene	YES	+ X X X	Uniform
60A	Bucureştene	YES	+ X X	Uniform
61A	Bucureştene	YES	+ X X X	non-uniform
61A1	Bucureştene	YES	+ X X X	Uniform
61B	Bucureştene	YES	+ X X	Uniform
61B1	Bucureştene	YES	+ X X	Average
61B2	Bucureştene	YES	+ X X X	non-uniform
62	Bucureştene	YES	- X X	Average
62A	Bucureştene	YES	- X	Uniform
62B	Bucureştene	YES	+ X X	Uniform
62C	Bucureştene	YES	+ X X X	Uniform
63	Bucureştene	YES	+ X X X	Uniform
63A	Bucureştene	YES	+ X X X	non-uniform
63B	Bucureştene	YES	+ X X	Uniform
64	Bucureştene	YES	+ X X	Average
64A	Bucureştene	YES	+ X X	Average
70	Bucureştene	YES	+ X X X	Uniform
74	Bucureştene	NO	-	-
76	Bucureştene	YES	+ X X	Uniform
77	Bucureştene	YES	+ X X	non-uniform
78	Bucureştene	YES	+ X X	Average
79	Bucureştene	YES	+ X X X	Uniform
10	Bucureştene	YES	+ X X X	Average
10A1	Bucureştene	YES	+ X X	Uniform

*- XXX = -30% production; - XX = -20% production; - X = -10% production; + X = 10% production; + XX = 20% production; + XXX = 30% production

In Table 3 the hybrid combinations resulting from crosses with the tester parent line 'Bucureștene' are presented.
Compatibility is marked as "YES" or "NO." This reflects the ability of the parent lines to cross successfully, which is essential for the success of any hybridization program.

- "YES" means that the cross is possible and genetically viable.
- "NO" (recorded in 8 hybrid combinations) indicates genetic incompatibility, which may result in the inability to produce viable or productive offspring.

The intensity of the heterosis phenomenon was calculated based on the percentage by which the F_1 hybrid exceeded the parental average in terms of yield (Figure 1).

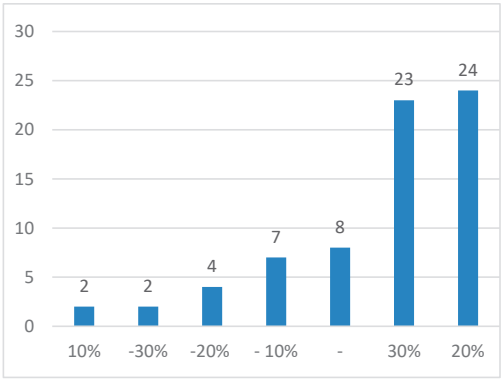


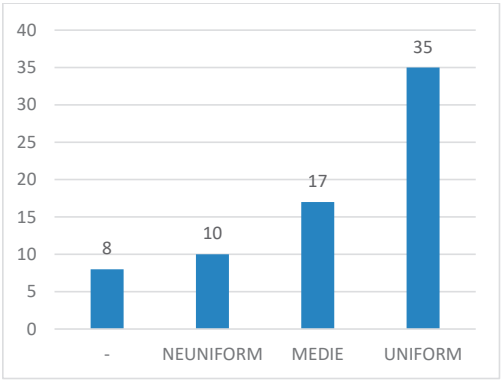
Figure 1. The intensity of the heterosis phenomenon for hybrid combinations with the 'Bucureștene' parent

The positive heterosis phenomenon is the most frequently observed and intense in hybrid combinations, with 30% and 20% heterosis values. In contrast, negative heterosis is rarer and less intense in this hybrid population.

- 24 hybrid combinations exhibited 20% heterosis;
- 23 hybrid combinations recorded a 30% increase in yield;
- Only 2 combinations showed negative heterosis (-30%).

This indicates that, in general, most tested lines exhibit a high heterosis intensity, suggesting good genetic compatibility between parents and a potential improvement in desirable traits.
Uniformity in F_1 offspring is an important indicator of the stability and predictability of traits expressed after crossing (Figure 2). The hybrid combinations were grouped into uniformity categories as follows:

- Uniform (35 hybrid combinations): All F_1 hybrids exhibit very similar traits, suggesting that the hybridization is consistent and stable.
- Non-uniform (10 hybrid combinations): The F_1 offspring are variable in traits, which may indicate genetic segregation due to the diversity of the parent lines.
- Average (17 hybrid combinations): This category suggests a moderate uniformity in F_1 offspring, neither perfectly uniform nor highly variable.



- Figure 2. Uniformity of F_1 offspring for hybrid combinations with the 'Bucureștene' parent

For example, lines 1C and 2C are marked as having non-uniform offspring, which could reflect greater variability in the inherited traits, impacting the stability of the F_1 generation. On the other hand, uniform lines in the offspring (e.g., 60, 62, 79, 70, 76, 10 A1, etc.) suggest stable genetic performance, favorable for the breeding process.
Line 58 represents a special case as it exhibits a high intensity of heterosis (30%) but shows non-uniform offspring. This could indicate significant potential for trait improvement and high variability, which might be more challenging to control in selection.
In general, the tested lines showed compatibility in hybridization, strong heterosis, and good uniformity in the offspring, suggesting that these crosses are viable and can lead to superior and high-performing hybrids.

In Table 4, the hybrid combinations resulting from crosses with the tester parent line 'Danubiana' are presented.

Table 4. Testing of general combining ability with 'Danubiana'

Crt. no.	Line G1	Genitor tester G2	Compatibility	F1 heterosis	F1 uniformity
1	1C	Danubiana	NU	-	-
2	2C	Danubiana	NU	-	-
3	3C	Danubiana	DA	-X X X	non-uniform
4	8B	Danubiana	DA	- X X	non-uniform
5	8C	Danubiana	DA	- X X X	non-uniform
6	12A	Danubiana	DA	- X	average
7	13B	Danubiana	DA	+ X X X	Uniform
8	20	Danubiana	DA	- X X	non-uniform
9	22A	Danubiana	DA	- X	non-uniform
10	23A	Danubiana	DA	+ X X X	Uniform
11	23A2	Danubiana	NU	-	-
12	23A3	Danubiana	DA	+ X X	Uniform
13	23F	Danubiana	DA	+ X	average
14	23F2	Danubiana	DA	- X	non-uniform
15	23N	Danubiana	NU	-	-
16	24	Danubiana	DA	+ X	non-uniform
17	25A	Danubiana	DA	+ X X	average
18	27B	Danubiana	NU	-	-
19	27B1	Danubiana	DA	- X X	non-uniform
20	27P	Danubiana	DA	- X X X	non-uniform
21	27P1	Danubiana	DA	+ X	non-uniform
22	51A	Danubiana	DA	+ X	non-uniform
23	51A1	Danubiana	DA	+ X X	Uniform
24	51A2	Danubiana	DA	+ X	average
25	51A3	Danubiana	DA	- X	non-uniform
26	58	Danubiana	DA	+ X X	average
27	58A	Danubiana	DA	+ X X X	Uniform
28	58B	Danubiana	DA	- X	average
29	58C	Danubiana	DA	+ X X	Uniform
30	28	Danubiana	DA	+ X X X	Uniform
31	29A	Danubiana	NU	-	-
32	29B	Danubiana	NU	-	-
33	29D	Danubiana	NU	-	-
34	30A	Danubiana	DA	- X X	average
35	30D	Danubiana	DA	- X	non-uniform
36	30D1	Danubiana	NU	-	-
37	30D2	Danubiana	DA	- X X	average
38	30E	Danubiana	DA	- X X X	non-uniform
39	31A	Danubiana	DA	+ X	Uniform
40	32A	Danubiana	DA	+ X X	average
41	33D	Danubiana	DA	+ X X X	Uniform
42	33D1	Danubiana	DA	- X X X	non-uniform
43	34A	Danubiana	DA	+ X X X	Uniform
44	40C	Danubiana	DA	- X	average
45	59	Danubiana	DA	+ X X	Uniform
46	59A	Danubiana	DA	+ X X	Uniform
47	60	Danubiana	DA	+ X X X	Uniform
48	60A	Danubiana	DA	+	Uniform
49	61A	Danubiana	DA	+ X X	average
50	61A1	Danubiana	DA	+ X X X	Uniform
51	61B	Danubiana	DA	+ X X X	Uniform
52	61B1	Danubiana	DA	+ X	non-uniform
53	61B2	Danubiana	DA	+ X X	Uniform
54	62	Danubiana	DA	- X	Uniform
55	62A	Danubiana	DA	+ X	average
56	62B	Danubiana	DA	+ X X X	Uniform
57	62C	Danubiana	DA	+ X	average
58	63	Danubiana	DA	+ X X	non-uniform
59	63A	Danubiana	DA	+ X X X	Uniform
60	63B	Danubiana	DA	+ X X	Uniform
61	64	Danubiana	DA	+ X X X	Uniform
62	64A	Danubiana	DA	+ X X X	Uniform
63	70	Danubiana	DA	+ X X X	Uniform
64	74	Danubiana	NU	-	-
65	76	Danubiana	DA	+ X X X	Uniform
66	77	Danubiana	DA	+ X	average
67	78	Danubiana	DA	- X	Uniform
68	79	Danubiana	DA	+ X X X	Uniform
69	10	Danubiana	DA	+ X X	Uniform
70	10A1	Danubiana	DA	+ X X X	non-uniform

*- XXX = -30% production; - XX = -20% production; - X = -10% production; + X = 10% production; + XX = 20% production; + XXX = 30% production.

Regarding compatibility, unlike the previous test where the tester parent 'Bucureștene' was used and 8 hybrid combinations were found to be incompatible, crossing with the tester parent 'Danubiana' resulted in 10 incompatible hybrid combinations.

The hybrid combinations 13B, 23A, 33D, 40C, 76 x 'Danubiana' recorded a 30% increase in yield, indicating a strong heterosis performance. These can be considered the best in terms of combining favorable traits from their parents (Figure 3).

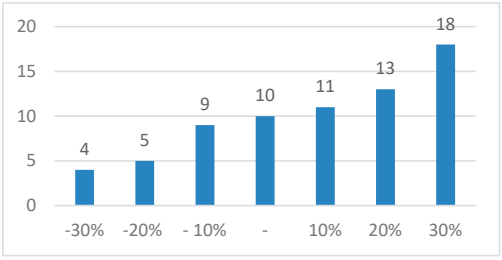


Figure 3. The intensity of the heterosis phenomenon for hybrid combinations with the 'Danubiana' parent

Hybrids with average performance, e.g., 12A, 17B, 30A x 'Danubiana', exhibit moderate heterosis.

In the case of hybrid combinations 1C, 2C, 8B x 'Danubiana', it can be observed that heterosis is not expressed uniformly across the entire offspring, and the traits are not distributed evenly.

Comparative with the results from the previous test, which involved the tester parent 'Bucureștene', where 30% heterosis was observed in 23 hybrid combinations, this time, only 18 combinations achieved this production increase. Additionally, it can be observed that the number of hybrid combinations exhibiting negative heterosis of -30%, -20%, -10% is very high, with 18 combinations, in contrast to the previous test where only 8 lines showed negative heterosis.

Regarding the uniformity of the offspring (Figure 4), the hybrid combinations were grouped as follows:

- Uniform (28 hybrid combinations), compared to 35 uniform hybrid combinations recorded in the test with the 'Bucureștene' tester parent.
- Non-uniform (18 hybrid combinations), a higher number than the 10 non-uniform

combinations recorded in the test with the 'Bucureștene' tester parent.

- Average (14 hybrid combinations), a lower value compared to the 17 hybrid combinations recorded in the previous test.

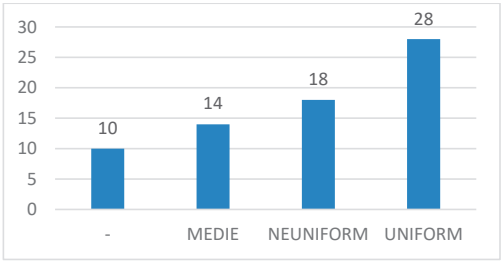


Figure 4. Uniformity of F_1 offspring for hybrid combinations with the 'Danubiana' parent

Lines with high heterosis are on row 1 – P1 (L20, 0.78 kg), P2 (L58, 4.13 kg), F1 Hybrid = 5.32 kg, resulting in average heterosis of 117.13% and BPH = 29%. This suggests that the resulting hybrid showed a significant increase in production compared to its parents. Similarly, the last entry (L10 A1 × L20) has heterosis of 246.8%, indicating an extremely successful combination (Figure 5).

Unfavorable combinations with negative heterosis, such as L76 × L61 A (-65%) or L58 C × L76 (-38%), indicate weaker hybrids than their parents. These combinations would not be suitable for further selection.

Hybrids with very high BPH:

- Row 16: L13 B × L58 C → 163.7%
- Row 31: L10 A1 × L20 → 246.8%

These could be good candidates for further testing or commercial production.

Crt. no.	P1	P1 kg/plant	P2	P2 kg/plant	M.P. kg/plantă	H F1 kg/plant	heterosis %	BPH %
1	L 20	0.76	L 58	4.13+E35E	2.45	5.32	117.13	29%
2	L 23 A2	4.26	L 10	8.31	6.29	9.78	55.62	56%
3	L 23 A3	1.75	L 61 A1	4.39	3.07	7.16	133.29	63%
4	L 23 F	3.92	L 61 B	7.65	5.78	5.84	0.94	-24%
5	L 27 P	1.64	L 79	11.1	6.37	7.2	13.08	-35%
6	L 51 A	10.47	L 64	10.67	10.57	11.96	13.15	12%
7	L 58	4.13	L 62 B	5.46	4.8	7.51	56.56	38%
8	L 58 C	3.28	L 76	11.3	7.29	6.95	-4.62	-38%
9	L 32 A	5.26	L 58 A	4.54	4.9	7.72	57.55	47%
10	L 59	7.11	L 70	10.73	8.92	14.98	67.8	40%
11	L 61 A	4.39	L 64 A	6.87	5.63	9.36	66.35	36%
12	L 61 B2	7.52	L 10 A1	6.08	6.8	5.75	-15.38	-23%
13	L 62 C	4.12	L 13 B	3.9	4.01	7.91	97.06	92%
14	L 63 B	6.07	L 63 A	4.83	5.45	9.68	77.75	60%
15	L 10	8.31	L 23 A	2.9	5.6	5.83	4.08	-30%
16	L 13 B	3.9	L 58 C	3.28	3.59	9.47	163.76	143%
17	L 23 A	2.9	L 51 A	10.47	6.69	8.93	33.62	-15%
18	L 58 A	4.54	L 28	5.57	5.05	9.86	95.02	77%
19	L 28	5.57	L 23 F	3.92	4.74	2.82	-40.6	-49%
20	L 34 A	4.1	L 27 P	1.64	2.87	3.75	30.84	-8%
21	L 60	7.75	L 59	7.11	7.43	11.74	57.96	51%
22	L 61 A1	4.39	L 62 C	4.12	4.26	7.83	84.05	79%
23	L 61 B	7.65	L 63	5.81	6.73	4.73	-29.8	-38%
24	L 62 B	5.46	L 32 A	5.26	5.36	6.98	30.22	28%
25	L 63 A	4.83	L 60	7.75	6.29	10.13	61.07	31%
26	L 64	10.67	L 23 A 3	2.9	6.78	5.23	-22.95	-51%
27	L 64 A	6.87	L 79	11.1	8.98	12.96	44.3	17%
28	L70	10.73	L 61B2	7.52	9.12	6.32	-30.74	-41%
29	L 76	11.3	L 61 A	4.39	7.84	3.92	-50.07	-65%
30	L 79	11.1	L 63 A	4.83	7.96	10.69	34.28	-4%
31	L 10 A1	6.08	L 20	0.76	3.42	11.86	246.8	95%

*The numerical positions on the Y-axis represent the serial number of the hybrid combination from Table 4

Figure 5. Testing of specific combining ability

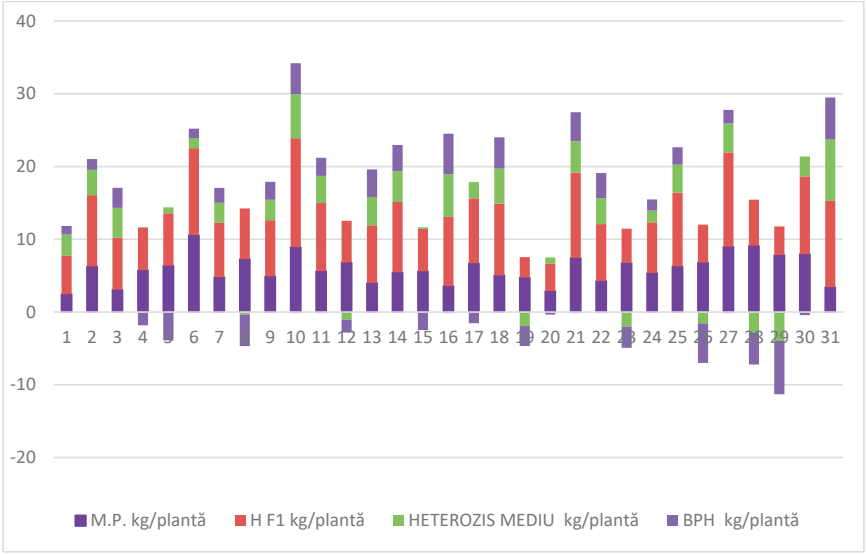


Figure 6. Graphical representation for combining ability testing

Figure 6 illustrates the performance of 31 hybrid combinations evaluated for yield-related traits, expressed in kilograms per plant. Four parameters are represented: the mean of the parents (M.P.) in purple, the F1 hybrid yield (H F1) in red, the mid-parent heterosis (green), and the best-parent heterosis (BPH) in light purple.

A notable number of hybrids display significant positive heterosis over both the mid-parent and the best parent, indicating favourable genetic interactions and superior hybrid vigour. For instance, 9, 11, and 31 combinations exhibit remarkably high F1 performance, suggesting strong specific combining ability.

On the other hand, a few combinations (e.g., 23 and 30) show negative BPH values, implying that their F1 performance fell below that of the better-performing parent. These results are critical for identifying the most promising hybrids for future selection and potential commercialization.

CONCLUSIONS

A total of 70 genitors were evaluated for general combining ability (GCA), of which 31 demonstrated favorable aptitude for hybridization.

Testing the specific combining ability (SCA) resulted in the development of 31 hybrid combinations.

The heterosis phenomenon manifested differently among the combinations, highlighting significant variations in yield, with certain hybrids showing superior performance.

Research will continue with field trials of the highest-yielding hybrids, alongside well-established control hybrids in Romania, aiming for the official registration and patenting of the most promising variants.

The seeds of the new hybrids were distributed free of charge across Romania to be tested under diverse soil and climate conditions, thus facilitating the zoning and microzoning process. The progenies resulting from both GCA and SCA testing will be further studied to identify new valuable genotypes, varieties, and hybrids.

REFERENCES

Birchler, J.A.; Yao, H.; Chudalayandi, S.; Vaiman, D.; Veitia, R.A. (2010). *Heterosis. Plant Cell*, 22, 2105–2112.

Hallauer, A.R., & Miranda Filho, J.B. (1988). *Quantitative Genetics in Maize Breeding.* Iowa State University Press.

Kaushik, P., Prohens, J., Vilanova, S., Gramazio, P., & Plazas, M. (2016). Phenotyping of Eggplant Wild Relatives and Interspecific Hybrids with Conventional and Phenomics Descriptors Provides Insight for Their Potential Utilization in Breeding. *Frontiers in Plant Science*, 7: 677. doi: 10.3389/fpls.2016.00677

Kalloo, G. (1993). *Eggplant, Solanum melongena L. - An important solanaceous vegetable crop.*

Knapp, S., Vorontsova, M. S., & Prohens, J. (2013). *Wild relatives of the eggplant (Solanum melongena L.: Solanaceae): a new understanding of species names in a complex group.* PLoS One, 8(2), e57039.

Kumar, A., Sharma, V., Jain, B.T., & Kaushik, P. (2020). Heterosis Breeding in Eggplant (*Solanum melongena* L.): Gains and Provocations. *Plants* (Basel), *9*(3), 403. doi: 10.3390/plants9030403

Muñoz-Falcón, J.E., Prohens, J., Vilanova, S., & Nuez, F. (2009). Diversity in commercial varieties and landraces of black eggplants and implications for broadening the breeders' gene pool. *Annals of Applied Biology*, 154: 453–465.

Prohens, J., et al. (2012, 2013). *EGGNET and IBPGR descriptors are used for evaluating segregating generations of interspecific crosses between eggplant and related species.*

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

GLOBAL DEVELOPMENT TRENDS AND STRATEGIC OUTLOOK OF VERTICAL AGRICULTURE

Qichang YANG[1], Florin STĂNICĂ[2], Yuxin TONG[3], Bateer BAIYIN[1]

[1]Institute of Urban Agriculture, Chinese Academy of Agricultural Sciences, Chengdu, China
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, Bucharest, Romania
[3]Institute of Environment and Sustainable Development in Agriculture, Chinese Academy of Agricultural Sciences, Beijing, China

Corresponding author email: yangqichang@caas.cn

Abstract

This paper aims to comprehensively understand the global development trends of vertical agriculture, analyze the key factors influencing its development, and then formulate forward-looking and feasible strategic plans. This research has both theoretical and practical significance. It can enrich the theories of agriculture and industrial development, guide enterprise decision-making, and assist in policy formulation, thereby ensuring global food security and promoting the sustainable development of agriculture. This paper systematically introduces the development history and trends of the vertical agriculture industry, comprehensively analyzes the key technologies and breakthroughs in the current vertical agriculture field, and examines the bottlenecks, challenges, and feasible countermeasures in industrial application. From a strategic perspective, it puts forward policy suggestions to promote the development of vertical agriculture, providing references and guidance for industry decision-makers and researchers.

Key words*: vertical agriculture, light efficiency, development suggestions, urban agriculture, smart agriculture.*

INTRODUCTION

In recent years, vertical agriculture has been emerging as a global development hotspot. Vertical agriculture is an advanced agricultural system. Through internal high-precision environmental control, such as using computers to automatically control environmental factors like temperature, humidity, light, CO_2 concentration, and nutrient solutions during the crop growth process, the production process is not restricted by natural conditions. It enables continuous and efficient crop production throughout the year and can significantly reduce labour costs (Yang, 2019).

Vertical agriculture makes full use of high-end technological means such as new materials, semiconductor light sources, biotechnology, hydroponics, intelligent decision-making, and even artificial intelligence and robots. This technology-intensive production method is internationally recognized as the most advanced stage of development in protected agriculture.

Vertical agriculture has a wide range of unique application scenarios. In urban environments, it can be established using idle urban spaces such as abandoned factories, rooftops of high-rise buildings, and underground garages to produce fresh, safe, and high-quality agricultural products like vegetables and herbs, meeting the urban residents' demand for green food and alleviating the shortage of urban land resources (Blom et al., 2023). In remote areas or places with harsh natural conditions, such as deserts, islands, and polar regions, vertical agriculture can overcome the limitations of extreme climates and soil conditions, providing a stable food supply for local residents and ensuring their basic living needs. In the aerospace field, vertical agriculture is one of the key technologies for long-term survival in space, providing astronauts with fresh vegetables and oxygen to maintain the balance of the life-support system. In addition, in the scientific research field, vertical agriculture can precisely control environmental factors such as light, temperature, humidity, and gas composition, providing an ideal experimental platform for research in plant physiology, genetic breeding, etc., which helps to accelerate the scientific research process and achieve innovative results.

There are numerous reasons why vertical agriculture has attracted global attention. Firstly, the global population is growing

rapidly while the available arable land is constantly decreasing, posing challenges to food security. According to the prediction of the Food and Agriculture Organization of the United Nations (FAO), by 2050, the global population will reach 9.1 billion, and the food demand will increase by more than 70% compared to the present, while the per-capita arable land will decrease by more than 20% (FAO 2009). This has led people to pay increasing attention to applying modern technologies and innovative methods such as vertical agriculture to improve agricultural production efficiency and meet the growing global food demand with limited arable land. Secondly, the high-precision environmental control in vertical agriculture can effectively reduce the use of pesticides and fertilizers, decrease environmental pollution, and provide safer and healthier agricultural products. It has become an important means to drive the transformation of healthy food production methods. In addition, the aging of the agricultural workforce and the reluctance of young people to engage in agriculture are becoming increasingly prominent. In some countries such as China and Japan, more than 60% of the agricultural labor force is over 60 years old. In this context, as a modern and high-tech agricultural production method, vertical agriculture provides more attractive agricultural employment opportunities for the younger generation and is expected to alleviate the problem of the aging agricultural workforce and promote the development of agricultural production in a more healthy and sustainable direction (Yang, 2019).

This paper conducts an in-depth analysis of the development process and innovative breakthroughs of vertical agriculture technology, and comprehensively investigates and summarizes the key technological breakthroughs in aspects such as improving production and resource utilization efficiency and facility automation. The paper also elaborates on the difficulties and challenges faced by the vertical agriculture industry in the process of achieving commercial application, including problems such as high costs, lack of market demand, and insufficient commercial profitability. It explores solutions from multiple aspects such as technology, funds, and policies, aiming to provide theoretical support and practical guidance for the healthy development of the vertical agriculture industry.

DEVELOPMENT PROCESS OF VERTICAL AGRICULTURE

Vertical agriculture has a history of over 60 years to date and has mainly gone through three stages. The early exploration stage began in the 1950s. Breakthroughs were made in “artificial simulated environment and climate control technology” and “nutrient solution cultivation technology”. Vertical agriculture started in developed countries and regions in Europe and America. In 1957, Denmark built the world's first vertical farm covering an area of 1,000 square meters. In 1960, the United States started to research and develop vertical farms using artificial light. In 1963, Austria built a 30-meter-high tower-type vertical farm. However, there were problems such as imperfect environmental control in technology, high costs, low automation levels, and a limited variety of applicable crops.

The rapid development stage spanned from the 1960s to the end of the 1990s. Numerous enterprises in countries like the United States, the Netherlands, and Japan carried out R & D and achieved remarkable technological results. The first real vertical farm using artificial light was established, and the industrial scale gradually expanded. Nevertheless, the application of light sources was restricted. High-pressure sodium lamps were initially used and later replaced by fluorescent lamps. The relatively high energy consumption limited large-scale promotion.

The stage of gradual maturity started in the 21st century. With the emergence of blue-light LED technology (Kusuma et al., 2020), the successful development of red-blue LED combined light sources, and the widespread application of intelligent control technology, the energy consumption of vertical agriculture has been reduced, and the space utilization rate has been improved. By 2022, there were about 300 vertical farms in Japan. South Korea has launched a variety of products and owns the world's largest tunnel-type vertical farm. The United States has carried out R & D and industrialization of various types of vertical agriculture, and some enterprises have received

substantial financing. In Europe, for example, the United Kingdom is also building vertical farms.

According to a 2023 prediction by Mordor Intelligence, a well-known international consulting firm, the global vertical agriculture industry will enter a period of rapid development in the next five years, with an average annual growth rate of over 10%. In Europe, the average annual growth rate of vertical agriculture will reach more than 22%, and its market size will reach $4.19 billion (approximately RMB 30.3 billion) in 2029 (Mordor Intelligence 2023). The New York-based fund company Insight Partners predicts that the global vertical agriculture market size will reach $19.864 billion (approximately RMB 143.8 billion) in 2028 (The Insight Partners 2021). Some well-known international enterprises (such as Siemens (Siemens vertical farming 2023), General Electric (General energy 2020), etc.) have also entered the field of vertical agriculture technology R & D and actively participated in industrialization promotion. The vertical agriculture-related industries globally are showing a booming development trend.

KEY TECHNOLOGIES IN VERTICAL AGRICULTURE

Vertical agriculture is an industry highly intensive in technology. Meanwhile, it incurs relatively high investment costs and operational energy consumption. Over the years, countries around the world have been conducting technological research to meet industrial demands such as significantly reducing energy consumption, enhancing the level of intelligence, and improving product quality, and have made important progress. In particular, a series of innovative achievements have been made in aspects such as the creation of plant light recipes and their LED light sources, energy-saving environmental control through light-temperature coupling, the improvement of vegetable nutritional quality, and AI-based intelligent management and control (Chang et al., 2021). These achievements have continuously enhanced the production efficiency of vertical agriculture and strongly supported the development of the vertical agriculture industry.

Vertical agriculture is technology-intensive, with high investment costs and high operational energy consumption. Over the years, countries have been working on technological breakthroughs to reduce energy consumption, improve the level of intelligence, and enhance product quality. Innovative achievements have been made in areas such as the creation of plant light recipes and LED light sources, which support the development of the industry. In terms of light source technology, the progress of new semiconductor materials has promoted the improvement of the luminous efficacy of LEDs in vertical agriculture. Light sources mainly composed of red and blue LEDs have been created, and the optimized parameters of light recipes have been mastered, enabling intelligent control of the dynamic light environment. Laser light sources have also been developed to reduce energy consumption.

Advances in environmental control technology have reduced the energy consumption of air-conditioning systems. The combination with clean energy has gradually made commercial profitability a reality. The expansion of vertical space has attracted attention. Multi-layer vertical farms have been built in countries such as China, Japan, the United Kingdom, and the United States. The Chinese Academy of Agricultural Sciences has built a 20-storey vertical farm (Figure 1).

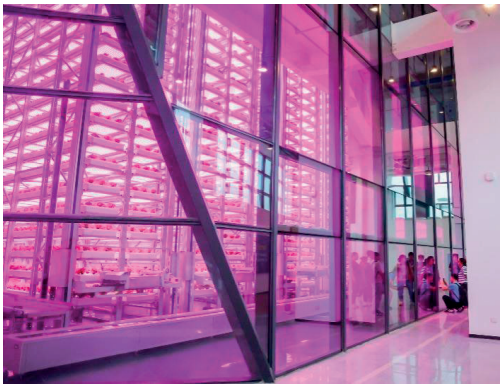


Figure 1. The vertical agriculture in Institute of Urban Agriculture, Chinese Academy of Agricultural Sciences

Building-type vertical agricultural systems can reduce energy consumption. The Chinese Academy of Agricultural Sciences has proposed the concept of a skyscraper plant

building (Zhang et al., 2023). Reducing labor costs and improving mechanization and automation are the research and development directions. Environmental monitoring and coordinated control are achieved through intelligent sensors, and complete sets of equipment and intelligent devices have been created.

Intelligent management and control technology has become an important direction, laying the foundation for intelligent unmanned operations. The combination of vertical agriculture and AI is an innovative approach. AI can monitor and adjust the growth environment, optimize decision-making, achieve personalized planting, and also automate harvesting and quality inspection, optimizing energy utilization efficiency and promoting the intelligent and sustainable development of agriculture (Chang et al., 2021). In addition, vertical agriculture provides support for space exploration and the development of remote areas. It can provide food and oxygen in the extreme environment of space. In harsh regions such as deserts and polar areas, it can efficiently utilize resources and provide a stable food supply.

THE INDUSTRIALIZATION OF VERTICAL AGRICULTURE

Vertical agriculture, as an important direction for the development of protected agriculture at present and in the future, has unique advantages such as significantly improving resource utilization efficiency, greatly increasing crop yield and quality, and attracting young people to engage in agriculture. However, it also faces many challenges in the development process (Van et al., 2021).

Its investment and operation costs include construction and equipment costs (the cost of buildings, artificial light sources, and cultivation systems accounts for over 70%, with artificial light sources accounting for 40-50%, and the average initial construction cost being 400-600 yuan per square meter) and production and operation costs (including electricity bills, materials, labor fees, etc., as well as equipment depreciation fees).

Energy consumption is also a limiting factor, accounting for about 28% of the total operating costs (Kozai et al., 2018). Artificial light illumination accounts for 60%, air-conditioning accounts for 35%, and other equipment accounts for 5%. Although there are energy-saving technologies, 7-10 kWh of electricity is still required for each kilogram of leafy vegetables.

The specialized market for vertical agriculture involves multiple entities and rich trading contents, and can bring important values from the economic, social, agricultural, and technological aspects. It can be cultivated through strategies such as products, prices, channels, promotions, market education, and cooperation alliances. Currently, due to large upfront investments, immature market cultivation, and operating models, the profitability level is not satisfactory. For example, about 20% of vertical farms in Japan are operating at a loss (Kozai et al., 2018).

To achieve profitability, it is necessary to improve yield and quality, precisely regulate the environment and nutrition, conduct phased cultivation, coordinately regulate light and nutrient supply, and select functional plants. However, with technological and business model innovation, it is estimated that by 2030, the construction investment cost will be reduced by 40%, the operating cost will be reduced by 60%, the added value of products will increase by 40%, and the comprehensive benefits will increase by over 120%. At that time, the economic benefits and large-scale application will be significantly improved.

SUGGESTIONS FOR THE DEVELOPMENT OF VERTICAL AGRICULTURE

As a “future scenario” for modern agriculture, vertical agriculture is crucial for ensuring food security and addressing climate change and resource constraints. It requires the coordinated promotion of policy guidance, technological innovation, international cooperation, and market cultivation. Countries should formulate differentiated strategies to promote its efficient, low-carbon, and sustainable development. The specific measures are as follows: Firstly, strengthen the cultivation of professional talents. Establish a talent cultivation system jointly formed by universities, research institutions, and enterprises, and formulate attractive talent policies to provide high-quality and interdisciplinary talents. Secondly, from a

strategic perspective, increase the intensity of innovation and R & D. Vertical agriculture is a key area in the international competition of agricultural high-tech. Finally, promote the industrial development in a step-by-step manner. Formulate long-term plans, start with small-scale demonstrations and then carry out large-scale promotions. Prioritize meeting the needs of special places and increase publicity to enhance public awareness and product recognition.

ACKNOWLEDGEMENTS

This research work was carried out with the support of Agricultural Science and Technology Innovation Program (ASTIP), and National Key Research and Development Program of China (2023YFF1001500).

REFERENCES

Blom, T., Jenkins, A., & van den Dobbelsteen, A. (2023). Synergetic integration of vertical farms and buildings: reducing the use of energy, water, and nutrients. *Frontiers in Sustainable Food Systems*, *7*, 1227672.

Chang, C. L., Chung, S. C., Fu, W. L., & Huang, C. C. (2021). Artificial intelligence approaches to predict growth, harvest day, and quality of lettuce (*Lactuca sativa* L.) in a IoT-enabled greenhouse system. *Biosystems Engineering*, *212*, 77-105.

Chang, C. L., Huang, C. C., & Chen, H. W. (2022). Design and implementation of artificial intelligence of things for tea (*Camellia sinensis* L.) grown in a plant factory. *Agronomy*, *12*(10), 2384.

Food and Agriculture Organization, "Global agriculture towards 2050", *Proceedings of the High Level Expert Forum - How to Feed the World*. 2009, 2050: 1-4.

General energy, Vertical agriculture, https://www.ge.com/news/reports/tag/Vertical%20Agriculture.

Kozai, T. (2018). Smart plant factory. *The Next Generation Indoor Vertical Farms;* Springer: Singapore, 238.

Kusuma, P., Pattison, P. M., & Bugbee, B. (2020). From physics to fixtures to food: Current and potential LED efficacy. *Horticulture Research*, *7*.

Mordor Intelligence, Vertical Farming Market Size & Share Analysis - Growth Trends & Forecasts (2024-2029), 2023. https://www.fortunebusinessinsights.com/industry-reports/vertical-farming-market-101958

Siemens vertical farming, "Vertical farming is flourishing thanks to digitalization", https://www.siemens.com/global/en/company/stories/industry/2023/vertical-farming-controlled-environment-agriculture-cea-digital-transformation-sustainability-80-acres-farms.html.

The Insight Partners, "Vertical Farming Market Forecast to 2028", 2021. https://www.researchandmarkets.com/reports/5516173/vertical-farming-market-forecast-to-2028-covid

Van Delden, S. H., SharathKumar, M., Butturini, M., Graamans, L. J. A., Heuvelink, E., Kacira, M., ... & Marcelis, L. F. M. (2021). Current status and future challenges in implementing and upscaling vertical farming systems. *Nature Food*, *2*(12), 944-956.

YANG Q C. (2019). *Plant factory*. Tsinghua University Press, Beijing.

Zhang, L., Huang, L., Li, T., Wang, T., Yang, X., & Yang, Q. (2023). The skyscraper crop factory: a potential crop-production system to meet rising urban food demand. *Engineering*, *31*, 70-75.

FLORICULTURE, ORNAMENTAL PLANTS, DESIGN AND LANDSCAPE ARCHITECTURE


EX·TERRA·AVRVM

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

THE BEHAVIOR OF *ARUM ITALICUM* MILL. IN IAŞI ECOLOGICAL CONDITIONS (NORTHEASTERN ROMANIA)

Lucia DRAGHIA[1], Maria CANTOR[2], Maria APOSTOL[1], Roberto-Renato BERNARDIS[1], Iulia MINEAȚĂ[3]

[1]"Ion Ionescu de la Brad" Iaşi University of Life Sciences,
3 Mihail Sadoveanu Alley, 700490, Iaşi, Romania
[2]University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca,
3-5 Calea Mănăștur Street, 400372, Cluj-Napoca, Romania
[3]Research and Development Station for Fruit Growing Iași,
175 Voineşti Street, 400015, 700620, Iaşi, Romania

Corresponding authors emails: marcantor@yahoo.com, mariabrinza2007@yahoo.com

Abstract

The genus Arum includes approx. 29 species and is the only genus of the family Araceae with representatives reaching the temperate zone of Europe. The present study aims to bring to attention a species of Arum native to the Mediterranean area (A. italicum Mill.), in order to establish the possibility of being cultivated in Iași, in the NE area of Romania. Phenological and biometric observations of plants in experimental crops were compared with data from the literature reported for plants in natural habitats. It was found that in the conditions of Iași, A. italicum wintered in the field without protection (it behaved like a rustic geophyte), but the foliage, formed in autumn, disappeared during the summers when very low temperatures occurred and reappeared in spring (March), and flowering began at least a month later. The biometric determinations on morphological characters of decorative interest were within the limits specified in specialized bibliographic references. The results obtained show a good ability of the species to adapt to a temperate continental climate with excessive variations and can be recommended for garden decoration.

Key words*: ecological adaption, italian lord-and-ladies, ornamental characteristics, photosynthetic pigments.*

INTRODUCTION

The genus *Arum* is a member of the Araceae family, a large family, which includes monocotyledonate, perennial geophytic plants, characterized by an impressive morphological diversity, which has led to reports of the number of genera and species ranging from 105-125 genera and 2000-4000 species (Alobaide et al., 2024; Alpinar et al., 2023; Espíndola et al., 2010; Henriquez et al., 2010; Linz et al., 2010; Sîrbu and Paraschiv, 2005; Zargoun et al., 2020), up to 140 genera and 4000-6000 species (Cole, 2024). Species native to tropical regions predominate within the family, although some can also be found in temperate or circumboreal regions, in a wide range of ecological habitats from sea level to altitudes above 3000 m, mainly terrestrial, epiphytic, climbing, less often aquatic (Cole, 2024; Espíndola et al., 2010; Henriquez et al., 2010; Linz et al., 2010). The members of the Araceae family are characterized by special inflorescences, of the spadix type, with protogynous hermaphrodite flowers, and by the presence in the tissues of calcium oxalate crystals with a much greater diversity than in other related families (Ağalar, 2016; Cole, 2024; Henriquez et al., 2010; Leguet et al., 2014; Sîrbu, 2020).

The genus *Arum* comprises species distributed in Central and Northern Europe, the Middle East, North Africa and Central and Western Asia, with the greatest diversity in the Mediterranean region. It is considered that the Aegean and Anatolian regions were the ones that sheltered, for a long time, the ancestral lines of the genus, the major dispersal to the current distribution areas being after the late Miocene (ca. 10 Mya). The expansion from the Aegean Sea and the colonization of Eastern Europe and the Arabian Plate is estimated to have taken place during the early Pliocene (5.3-2.6 Mya), while the colonization of the North African region, took place later, during the Pleistocene era (Espíndola et al., 2010).

Arum is one of the few genera of the family Araceae that reaches the temperate region of Europe (Riedl, 1980). In the flora of Romania, two species are mentioned: *A. maculatum* (with subsp. *maculatum*) and *A. orientale* (with subsp. *orientale*), together with three inclusive taxa, they are part of the affinity circle of the previous species and require further studying: *A. alpinum, A. intermedium* and *A. maculatum* subsp. *besseranum* (Oprea, 2005).

The number of circumscribed species ranged from 26-29 (Alobaide et al., 2024; Al-Qudah, 2016; Azab, 2017; Cole, 2024; Boyce, 1993, 2006; Espíndola et al., 2010; Linz et al., 2010; Ollerton and Diaz, 1999; Urru et al., 2011; Zargoun et al., 2020), taxonomic controversies being determined by different causes: the exclusive description of plants according to herbarium specimens, with less obvious characteristics; the simultaneous description of some taxa spread on larger areas and assigned to different denominations; high levels of intraspecific polymorphism (Espindola et al., 2010.

Morphologically, plants of the genus *Arum* have rhizomes or tubers, sagittate or hastate leaves, unisex flowers separated by sterile, filamentous flowers, fruits with juicy pericarp and 1-6 seeds (Ağalar, 2016; Cole, 2024). The special organization of the inflorescences, the emission of scents that attract insects and the thermogenic reaction occurring during the flowering period are strategies that ensure pollination in *Arum* (Albre et al., 2003; Barabé et al., 2003; Boyce, 1993; Bröderbauer et al., 2013; Chartier et al., 2016; Espíndola et al., 2010; Linz et al., 2010; Quilichini et al., 2010; Seymour et al., 2009).

Due to the high content of calcium cyanoglycosids and oxalates, *Arum* plants are recognized for their toxicity (Alobaide et al., 2024). However, its use for medicinal or food purposes has been mentioned since ancient times (Azab, 2017; Espíndola et al., 2010). *A. maculatum* is mentioned in the pharmacopoeia written by Dioscorides (20-70 AD) (Kochmarov et al., 2014), the tubers being used in traditional medicine, some of which have also been confirmed by clinical trials (Alobaide et al., 2024; Kochmarov et al., 2014a; Kozuharova et al., 2014; Kozuharova et al., 2018). Other species of *Arum* (*A. italicum, A. palestinum, A. dioscorides, A. cyrenaicum, A. korolkowii*) also have medicinal recommendations (Alobaide et al., 2024; Al-Qudah, 2016; Kochmarov et al., 2014; Rechek et al., 2023; Zargoun et al., 2020; Yeginbay et al., 2024). Heat-treated, the leaves and tubers of *A. palestinum, A. dioscorides, A. italicum, A. korolkowii* lose their toxicity and are used in traditional Asian or Mediterranean cuisine (Alobaide et al., 2024; Al-Qudah, 2016; Karahan et al., 2014; Rechek et al., 2023; Yeginbay et al., 2024).

The ornamental quality of the arum mostly comes from the interesting appearance of the inflorescences, but also from the elegance of the foliage or the intense colour of the ripe fruits (Șelaru, 2007). Many species of this genus are cultivated and marketed as ornamental plants, due to their colourful spathe and spadix (Ağalar, 2016).

The present paper aimed to analyze the behaviour in the ecological conditions of Iași (NE Romania) of the Italian arum (*Arum italicum* Mill.), a herbaceous geophyte (horizontal rhizomatous tubers), with its origin from the Caucasus, the Balkans and western Asia, through southern Europe and northern Africa, to the Atlantic coast and the British Isles. Naturalized, it can also be found in other areas of Europe, New Zealand, and South and North America (Boyce, 1993; Méndez, 1998; 1999; Rechek et al., 2023; Turco et al., 2014). Although it prefers shady places with constant humidity and mild winters, specific to the Mediterranean climate, it is tolerant of low temperatures and dryness. In areas where it finds favourable conditions, it becomes invasive and often appears on the list of harmful weeds that are difficult to eliminate (e.g., in the states of Washington and Oregon in the USA) (https://www.nwcb.wa.gov/weeds/italian-arum).

Studies on some species of *Arum* (*A. italicum, A. creticum*) have also been carried out in Iași, and the results have confirmed a good adaptation of the plants, with possibilities of extension in the culture (Arhip, 2015).

MATERIALS AND METHODS

Plant material. *A. italicum* Mill. has leaves sagittate-hastate, and long petiolate, glossy, dark green lamina, with lighter-coloured veins. The spathe is yellowish-green, and the spadix is yellow. Apart from the fertile flowers, two groups of sterile, filiform flowers are also present on the spadix: female (pistillodes) and male (staminodes). The fruits (berries) reach

maturity in August-September, when they acquire a red-orange colour (Alpinar, 2023; Boyce, 1993; Méndez, 1998, 1999). The species is polyploid (2x = 84) and shows a high variability of characters (Lendel et al., 2003).

Local conditions. The experimental crops were established in the field of the Floriculture Discipline within the Iasi University of Life Sciences (IULS), in the NE area of Romania (47°11'39"N 27°33'16"E). The climate is temperate-continental with excessive nuances, and the altitude of approx. 60 m. The soil, of the cambic chernozem type, has a sandy-clay texture and pH approx. 7.8. The meteorological conditions in the analyzed period were characterized by average annual temperatures between 10.8-11.8^{0}C and annual rainfall of 322-546 mm.

The crops were established in the autumn of 2013 with tubers purchased from a Dutch company supplying flower seedlings. The planting was done at distances of 30/40 cm. The experiments were organized in randomized blocks with three repetitions (10 plants/repetition). All the indicators within the experiment have been analyzed during the period 2017-2024.

Phenological and biometrical investigations. To phenological observations were made from the start in vegetation, the appearance of the first inflorescences, beginning and end of flowering, fruiting, entry into dormancy and biometric determinations regarding the height of the plant, number of leaves/plant, size of leaves and inflorescences, number of inflorescences/plant.

Physiological analysis. In the mature leaves, harvested during the flowering period, the extraction and determination of the content of assimilatory pigments was carried out using the spectrophotometric method according to Current Protocols in Food Analytical Chemistry (Lichtenthaler and Buschmann, 2001). Reading of the extracted samples was performed at an absorbance of E661.6 nm for chlorophyll *a* (Chl *a*), E644.8 nm for chlorophyll *b* (Chl *b*), and E470 nm for carotenoid pigments (*x*+*c*) using UV-VIS spectrophotometer (T70 UV/VIS spectrophotometer PG Instruments Ltd.).

RESULTS AND DISCUSSIONS

The phenological study

Because italian arum is not mentioned in the spontaneous flora of Romania, the results obtained in the experimental field in Iasi were compared with those in the literature, corresponding to the natural habitats of the species (Italy, France, Spain, Turkey).

The phenology of the plants was monitored throughout the year and the data are presented graphically, in the form of a diagram (Figure 1), by months and weeks, each phenophase being marked with a specific colour.

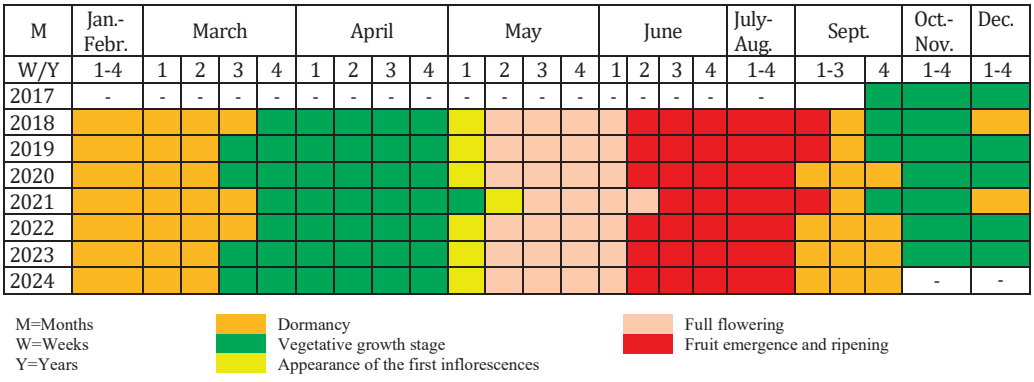


Figure 1. Phenophase diagram in *A. italicum*

It can be seen that in the analysed period (September 2017 - September 2024) the start of vegetation, respectively the appearance of leaves, took place in autumn (September - October) when temperatures began to drop. There was a delay of approximately one week in the years 2022-2024, when temperatures were higher in September (Figure 1).

In October-November, the foliage was fairly well developed (Figure 2 a, b), but in in January - February or in December (2018 and 2021), when temperatures dropped below -10…-15^{0}C, the

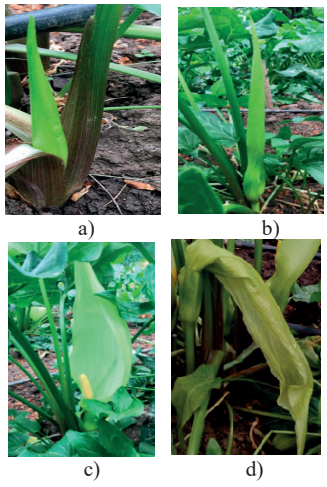
foliage was destroyed and reappeared in spring. This phenomenon, specific to areas with colder winters, is recorded in the literature (https://www.missouribotanicalgarden.org). In 2018, the leaves reappeared in the last week of March, except 2019, 2020, 2023 and 2024, when temperatures were higher and the start of vegetation was 7-10 days earlier (Figures 1 and 2. c).



a) b) c)

Figure 2. Appearance of plants in autumn and spring (original): a) October 2017; b) November 2017; c) March 2018

A. italicum is a spring-flowering plant, the flowering capacity being influenced by the degree of maturity and the weight of the tuber (Albre and Gibernau, 2008). The phenophase of the appearance of the first inflorescences was recorded when they emerged from the leaf sheath (Figure 3 a). The first inflorescences appeared at the end of April - beginning of May, but with a delay of approx. one week in 2021. Figure 3 shows the flowering stages, from the appearance of the inflorescences (a) and the closed turbinate phase (b), to the complete opening (c) and the end of flowering (d).



a) b)

c) d)

Figure 3. Flowering stages (original):
a) the appearance of the inflorescence; b) inflorescence before opening (closed cone); c) inflorescence completely open; d) end of flowering

Reported individually, for each inflorescence, the duration from the appearance of the inflorescence to the end of flowering was reduced, but the successive formation of several inflorescences in mature and well-developed plants extended the total flowering period in the experimental culture to 30-35 days, until the mid- June (Figure 1). As the anthesis ended, the inflorescences at which pollination took place began to form fruits at the base of the spadix. In the conditions of Iași, the end of flowering and the beginning of fruit formation corresponded to the last two weeks of June (Figure 1). The summer rest sets in gradually, after the formation of fruits, when the foliage declines, the only above-ground parts being the stems with fruiting that remain until full maturity, in August - September. In autumn (September) the plants resumed their vegetation (Figure 1). The presence of leaves on the plant partially coincided with the end of flowering and the formation of fruits, as confirmed by the specialty literature (Méndez, 1999).

The phenological data recorded in the experimental field in Iași were compared with those reported by studies carried out on *A. italicum* in natural habitats (Table 1).

Table 1. The periods of development of phenophases in *A. italicum*

Phenophases	Periods	
	Iași	Bibliography (author)
Occurrence of leaves	I. end of September	Autumn (Albre and Gibernau, 2008) End of autumn - beginning of winter (Alpinar et al., 2023) September - October (Méndez, 1998, 1999)
	II. March (the resumption of vegetation after the winter interruption)	Not typical for natural habitats Mentioned for areas with frosty winters (https://www.missouribotanicalgarden.org)
Flowering	May – beginning of June	Beginning of March - end of May (Albre and Gibernau, 2008) February - May (Méndez, 1999)
Leaf wilting/ fruit formation	June - July	June (Méndez, 1999) July (Alpinar et al., 2023)
Fruit ripening	End of July – beginning of September	August - September (Méndez, 1999)

It can be seen that the large differences in the development of the phenophases appeared only in the case of the appearance of leaves (in the climate of frosty winters in Iași, the leaves that appeared in autumn are destroyed during the winter, but reappear in the spring of the following year) and flowering (in the literature

it is mentioned the period February-May, while in Iași from May to the beginning of June). The delay in the onset of flowering in Iasi can be explained by the need to resume vegetation in spring and the cold, less favorable periods of late winter - early spring.

Study of morpho-decorative characteristics

During the analyzed period, the evaluation of the ability of *A. italicum* plants to adapt to the ecological conditions of Iași was also carried out by determinations of morpho-decorative characteristics that were compared with the related data in the literature.

The main morphological characters that ensure the ornamental appearance of the plants are presented in Table 2, the results of the determinations being compared with the data from specialized publications. The data corresponding to the crops in Iasi represent the average values of the 30 plants analyzed.

In general, when the aesthetic quality of plant foliage is analysed, there are considered such characteristics that provide a visually attractive appearance through colour, shape, size, arrangement, the presence of certain specific formations, etc. (Cantor et al., 2021). The appearance of the foliar apparatus in *A. italicum* is one of the very important decorative elements, if we take into account not only the beautiful, marbled appearance of the blade but also the long period of decoration, starting in autumn, partly in winter, then from early spring to early summer. The determinations took into account both the number of leaves and their dimensions. As for the average number of leaves/plant, it can be seen that it fell within the limits of the literature. For the characterization of leaf size (petiole length, leaf blade length, and width), measurements were made only on mature leaves. The mean petiole length was 32 cm, compared to 26-40(50) cm (Davis, 1984, cited by Ağalar, 2016) or 15-35(40) cm according to Boyce (1993) and Alpinar et al. (2023). The records from Iasi showed a leaf lamina length of 25.8 cm, falling within the range of values of 9(11.5)-30(35) mentioned by the same authors. The studies made by Prime et al. (1961) are also confirmed, which specify that the petiole is longer than the lamina. Also, the basal width of the lamina, 22.4 cm, ranged from a minimum of 9 cm to a maximum of 35(40) cm in other studies. The maximum height of the plants was also evaluated, measured from ground level to the tip of the blade of the longest leaf of each plant. The average values were 62.7 cm, and even if no comparison data were found for this characteristic, it can be associated with the sum of the maximum values from the length of the petiole and lamina, respectively 70-90 cm.

Table 2. Morphologic-decorative characteristics in *A. italicum*

Characters	Rezults		
	Iași	Bibliography/author	
No. of leaves/plant (pcs.)	7.30	5-8	Albre and Gibernau, 2008
Length of petiole(cm)	32.0	26-40(50)	Davis, 1984, quoted by Ağalar, 2016
		15-35	Boyce, 1993
		15-40	Alpinar *et al*., 2023
Length of blade (cm)	25.80	11.5-30	Davis, 1984, quoted by Ağalar, 2016
		9-35	Boyce, 1993
		9-35(40)	Alpinar et al., 2023
Width base to blade (cm)	22.40	10-23.5	Davis, 1984, quoted by Ağalar, 2016
		2-29	Boyce, 1993
		2-29	Alpinar et al., 2023
Maximum plant height (cm)	62.70	-	-
Length of floral peduncle (cm)	5.80	12-20	Davis, 1984, quoted by Ağalar, 2016
		4.6 - 16	Boyce, 1993
Total spathe length (cm)	23.60	(13)20-40	Davis, 1984, quoted by Ağalar, 2016
		11-27(38)	Boyce, 1993
Total spadix length (cm)	6.70	(5.5)6.5-13.5	Davis, 1984, quoted by Ağalar, 2016
		4.4-14	Boyce, 1993
		5.2-18	Barabé et al., 2003
		8.8-9.8	Chartier et al., 2016
Appendix length (cm)	3.90	3-7.5	Davis, 1984, quoted by Ağalar, 2016
		3-10	Boyce, 1993
		3-14	Barabé et al., 2003
		3.6-4.2	Chartier, 2016
		4.7-5.3	Mendez and Diaz, 2001
No. of inflorescences/ plant (pcs.)	1.70	1.96	Albre and Gibernau, 2008
		1-3	Méndez, 1998

Another category of determinations took into account the size of the inflorescences and the number of inflorescences/plants (Table 2). Although the fully open inflorescence of *A. italicum* does not have a long decorative period (approx. two days), it attracts attention with its interesting appearance (Figure 3 c), and the successive flowering of 2-3 inflorescences in mature plants, with vigorous tubers, extends the total flowering time to approx. a month, which also justifies the interest in the flowering of this plant.

The total length of the two main components, as measured from the level of the insertion on the

peduncle to the tip of the spathe, respectively of the spadix, was within the values mentioned in the bibliographic references (23.6 cm spathe length and 6.7 cm spadix length), also respecting the proportion between spathe and spadix of 1/3-1/4 (Prime et al., 1961). The decorative effect of the inflorescences is also completed by the yellow colour of the appendix (the terminal portion of the spadix), of approx. 4 cm (Figure 3 c). The number of inflorescences/plant ranged from 1-3 (47% of plants with one inflorescence, 37% with two, and 15% with three), with an average of 1.7. In natural habitats, the average reaches 1.96, with most plants forming two inflorescences (Albre and Gibernau, 2008).

Content of photosynthetic pigments

The study of the adaptation of *A. italicum* plants to the growing conditions in Iași was also completed with the analysis of the content of photosynthetic pigments (chlorophylls and carotenoids) in mature leaves. These data provide information on the level of physiological stress in plants determined by less favourable environmental conditions, manifested by the modification of the balance in favor of carotenoids, concomitant with the degradation of chlorophyll (Bonora et al., 2000). Chlorophylls are essential for capturing light energy, and carotenoids help absorb light from the blue-green spectrum and protect against photo-oxidation by dispersing excess light energy. Studies on the content of photosynthetic pigments in *A. italicum* have been carried out both on the leaf (La Rocca et al., 2011; Konoplyova et al., 2008), as well as fruits in different stages of ripening (Bonora et al., 2000; Ferroni et al., 2003).

The results obtained in the plants of *A. italicum* cultivated in Iași (average 2018-2024) indicate a total content of photosynthetic pigments of 3.19 mg/g^{-1} F.W., the largest amount of which is chlorophyll *a* (Chl *a*), 1.74 mg/g^{-1} F.W., followed by chlorophyll *b* (Chl *b*), 1.06 mg/g^{-1} F.W., and total carotenoids (*x*+*c*) 0.39 mg/g^{-1} F.W. (Table 3).

It was found that the Chl *a/b* ratio was relatively low (1.64) as compared to the data in the literature indicating the value of this ratio of 2.6 in *A. italicum* plants from Patras, Greece (Konoplyova et al., 2008) or 2.7-2.9 in plants from Bologna, Italy (La Rocca et al., 2011).

Table 3. Average content of photosynthetic pigments (mg/g^{-1} F.W.)

Chl *a*	Chl *b*	(*x*+*c*)	Σ	Chl *a/b*	(*x*+*c*)/(*a*+*b*)
1.74±0.07	1.06±0.07	0.39±0.06	3.19	1.64	0.13

Each value is shown as the mean ± S. D.; F.W.-fresh weight

The negative change in chlorophyll content (Chl *a*, Chl *b*, Chl *a*+*b* and Chl *a/b*) characterizes the response of plants to adapt to the less favourable environment (probably to lower light and temperature levels), in order to optimize their photosynthesis (Li et al., 2018). Lower values were also recorded in the ratio between the content of carotenoid pigments and chlorophyll pigments (0.13), compared to 0.16-0.17 by the aforementioned studies (Konoplyova et al., 2008; La Rocca et al., 2011). The decrease in the value of the carotenoid/chlorophyll pigment ratio ((*x*+*c*)/(*a*+*b*)) suggests that the content of chlorophyll pigments has increased in the arum plants cultivated in Iasi, possibly due to low light, which decreases the carotene content.

The determination of the content of photosynthetic pigments, in order to assess the degree of adaptation to the conditions in Iași, was also done in other rustic geophytes (*Eremurus*, *Gladiolus*) (Amisculesei et al., 2021; Bahrim et al., 2021).

CONCLUSIONS

The results of phenological and morphological observations conducted on *A. italicum* plants cultivated in Iasi are in accordance, to a large extent, to bibliographic references data recorded in the natural areas of the species. The plants started to grow in autumn, bloomed in spring and rest in the summer, but unlike the habitats with mild winters, the foliage formed in autumn was destroyed by winter frosts and reappeared in spring, and the onset of flowering registered delays of approx. a month. The biometrics of the main morphologic-decorative characteristics were within the limits specified in the bibliography. The lower content of photosynthetic pigments in mature leaves indicates a slight stress of the plants, but does not influence the aesthetic appearance of the plants.

The results obtained confirm the good adaptability of *A. italicum* plants to the conditions of a colder climate, specific to Iasi, and the possibility of cultivation in the field,

without protection in winter, in accordance with the chronology of the rustic geophytes.
From an ornamental point of view, *A. italicum* is recommended for garden decoration, due to its beautiful foliage, with a long duration of decoration, and interesting inflorescences. The leaves can also be used in floral art. Regardless of the ways of use, the toxicity of the plant must be taken into account.

ACKNOWLEDGEMENTS

This research work was carried out with the support of Horticultural Research Center of Iasi University of Life Sciences (IULS)

REFERENCES

Amişculesei, P., Apostol, M., Chelariu E.L., Rotaru L., & Draghia L. (2021). Ornamental, physiological and enzymatic evaluation of some *Gladiolus* species. *Scientific Papers. Series B, Horticulture*, LXV (2), 177–186.

Ağalar, H.G. (2016). *Arum italicum Miller üzerinde farmakognozik araştirmalar.* Doktora tezi, Anadolu Üniversitesi, pp. 2–18.

Albre, J., Quilichini, A., & Gibernau, M. (2003). Pollination ecology of *Arum italicum* (Araceae). *Botanical Journal of the Linnean Society*, 141, 205–214.

Albre, J., & Gibernau, M. (2008). Reproductive biology of *Arum italicum* (Araceae) in the south of France. *Botanical Journal of the Linnean Society*, 156, 43–49

Alobaide, Z.N., Habel, A., & Awin, T., Buzgaia, N., Elamari, A., Elteera, F. & Elabbar, F. (2024). *Arum cyrenaicum*: A comprehensive review of Phytochemistry and Bioactivity. *Scientiae Radices*, 3(3), 218–227

Alpinar, K., Aslan, S.S., Piskin, B.K., Nesetoglu, N., Danis, I., & Unal, D.S.O. (2023). Comparison of Fatty Acid Contents of Wild and Cultivated *Arum italicum* Mill. Seed Oils from Kırklareli in Turkey by Gas Chromatography-Mass Spectrometry. *European Journal of Biology,* 82(2): 239–242, DOI: 10.26650/EurJBiol.2023.1282708.

Al-Qudah, M.M.A. (2016). Histological and Biochemical Studies on Liver of Female Rats Treated with Different Concentrations of Ethanolic Extract of *Arum palaestinum. Journal of Appl. Environmental and Biol. Sciences,* 6(7) 1-1. https://www.researchgate.net/publication/306118236

Arhip (Însurățelu), I.C. (2015). *Agrobiological study of certain species of Zantedeschia Spreng. and Arum L. in different culture conditions*. Doctoral thesis, Univ. of Agr. Sciences and Veterinary Medicine Iași, Romania.

Azab, A. (2017). *Arum*: a plant genus with great medicinal potential. *European Chemical Bulletin*, 6(2), 59–68

Bahrim, C., García–Breijo, F.J, M., Apostol, Asănică, A.C., Teliban, G.C., Munteanu, N., Rotaru, L., & Draghia, L. (2021). Study of some foxtail lilies species (*Eremurus* M. Bieb.) grown in the North-East of Romania. *Romanian Biotechnological Letters*, 26(2), 2489–2498.

Barabé, D., Lacroix, C., & Gibernau M. (2003). Development of the flower and inflorescence of *Arum italicum* (Araceae). *Canadian Journal of Botany*, 81, 622–632, DOI: 10.1139/b03-060.

Bonora, A., Pancaldi, S., Gualandri, R., & Fasulo, M.P. (2000). Carotenoid and ultrastructure variations in plastids of *Arum italicum* Miller fruit during maturation and ripening. *Journal of Experimental Botany,* 51(346), 873–884.

Boyce, P.C. (1993). The Genus *Arum*. The Royal Botanic Gardens, Kew. *A Kew Magazine Monograph*, pp: 69–73.

Boyce, P.C. (2006). *Arum*-A decade of change. AROIDEANA, *Journal of the International Aroid Society*, 29: 132–137.

Bröderbauer, D., Weber, A., & Diaz, A. (2013). The design of trapping devices in pollination traps of the genus *Arum* (Araceae) is related to insect type. Botanical *Journal of the Linnean Society*, 172:385–397.

Cantor, M., Buta, E., & Buru, T. (2021). *Growing of ornamental plants in controlled climate (Cultura plantelor ornamentale în climat controlat).* Cluj-Napoca, RO. AcademicPres Publishing House. pp. 297–298.

Chartier, M., Liagre, S., Weiss-Schneeweiss, H., Kolano, B., Bessière, J.M., Schönenberger, J., & Gibernau, M. (2016). Floral traits and pollination ecology of European *Arum* hybrids. *Oecologia*, 180:439–451, DOI 10.1007/s00442-015-3498-9.

Cole, T.C.H. (2024). Araceae Phylogeny Poster (Essential Aroids), https://www.researchgate.net/publication/337090323_ARACEAE_Phylogeny_Poster_Essential_Aroids_2024.

De Keyser, E., Dhooghe, E., Christiaens, A., Van Labeke, M.C., & Van Huylenbroeck, J. (2019). LED light quality intensifies leaf pigmentation in ornamental pot plants. *Scientia Horticulturae*, 253: 270–275

Espíndola, A., Buerki, S., Bedalov, M., Küpfer, P., & Alvarez, N. (2010). New insights into the phylogenetics and biogeography of *Arum* (Araceae): unravelling its evolutionary history. *Botanical Journal of the Linnean Society*, 163:14–32.

Ferroni, L., Pantaleoni, L., Baldisserotto, C., Arob, E.M., & Pancaldi, S. (2003). Low photosynthetic activity is linked to changes in the organization of photosystem II in the fruit of *Arum italicum*. *Plant Physiology and Biochemistry*, 63: 140–150.

Henriquez, C.L., Arias, T., Pires, J.C., Croat, T.B., & Schaal, B.A. (2014). Phylogenomics of the plant family Araceae. Molecular *Phylogenetics and Evolution*, 75: 91–102.

Karahan, F., Kulak, M., Urlu, E., Gözüacik, H.G., Böyümez, T., Şekeroğlu, N., & Doganturk, I.H. (2014). Total phenolic content, ferric reducing and DPPH scavenging activity of *Arum dioscoridis*. *Natural Product Research*. http://dx.doi.org/10.1080/14786419.2014.991320

Kochmarov, V., Kozuharova, E., Naychov, Z., Momekov, G., & Mincheva, I. (2014). Ethnobotany and Ethnopharmacology of *Arum maculatum* L. (Araceae) in Bulgaria with an Emphasis on its Effect Against Haemorrhoids. *International Journal of Pharmaceutical, Chemical and Biological Sciences*, 5(2): 394–402.

Konoplyova, A., Petropoulou, Y., Yiotis, C., Psaras, G.K., & Manetas, Y. (2008). The fine structure and photosynthetic cost of structural leaf variegation. *Flora*, 203: 653–662.

Kozuharova, E., Kochmarov, V., Kachaunova, E., Espíndola, A., Aleksandrov, B., & Mincheva, I. (2014). Distribution of *Arum* (Araceae) in Bulgaria. *Flora Mediterranea*, 24: 51–62, doi: 10.7320/FlMedit24.051.

Kozuharova, E., Kochmarov, V., Semerdjieva, I., Mincheva, I., & Gibernau, M. (2018). Potential Resource of Wild Populations of *Arum maculatum* L. (Araceae) in Bulgaria a Prospective Medicinal Plant. *Comptes rendus de l'Academie bulgare des Sciences*, 71(2): 193–200.

La Rocca, N., Rascio, N., & Pupillo, P. (2011). Variegation in *Arum italicum* leaves. A structurale functional study. *Plant Physiology and Biochemistry*, 49: 1392–1398.

Leguet, A., Gibernau, M., Shintu, L., Caldarelli, S., Moja, S., Baudino, S., & Caissard, J.C. (2014). Evidence for early intracellular accumulation of volatile compounds during spadix development in *Arum italicum* L. and preliminary data on some tropical Aroids. *Naturwissenschaften*, 101: 623–635. DOI 10.1007/s00114-014-1197-8.

Lendel, A., Bedalov, M., Sabo, M., Bačić, T., Kristin, L., & Marček, T. (2006). Comparative chromosome and molecular studies of some species of genus *Arum* from Eastern Slavonia and Baranya Region in Croatia. *Acta Societatis Botanicorum Poloniae*, *75*(4): 285–292. https://doi.org/10.5586/asbp.2006.034.

Li, Y., He, N., Hou, J., Xu, L., Liu, C., Zhang, J., Wang, Q., Zhang, X., & Wu, X. (2018). Factors Influencing Leaf Chlorophyll Content in Natural Forests at the Biome Scale. *Frontiers in Ecology and Evolution*, 6, article 64, https://doi.org/10.3389/fevo.2018.00064.

Lichtenthaler, H., & Buschmann, C. (2001). *Extraction of Photosynthetic Tissue: Chlorophylls and Carotenoids* (Unit F.4., F.4.2.1. – F.4.2.6.). In Current Protocols in Food Analytical Chemistry. DOI: 10.1002/0471142913.faf0402s01.

Linz, J., Stökl, J., Urru, I., Krügel, T., Stensmyr, M.C., & Hansson, B.S. (2010). Molecular phylogeny of the genus *Arum* (Araceae) inferred from multi-locus sequence data and AFLPs. *Taxon*, 59(2): 405–415.

Méndez, M. (1998). Modification of phenotypic and functional gender in the monoecious *Arum italicum* (Araceae). *American Journal of Botany*, 85(2): 225–234.

Méndez, M. (1999). Effects of Sexual Reproduction on Growth and Vegetative Propagation in the Perennial Geophyte *Arum italicum* (Araceae). *Plant biology*, 1: 115–120.

Ollerton, J., & Diaz, A. (1999). Evidence for stabilising selection acting on flowering time in *Arum maculatum* (Araceae): the influence of phylogeny on adaptation. *Oecologia*, 119: 340–348.

Oprea, A. (2005). *Critical list of vascular plants in Romania. (Lista critică a plantelor vasculare din România)*. Iasi, RO. "Al. I. Cuza" University Publishing House.

Prime, C.T. (1961). Taxonomy and nomenclature in some species of the genus *Arum* L. *Watsonia*, 5(2): 106–109.

Quilichini, A., Macquart, D., Barabe, D., Albre, J., & Gibernau, M. (2010). Reproduction of the West Mediterranean endemic *Arum pictum* (Araceae) on Corsica. *Plant Syst. Evol.,* 287: 179–187. DOI 10.1007/s00606-010-0312-3.

Rechek, H., Haouat, A., Pinto, D.C.G.A., Pereira, D.M., Pereira, R.B., Válega, M.S.G.A., Cardoso, S.M., & Silva, A.M.S. (2023). A Comparative Analysis between the Phenolic Content, Key Enzyme Inhibitory Potential, and Cytotoxic Activity of *Arum italicum* Miller in Two Different Organs. *International Journal Plant Biology*, 14: 520–532. https:// doi.org/10.3390/ijpb14020041.

Riedl, H. (1980). The importance of ecology for generic and specific differentiation in the Araceae-Aroideae. *Aroideana*, 3: 49–55.

Seymour, R., Gibernau, M., & Pirintsos, S. (2009). Thermogenesis of three species of *Arum* from Crete. *Plant, Cell and Environment*, 32: 1467–1476. doi: 10.1111/j.1365-3040.2009.02015.x.

Sîrbu, C., & Paraschiv, L.N. (2005). *Systematic botany (Botanică sistematică)*, Iași, RO. "Ion Ionescu de la Brad" Publishing House. pp. 360

Sîrbu, C. (2020). *Botanica. Plants morphology and anatomy. (Botanica. Morfologia și anatomia plantelor)*. Iași, RO. "Ion Ionescu de la Brad" Publishing House. pp. 237.

Șelaru, E. (2007). *Cultura florilor de grădină*. Editura Ceres, București, pp. 197–198.

Turco, A., Medagli, P., Albano, A., & D'Emerico, S. (2014). Karyomorphometry on three polyploid species of *Arum* L. (Araceae, Aroideae). *Comparative Cytogenetics*, 8(1): 71–80.

Urru, I., Stökl, J., Linz, J., Krügel, T., Stensmyr, M.C., & Hansson, B. (2010). Pollination strategies in Cretan Arum lilies. *Biological Journal of the Linnean Society*, 101: 991–1001.

Urru, I., Stensmyr, M.C., & Hansson, B. (2011). Pollination by brood-site deception. *Phytochemistry*, 72: 1655–1666.

Yeginbay, A., Aripova, S., Abubakirova, A., Mutalova, M., Aitkulova, R., Akhmadaliev, B.J., Burabaev, Al., Burabaev, As., & Narimanov, A.A. (2024). Biology of the medicinal plant *Arum korolkowii* Regel (Arum). *Plant Science Today*, 11(1): 602–605. https://doi.org/10.14719/pst.3137

Zargoun, A.H., Mohammed, A.M.A., Abdelshafeek, K.A., Ibansharrada, A.A., & Alomari, A.A. (2020). GC/MS analysis of lipid constituents and antimicrobial activity of *Arum cyrinaicum* extracts. *Egyptian Journal of Chemistry*, 63, No., DOI: 10.21608/ejchem.2020.30199.2647, https://www.researchgate.net/publication/348743588

WA State Noxious Weed Control Board. *Arum italicum*. A toxic, noxious weed in Washington. https://www.nwcb.wa.gov/weeds/italian-arum.

https://www.missouribotanicalgarden.org

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

MORE THAN JUST AESTHETICS: QUANTIFYING THE MONETARY VALUE OF TREE ALIGNMENTS IN "CATACUZIONO" PARK FROM FLOREŞTI – PRAHOVA COUNTY

Cristina DRĂGHICI[1], Diana Lavinia CULESCU[2]

[1]Department of Silviculture, Faculty of Silviculture and Forest Engineering, Transilvania University of Brașov, 1 Sirul Beethowen Street, 500123, Brașov, Romania
[2]RPR Contemporary Studies Office, 30 Maica Alexandra Street, District 1, 011243, Bucharest, Romania

Corresponding author email: dianaculescu@gmail.com

Abstract

Floresti Domain embeds a historic park designed by landscape architect Karl Friedrich Wilhelm Meyer, that features tree lines that contribute significantly to its aesthetic and ecological value. This study quantifies the economic value of these trees using the BEVA (Bareme d'Evaluation de la Valeur d'un Arbre) method. This method considers factors like tree size, species value, aesthetic contribution, health, and location to determine a monetary value for the presence of each tree within the general setup. The research relies on a smart tool used to make the tree inventory and to analyse data from three main tree lines: Aesculus hippocastanum L. (horse chestnut), Juglans regia L. (walnut) and Tilia tomentosa Moench (silver linden). The paper describes the evaluation process and provides an estimation for the total value of the targeted tree population. The study also reveals that inadequate management and vandalism have resulted in a 26% reduction in the potential value of these trees. The BEVA method provides a practical tool for assessing the financial value of trees in urban landscapes, facilitating informed management decisions and promoting better tree care.

Key words*: tree value, BEVA, TreePlotter, Cantacuzino Domain, Karl Friedrich Wilhelm Meyer.*

INTRODUCTION

Public parks and gardens emerged in Europe in the 19th century. Representing a new cultural phenomenon at the time, many of them have been appreciated and extensively used by the public ever since.

Today, in most countries that have reached an unprecedented level of industrialization, technology, and urbanization, the conservation of existing parks and gardens, as well as the development of green areas serves as an important mean of protecting both people and their living environment.

In the case of historic gardens and parks, the vegetal compositional elements are at least as important as the built one for defining the character of these heritage green spaces. While the monetary value of the built components can be easily assessed, allowing for example for the estimation of damages or restoration costs, in Romania there is no established practice for evaluating the green components, particularly trees in historic parks and gardens.

The urban forests and trees are playing within our living environment primarily by regulating the microclimate, providing ecological benefits and reducing pollution. In this respect, there are already countless studies highlighting the benefits that urban green spaces provide for inhabitants, both at national and international level, such as Dwyer et al., 1991; Dwyer et al., 1992; Chiesura, 2004; Georgi & Zafiriadis, 2006; Nowak & Heisler, 2010; Millward et al., 2011; Hanspach et al., 2011; Shackleton et al., 2015; Duinker et al., 2015; Mexi & El-Shamali 2015 and Culescu 2015, to name just a few examples. Further more, Georgi & Zafiriadis (2006) lean over the impact of vegetation on the urban microclimate, observing how trees influence summer conditions, including air temperature, relative humidity, and solar radiation in Thessaloniki, Greece. Nowak and Heisler (2010) studied expensively the air quality in parks and urban areas. A similar research was conducted in Poland, in Łódź, the value of street trees in the city centre being assessed in relation with their contribution to

urban ecosystem services (Giergiczny & Kronenberg, 2014).

The value of trees is a constant topic of discussion: how much they worth in relation to different features like the oxygen they produce, their aesthetic contribution to a landscape, or their importance to a specific community.

However, in Romanian context, these are often highly subjective discussions about aspects that are difficult to quantify and evaluate. In the recent years, among the earliest recorded approaches to evaluating historic landscapes in Romanian heritage parks and gardens is proposed El-Shamali et al. (2010). In this research, the maximum aesthetic value was attributed by the authors to "fallen ancient trees, whose decaying trunks dramatically and spectacularly shaped the landscape". Also in the Romanian context, Dobrescu (2009) described a rather narrow approach of the topic within the national literature, while Ciupa et al. (2005) proposed a complex 28-criterion method for evaluating vegetation, which, even from the authors' perspective, remains highly laborious.

At international level, several well-established methods are currently used to estimate the value of the vegetal features within a green space. Among the ones considered for this research there were:

- **The BEVA** - Bareme d'Évaluation de la Valeur d'un Arbre **method** (Conseil general des Hauts-de-Seine, 2004; Culescu, 2018; Département du Loiret, 2020; Mairie de Metz, 2015; Ville de Rouen - Direction des Espaces Publics et Naturels, 2015)
- **The BURNLEY** (Burnley, 1967; Culescu, 2018; Kielbaso, 1979; McGarry, 1988; Moore, 1991; Watson, G. , 2002)
- **The CAVAT** - Capital Asset Value for Amenity Trees **method** (CAVAT Group, 2023-1; CAVAT Group, 2023-2; Culescu, 2018; Doick et al., 2018);
- **The CTLA** - Council of Tree and Landscape Appraisers **method** (Council of Tree and Landscape Appraisers, 2020; Culescu, 2018)
- **The HELLIWELL method** (Culescu, 2018; Hellis Solutions Limited, 2019; Helliwell, 1967; Negrutiu, 1980; Padlet, 2020; Watson G., 2002)

However, due to evaluation criteria, only BEVA method could be retained for this research. It is important to note here that the other methods include parameters that cannot be provided in Romanian context due to the lack of public data or the shortage of specialized workforce. The main exclusion reason were:

For Burnley method:

- The differences in the Romanian vegetation profile compared to the Australian flora on which the method is calibrated;
- The need for an accurate determination for the following: a tree's volume (in cubic meters, including foliage and not restricting the measurements only to woody parts as in forestry); the useful lifespan of the analysed specimen; the extent to which the analysed specimen fits within the space where it is located.

For CAVAT method:

- The need to determine the social value of a specimen;
- The requirement to assess how the species and the analysed specimen fit within the space where it is planted;
- The need to evaluate of how a tree is functioning from the biological point of view.

For CTLA method:

- The need have information regarding the replacement costs for each species for the largest specimen that can be transplanted from a local nursery;
- The need to use a coefficient assigned to each species based on its physical characteristics, rarity, and the degree of difficulty regarding its propagation and development in a nursery;
- The difference between the vegetal species profile between Romana and US (for examples species considered native in US are exotic for Romania and, thus, the degree of rarity is different).

For the Helliwell method:

- The need to determine the tree's expected useful lifespan, the significance of its placement within the landscape, and its relationship with the surroundings (all requiring highly specialized workforce for the evaluation);
- The requirement to correlate the above mentioned factors with the tree's impact on increasing property value (an aspect for which there are no studies or reliable data in Romania).

The exclusions of this methods was also based on the fact that, although they are mentioned in the national literature almost half a century ago (see for example Negrutiu, 1980), they have never been used in official manner (by professionals, by the public administration, in litigious situations etc.).

Lastly, the research is based on BEVA method and not on its newer version, VIE (Freytet et al., 2025, because this enhance version draws on data predetermined for each French city and, at this time, no similar data could be found for Romanian cities.

Therefore, this study aims to present a monetary valuation for tree lines embedded within the historic park of Cantacuzino Domain in Floresti - Prahova County from Romania (code PH-II-m-A-16389.04 according to Ministry of Culture, 2015. The paper is presenting the 2023 situation for the tree lines of *Aesculus hippocastanum* L. (horse chestnut), *Juglans regia* L. (walnut) and *Tilia tomentosa* Moench (silver linden) that are bordering the main north-south pathways within the hunting area of this historic domain. The goal of this demarche is to establish the current value of these green compositional elements and to provide an important stepping-stone for future restauration activities.

MATERIALS AND METHODS

In terms of material and methods, this research rests on two main pillars: **the TreePlotter software and BEVA** (Bareme d'Evaluation de la Valeur d'un Arbre) method.

The TreePlotter Inventory is a GIS-based green tree management system that allows users to collect and update data, to evaluate trees and to monitor the maintenance process and trees' evolution over time and preserves the maintenance work history for tree population. The software was built by certified arborists and foresters (Hanou, 2014; TreePlotter, 2019; Morar et al., 2019) and it is extensively used in US and Western Europe to tackle tree evaluation and maintenance activity. Its cloud-based functionality enables navigation, data filtering, reporting, and export options, ensuring seamless management and live data access.

To develop this tree inventory, field data were collected and processed during the summer of 2023 using the TreePlotter software (see Figures 1, 2 and 3). Data collection targeted the most important alignments bordering the roads within the park of the Cantacuzino Domain from Floresti - Prahova County. The research leaned over the following species: *Aesculus hippocastanum* L. (horse chestnut), *Juglans regia* L. (walnut) and *Tilia tomentosa* Moench (silver linden).

For each tree were collected information regarding the location, the species and the tree trunk diameter at 1.00 meters, according to the European technical & quality standards for nurserystock for amenity trees in standard form (ENA, 2010). This height for diameter measurements aims to establish a connection for a potential replacement of an individual tree with nursery stock material produced for urban environment plantations. In this case, the diameter measurements does not aim to establish the wood quantity and value as it is the case for DBH, measured at 1.20 meters or 1.30 meters, according to the norms establisher in this respect in each country, in the forestry field.

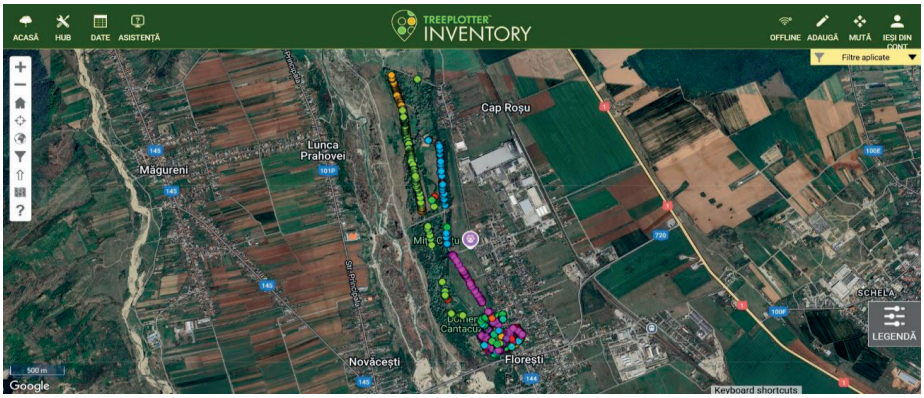


Figure 1. General view for the tree inventory in Cantacuzino Domain



Figure 2. Detailed view from the tree lines inventory in Cantacuzino Domain:
(with blue) specimens of *Juglans regia* L.; (with magenta) specimens of *Tilia tomentosa* Moench.

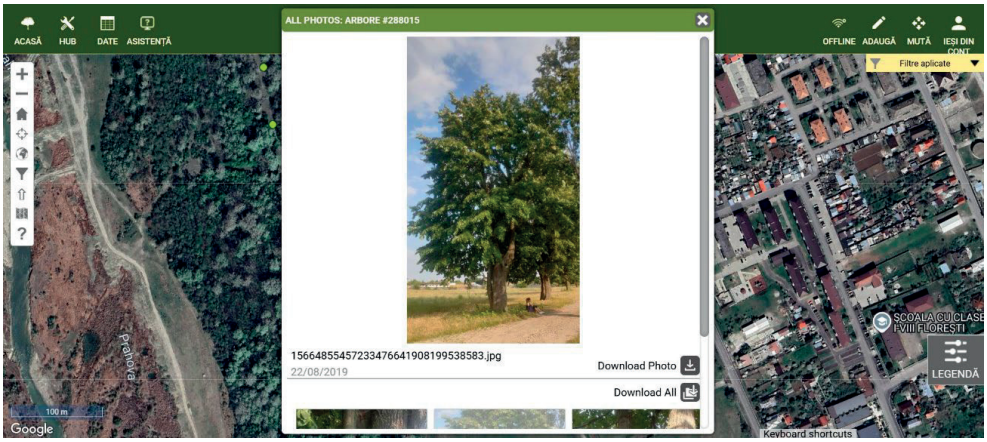


Figure 3. Detailed view for a tree file embedded in TreePlotter Inventory for Cantacuzino Domain

BEVA Method (Bareme d'Evaluation de la Valeur d'un Arbre, roughly translated in English as: Scale for the Evaluation of a Tree's Value) is a simple way to calculate the value of trees as part of an urban landscape Département du Loiret, 2020).

This method was developed by the French Society of Arboriculture in collaboration with several public administrations from France.

Compared to other internationally recognized tree evaluation methods, BEVA is significantly simpler and easy to use both by public administrations and less specialized professionals. It is important to highlight here the fact that this method does not require:

- Special measurements using relatively expensive equipment;
- The involvement of professionals with highly specialised expertise;
- The use of very specific data, such as the maintenance cost of the analysed specimen.

The evaluation principles of this method are straightforward, beginning with an individual assessment of each tree.

For this purpose, four key features have been established to determine the value of each tree:

(1) *Tree Size Index*

To determine this index, the tree's circumference (in centimetres) is measured at 1.00 meter above ground level.

An index is assigned to each tree according to the following scale:

Table 1. Tree Size Index for BEVA method

Circumference	Index	Circumference	Index
10-14 cm	0.5	181-190 cm	19
15-22 cm	0.8	191-200 cm	20
23-30 cm	1	201-220 cm	21
31-40 cm	1.4	221-240 cm	22
41-50 cm	2	241-260 cm	23
51-60 cm	2.8	261-280 cm	24
61-70 cm	3.8	281-300 cm	25
71-80 cm	5	301-320 cm	26
81-90 cm	6.4	321-340 cm	27
91-100 cm	8	341-360 cm	28
101-110 cm	9.5	361-380 cm	29
111-120 cm	11	381-400 cm	30
121-130 cm	12.5	401-420 cm	31
131-140 cm	14	421-440 cm	32
141-150 cm	15	441-460 cm	33
151-160 cm	16	461-480 cm	34
161-170 cm	17	481-500 cm	35
171-180 cm	18	501-600 cm	44 etc.

(2) ***Species Value Index***

This index is based on the selling price of a specimen from each species included in the study and is determined usually according to the selling prices of local nurseries.

The index is equal to 10% of the selling price of a tree with a circumference of 10-12 cm for deciduous species or a height of 150-175 cm for coniferous species. Seeing that Romania does not have that many local nursery that are producing amenity tree standards, a correlation was made between available resources at national level (Pepiniera Silva Periland, 2021; 2023; 2025) and the ones at European level (Bruns Pflanzen, 2011; 2021; 2023) that are usually providing planting resources for this country.

(3) ***Aesthetic and Health Value Index***

This index is represented by a coefficient ranging from 1 to 10, depending on the tree's placement within the landscape (whether it is a solitary tree, part of a group, or an alignment) and the specimen's vigour.

The values for this index are:

- 10 for a healthy, vigorous, solitary, and remarkable tree;
- 9 for a healthy, vigorous and remarkable tree, placed in groups of 2-5 specimens;
- 8 for a healthy, vigorous tree, placed in a group, a protection plantation or in a tree alignment;
- 7 for a healthy tree with medium vigor, placed as a solitary specimen;
- 6 for a healthy tree with medium vigor, placed in groups of 2-5 specimens;
- 5 for a healthy tree with medium vigor, placed in a group, a protection plantation or in a tree alignment;
- 4 for a tree with very low vigor, old, placed as a solitary specimen;
- 3 for a tree with very low vigor, deformed, placed in a group or in a tree alignment;
- 2 for a tree without vigor, diseased;
- 1 for a tree without amenity value (dead).

(4) ***Placement Index***

This index is represented by a coefficient that reflects the tree's value in relation to its location within the city. This index can have the following values:

- 10 for a tree located in the city centre;
- 8 for a tree located on the outskirts of the city;
- 6 for a tree located in a rural area.

According to BEVA method, the value of the tree is obtained by multiplying these four indices together:

Index 1 x Index 2 x Index 3 x Index 4.

The **statistical analysis** was performed using the **ANOVA** calculation software.

The focus point of this research is the **Cantacuzino Domain from Prahova County**. At Florești, nearly 80 km from Bucharest, lies one of the most impressive noble estates of the Old Kingdom, spanning over 150 hectares. The estate includes a romantic park, utilitarian areas, and a hunting park, landscaped in the 19th - early 20th century by Carl Friedrich Wilhelm Meyer and Emile Pinard.

After the death of Gheorghe Grigore Cantacuzino in 1913 and the events of World War I and World War II, the estate's vegetation composition gradually deteriorated. Communist and post-communist interventions led to the disappearance of garden furniture and the loss of important tree specimens. Nevertheless, the estate remains a landmark on the European map of extra-urban noble gardens.

In terms of vegetation, the site is still emending at this time several spectacular specimens of *Juglans nigra* L. (black walnut), *Pinus strobus* L. (Eastern white pine), *Platanus* x *acerifolia* (London plane), *Populus alba* L. (white poplar) and the rare *Pterocarya fraxinifolia* Spach (Caucasian wingnut). Among the trees preserved from the historical setup there are also parts of the tree lines composed of *Aesculus hippocastanum* L., *Juglans regia* L. and *Tilia tomentosa* Moench. (see Figures 4, 5 and 6).



Figure 4. View of *Aesculus hippocastanum* L.tree line bordering the South pathway, toward the Western part of the hunting park: (above) in July 2022; (down) in august 2023



Figure 5. View of *Juglans regia* L. tree line bordering the North pathway, toward the middle part of the hunting park: (above) in August 2019; (down) in august 2023



Figure 6. View of *Tilia tomentosa* Moench. tree line bordering the access on the hunting park: (above) in august 2021; (down) in august 2023

RESULTS AND DISCUSSIONS

For this research, 98 *Aesculus hippocastanum* L., 51 *Juglans regia* L. and 36 *Tilia tomentosa* Moench. were inventoried.

Based on the measurements, the value of each tree was calculated. This value reflects the tree's actual presence within the green infrastructure and its contribution from social, aesthetic, ecological, and cultural perspectives.

The value was obtained by multiplying the four indexes described above (tree size index, species value index, aesthetic and health value index and placement index). The result is representing the

monetary value in euros for the analysed specimens. Essentially, this value is equivalent to the cost required to replace a lost or removed tree with another specimen of similar significance within the landscape.

For data processing, a mono-factorial experience was conducted, where the analysed factor was the species, with three levels: a1 for *Aesculus hippocastanum* L., a2 for *Tilia tomentosa* Moench and a3 for *Juglans regia* L. The analysed parameter was the average value of each species (see Table 2). Compared to the species *Aesculus hippocastanum* L. (see "castan" in the chart below), the species *Tilia tomentosa* Moench. (see "tei" in the chart below) showed a higher value (€10,246.67), with a positive but statistically insignificant difference (€8,363.36). In contrast, the species *Juglans regia* L (see "nuc" în the chart below) had a lower value (€7,989.99), recording a statistically assured but insignificant negative difference.

The value determined for each tree can be observed in detail in Figure 7.

Table 2. The average value (in euros) of each species

Species	Average value (in euros)	Difference (in euro)	Significance
Aesculus hippocastanum L. | castan	9,410.33	-	-
Tilia tomentosa Moench. | tei	10,246.67	8,363.36	ns
Juglans regia L. | castan	7,989,99	-1,420.34	ns

DL (p 5%) = 0.044 | DL (p 1%) = 0.103 | DL (p 0.1%) = 0.393

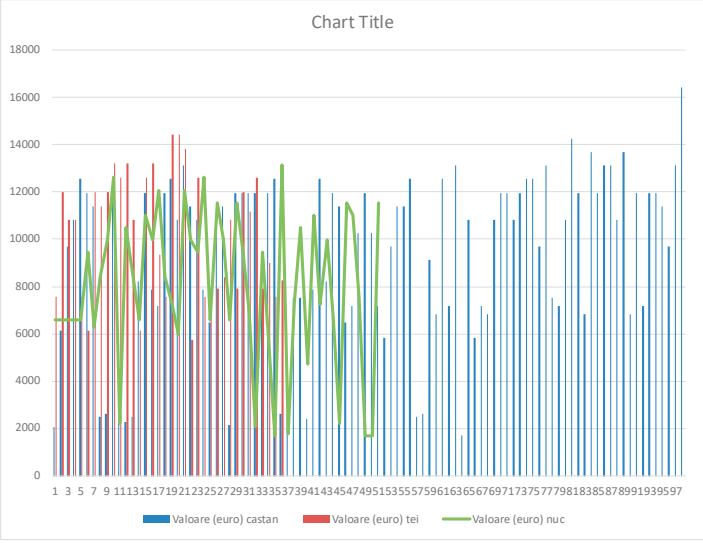


Figure 7. Value determined for each tree inventoried for this research

The total value of the trees currently part of the tree lines embedded by Cantacuzino Domain is is **€1,696,935** (Table 3) - broken down as follows:

- €920,550 for *Aesculus hippocastanum* L.;
- €407,505 for *Juglans regia* L.;
- €368,880 for *Tilia tomentosa* Moench.

It is important to note that, probably due to inadequate maintenance and various acts of vandalism, this compositional element has significantly lost its value.

For example, if in the case of the aesthetic and health value index **the specific value (5)** could be assigned for "healthy tree with medium vigor, placed in a group, a protection plantation or in a tree alignment", the inventoried trees from Floresti Domain would be worthing **€2,145,045** today (Table 3). Thus, due to poor maintenance and vandalism actions, this tree lines from this historic domain **lost approximately €448,110** (26%) of the value it could have had under proper management.

Table 3. The current value (in euros) of each species

Species	Number of specimens	Current value (in euro)	Lost value (in euro)
Aesculus hippocastanum L.	98	920,550	1,157,670
Juglans regia L.	51	407,505	534,975
Tilia tomentosa Moench.	36	368,880	452,400
TOTAL	**185**	**1,696,953**	**2,145,045**

CONCLUSIONS

This study assesses the monetary value of tree alignments in "Cantacuzino" Park from Florești (Prahova County in Romania), using the BEVA (Bareme d'Evaluation de la Valeur d'un Arbre) method, which evaluates trees based on size, species, aesthetic and health condition, and placement within the built landscape. The research focused on 98 *Aesculus hippocastanum* L. (horse chestnut), 51 *Juglans regia* L. (walnut) and 36 *Tilia tomentosa* Moench. (silver linden), providing a financial estimate for their contribution to this historic park's green infrastructure.

The results indicate that the total value of the inventoried trees amounts to €1,696,935. Yet inadequate management and vandalism have led to a 26% loss in potential value, equating to approximately €448,110. This loss highlights the urgent need for improved tree care and strategic conservation efforts. Had proper maintenance been implemented, the total estimated value of these trees could have reached €2,145,045.

By assigning a financial worth to trees, the BEVA method provides a practical and accessible tool for evaluating green heritage elements in historic parks and urban landscapes. This valuation is essential for informing decision-making processes related to tree preservation, maintenance planning, and future restoration initiatives. The study highlights the importance of incorporating monetary assessments into conservation strategies to ensure the long-term sustainability of historic green spaces.

ACKNOWLEDGEMENTS

We extend our gratitude to the Bucharest Branch of the Romanian Landscape Architects Asscotiation, to Arche Association and to Cantacuzino Floresti Foundation for their support in conducting this research and for granting access to the study site and facilitating data collection.

We also appreciate the valuable insights and collaboration in gathering the data of all the professionals and students participating in the summer school events organised at Cantacuzino Domain over last years.

At the same time, we acknowledge the contribution of specialists and researchers who have previously explored tree valuation methods, thus providing a solid foundation for our study.

REFERENCES

Bruns Pflanzen (2011). *Catalogue of trees and shrubs 2011/2012*.

Bruns Pflanzen (2021). *Catalogue of trees and shrubs 2021/2022*.

Bruns Pflanzen (2023). *Catalogue of trees and shrubs 2023/2024*.

CAVAT Group (2023-1). CAVAT Full Method - A Guide for Practitioners. Online. . Retrieved November 11, 2024, from https://www.ltoa.org.uk/documents-1/capital-asset-value-for-amenity-trees-cavat/309-cavat-full-method-a-guide-for-practitioners/file.

CAVAT Group (2023-2). *Capital Asset Value for Amenity Trees Calculator*. Online. Retrieved November 11, 2024, from https://www.ltoa.org.uk/resources/cavat.

Chiesura, A. (2004). *The role of urban parks for the sustainable city*. Landscape and Urban planning.

Ciobota, A., Rusu, R, Culescu, D. L., Boanca, P., Condoroș, A., Bodea, S. Bedelean, R. I., Raducu-Lefter, A., Morar, T., Sirca, M., Mihalciuc, I., & Weber, C. (2017). *Ghid de bună practică pentru administrarea spațiilor verzi*. Universitatea de Vest publishing house - Agora Collection, ISBN 978-973-125-532-3.

Ciupa, V., Radoslav, R., Oarcea, C. & Oarcea Z. (2005). *Timișoara verde: Sistemul de spații verzi al Timișoarei*. Timișoara: Editura Marineasa.

Council of Tree and Landscape Appraisers (2020). *Guide for Plant Appraisal*. 10th Edition (revised). International Society of Arboriculture.

Conseil general des Hauts-de-Seine - Direction des parcs, jardins et paysages (2004). *Guide de gestion contractuelle de l'Arbre des Hauts-de-Seine*. Online. Retrieved November 11, 2024, from https://applis.hauts-de-seine.fr/v3fichiers/00_guide_arbre_complet.pdf.

Culescu, D. L. (2009). Landscape maintenance - Aviatorilor Boulevard - Bucharest: Assessment of the vegetal components, analysis of the current situation and guidelines setup for a rehabilitation strategy. *Scientific Papers. Series B, Horticulture. Vol. LIII*, Online ISSN 1222-5312.

Culescu, D. L. (2015). *Elaborarea unei metode de analiză a calității spațiilor verzi urbane* (PhD thesis within the Univerity of Agronomic Sciences and Veterinary Medicine of Bucharest).

Culescu, D. L. (2018). *Cat valoreaza un arbore?* Online Article. Retrieved November 11, 2024, from https://www.dianaculescu.ro/2018/09/01/cat-valoreaza-un-arbore/.

Département du Loiret (2020). Bareme d'Evaluation de la Valeur d'un Arbre. Online. Retrieved November 11, 2024, from https://www.loiret.fr/sites/loiret/files/media/documents/2020/05/bareme-evaluation-valeur-arbre-05042020.pdf.

Dobrescu, E. (2009). *Studii privind revitalizarea și restaurarea unor grădini din România realizate în sec. XIX-XX, sub influența modelelor francez și italian* (PhD thesis within the Univerity of Agronomic Sciences and Veterinary Medicine of Bucharest).

Doick, K. J., Neilan, C., Jones, G., Allison, A., McDermott, I., Tipping, A., & Haw, R. (2018). CAVAT (Capital Asset Value for Amenity Trees): valuing amenity trees as public assets. *Arboricultural Journal, 40*(2), 67–91.

Duinker, P. N., Ordóñez, C., Steenberg, J. W., Miller, K. H., Toni, S. A., & Nitoslawski, S. A. (2015). Trees in Canadian cities: Indispensable life form for urban sustainability. *Sustainability, 7*(6), 7379-7396.

Dwyer, J. F., Schroeder, H. W., & Gobster, P. H. Gobster. (1991). The significance of urban trees and forests: toward a deeper understanding of values. *Journal of Arboriculture,* 17.10, 276-284.

Dwyer, J. F., McPherson, E. G., Schroeder, H. W., & Rowntree, R. A. (1992). Assessing the benefits and costs of the urban forest. *Arboriculture & Urban Forestry (AUF)*, 18.5, 227-234.

El-Shamali, S. A., Streza, I. C., Dobrescu, E., Iliescu, A.F., & Ionescu, R. (2010). *Carl Friederich Meyer - Contributions to the Cultural Landscape of Bucharest*. Istanbul: ECLAS Conference Proceedings.

ENA - European Nurserystock Association. (2010). European technical & quality standards for nurserystock. 35 (section 9.2.5. Standard trees). Retrieved November 11, 2024, from https://www.enaplants.eu/_files/ugd/6336a3_ccb04222134f492fb300ad9f21e862b0.pdf.

Freytet, F., Bonnardot, A., & Laïlle, P. (2025). *VIE - Valeur Intégrale Evaluée de l'arbre*. Copalme & CAUE 77, Retrieved November 11, 2024, from https://www.baremedelarbre.fr/.

Georgi, N. J., & Zafiriadis K. (2006). The impact of park trees on microclimate in urban areas. *Urban Ecosystems*, 9, 195-209.

Giergiczny, M., & Kronenberg, J. (2014). From valuation to governance: using choice experiment to value street trees. *Ambio*, 43, 492-501.

Hanspach, J., Fischer, J., Stott, J., & Stagoll, K. (2011). Conservation management of eastern Australian farmland birds in relation to landscape gradients. *Journal of Applied Ecology*, 48(3), 523-531.

Hanou, I., Thurau, R., & Beck, B. (2014). *Virginia Locates New Urban Forest Benefits*. ESRI News for Forestry.

Hellis Solutions Limited (2019). An Introduction to Visual Amenity Valuation of Trees and Woodlands. Online. Retrieved November 11, 2024, from https://www.hellis.biz/advice-centre/general/an-introduction-to-visual-amenity-valuation-of-trees-and-woodlands/.

Helliwell, D. R. (1967). The Amenity Value of Trees and Woodlands. *Scottish Forestry*, Vol. 21, 109-112.

Kielbaso, J. J. (1979). Evaluating trees in urban areas. *Journal of Arboriculture*, 5, 70–72.

Mairie de Metz (2015). *Comte Rendu d'Infraction Initial*. Online. Retrieved November 11, 2024, from https://metz.fr/fichiers/2015/04/14/Arbres_decoupes_brules_r_Y__Goll_16_17032015.pdf.

McGarry, P. J., & G.M. Moore, G. M. (1988). The Burnley method of amenity tree evaluation. *Journal of Arboriculture*, 1(1), 19–26

Mexi, A., & Culescu, D. L. (2018). Cismigiu Garden and the beautification of culture. The role of vegetation in the coherence of landscaped ensemble. In *Caiete ARA - Arhitectura, Restaurare, Arheologie*, No. 9, Bucharest: "Arhitectura, Restaurare, Arheologie" publishing house, Bucharest, ISSN: 2068-0686, 223-230.

Mexi, A. & El-Shamali, S.A. (2015). Cismigiu garden in between original design and further transformations - a comparative study on continuously redesign process. *Scientific Papers. Series B, Horticulture.* Vol. LIX, Online ISSN 2286-1580.

Ministry of Culture (2015). *LMI - Lista Monumentelor Istorice, Judetul Prahova*. Online. Retrieved November 11, 2024, from https://www.cultura.ro/sites/default/files/inline-files/LMI-PH.pdf.

Moore, G.M. (1991). Amenity tree evaluation: A revised method. *Scientific Management of Plants in the Urban Environment*. Australia: Proceedings of the Burnley Centenary Conference, Centre for Urban Horticulture, Melbourne, 166–171.

Morar, T., Luca, E., Mornea Petrache, A., & Culescu D. L. (2019). Tree Inventory in historical garden of Teleki Castel using TreePlotter Software. *Agricultura*, no. 3-4(111-112), 418-422.

Negrutiu, F. (1980). *Spatii Verzi*. Bucharest: Editura Didactica si Pedagogica, 67-75.

Nowak, D., & Heisler, G. (2010). Air quality effects of urban trees and parks. Research Series Monograph. Ashburn, VA: National Recreation and Parks Association Research Series Monograph, 1-44.

Padlet (2020). Helliwell System – Advantage / Disadvantages. Online. Retrieved November 11, 2024, from https://padlet.com/whall16/helliwell-system-advantage-disadvantages-2020-vlo6fzb1bhhn079t.

Pepiniera Silva Periland (2021). *Oferta arbori si arbusti ornamentali – primavara 2021*.

Pepiniera Silva Periland (2023). *Oferta arbori si arbusti ornamentali – primavara 2023*.

Pepiniera Silva Periland (2025). *Oferta arbori si arbusti ornamentali – primavara 2025*.

PlanIT Geo (2025). TreePlotter Inventory for Cantacuziona Domain from Floresti – Prahova County (Romania). Online data base. Retrieved November 11, 2024, from https://uk.pg-cloud.com/RPR/.

Shackleton, S., Chinyimba, A., Hebinck, P., Shackleton, C., & Kaoma, H. (2015). Multiple benefits and values of trees in urban landscapes in two towns in northern South Africa. *Landscape and Urban Planning*, 136, 76-86.

Ville de Rouen - Direction des Espaces Publics et Naturels (2015). *Charte de l'arbre urbain à Rouen*. Online. Retrieved November 11, 2024, from https://rouen.fr/sites/default/files/publication/arbres_double.pdf.

Watson, G. (2002). Comparing formula methods of tree appraisal. *Journal of Arboriculture*, 28(1), 11-18.

Wycherley, P. R. (1976). *Towards a National Method of Valuing Trees*. Aust. Parks and Rec., 41-43, May.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

CENOLOGY, ECOLOGY AND DISTRIBUTION OF POPULATIONS OF *VIOLA HYMETTIA* BOISS. & HELDR. (VIOLA, SECT. MELANIUM) ON THE SANDS OF SOUTHERN OLTENIA, ROMANIA

Paula IANCU, Mariana NICULESCU

University of Craiova, Faculty of Agronomy, 19 Libertății Street, Craiova, Romania

Corresponding author email: mniculescum@yahoo.com

Abstract

The thematic area is located between Târburești and Sadova localities, in the southern part of Oltenia, south-east of Dolj County, in an area with sandy soil, also called "Sahara of Oltenia". The relief consists in plains and meadows covered by sand dunes. During the geobotanical research carried out in this area in the 2023-2024 period, several populations of Viola hymettia Boiss. & Heldr. (Viola, sect. Melanium) were identified. As a result of field studies, the presence of this rare species was found in the floristic structure of the plant communities specific to the sandy soils from south Oltenia. These populations were analyzed from a chorological, ecological and phytocenotic point of view in close correlation with the climatic changes of recent years and the degree of expansion of the surface of sandy soils in this part of Romania. Also, the species under study itself edify a community of plants Violaeto hymettiae-Cynodontetum Cîrțu 1973. The importance of this association lies in the fact that the participating species contribute to the stabilization of the sands and the enrichment of their humus content.

Key words*: coenology, chorology, ecology, sandy soil, Viola hymettia.*

INTRODUCTION

The floristic and faunal biodiversity of the sandy area of the Oltenia Plain, called the "Sahara of Oltenia", has been very little researched in the last 30 years. The growth and development of spontaneous and cultivated plants on sandy soils, unlike other lands, presents something specific to them (Buia & Păun, 1964). On the sandy soils of Oltenia, the specific biotic conditions allow the establishment of special plant communities (Păun et al., 1971; Păun & Popescu, 1972, 1983, 1985). Also, the natural habitats present a specific structure and shelter a special fauna (Niculescu, 2014). Large areas of forest vegetation, especially acacia, have been deforested, the area of sandy lands have expanded, the desertification phenomenon in Oltenia has taken on an even greater scale compared to the last century, a series of invasive species have appeared, especially in agricultural crops. The latter cause great damage and are very difficult to combat, as not much is known about their biology and ecology.

Viola hymettia, a flowering plant species in the violet family (*Violaceae*) is an annual pansy widespread in the South and East Europe to Caucasus, southern Balkans and the Aegean Islands, quite rare in central and southern Italy and Sicily, and in central-oriental part of Portugal, linked to stony pastures, dry open habitats and shrub fringes, mostly on sub-acid soils (Scoppola & Magrini 2019).

The *Viola* genus is represented, in the European Flora, by 123 species, among which 24 species are to be found in the Romanian Flora. *Viola hymettia* has the fallowing synonymies: *Viola kitaibeliana* f. *hymettia* (Boiss. & Heldr.) W. Becker in Beih. Bot. Centralbl. 26(2): 339 (1910); *Viola tricolor* subvar. *hymettia* (Boiss. & Heldr.) Gams in G. Hegi, Ill. Fl. Mitt.-Eur. 5: 601 (1926); *Viola tricolor* var. *hymettia* (Boiss. & Heldr.) Boiss. in Fl. Orient. 1: 466 (1867).

Viola hymettia is a therophyte species that blooms in spring from March to June whose habitat includes: grasslands, rocky terrains, river gorges. Due to its limited habitat and threats such as overgrazing and illegal collection, this species is classified as vulnerable on the IUCN Red List.

Information about the distribution of the species *Viola hymettia* in Romania is limited. However, this species is known to prefer mountainous habitat and is found especially in hilly and mountainous regions of the country, such as the Carpathians. *Viola hymettia* is found in forests and wet meadows at higher altitudes where moisture conditions and soil rich in organic matter are favorable. The Apuseni region and other mountainous areas in the west of the country are also places where this species can be frequently encountered. It is also found in the hilly areas of Maramureș and Bucovina, where the ecological conditions are favorable for its development. *Viola hymettia* is often found in rich plant communities that include herbaceous and shrubby species adapted to moist and shady conditions. Association with other species may vary depending on habitat specifics such as altitude, soil type and humidity.

Among the mentions related to the presence of the species *Viola hymettia*: GL: Hanu Conachi, 21.06.1993, leg. A. Oprea [I 97444]; Hanu Conachi, 14.05.1994, leg. A. Oprea [I 97451, 97455]; Hanu Conachi, 30.04.1995, leg. A. Oprea [I 97445, 97449]; Hanu Conachi, 12.06.1995, leg. A. Oprea [I 97446-97448]; Hanu Conachi, 19.05.1996, leg. A. Oprea [I 97454]; Şerbăneşti, 13.05.1994, leg. A. Oprea [I 97450]; Şerbăneşti, 14.05.1994, leg. A. Oprea [I 97453] (Irimia & Bartok, 2017).

Niculescu et al., 2014, identified this specie in the sandy soils from Dăbuleni surroundings placed in the southern part of Oltenia, Dolj County. Few years later, Simeanu et al., 2019 declared numerous modifications regarding the reduction of the area or the disappearance of some species or vegetal associations, the identification of some new places for some rare psamophilous taxa in Romania's flora (ex. *Silene borystenica, Achillea ochroleuca*) or by disappearance of some plant association (ex. *Violo hymettiae-Cynodontetum*). This species is also adapted to higher altitudes, where the climate is colder and humidity is higher.

The diversity of climatic, relief and soil conditions of Oltenia region has led to high phytodiversity and allow to many of the spontaneous plants to vegetate among Fam. Molluginaceae: *Mollugo cerviana* (L.) Ser. or Fam. Violaceae, with *Viola tricolor* L., *V. canina* L., *V. odorata* L., *V. alba* Bess. (Răduțoiu, 2020) and others.

This study presents a chorological, ecological and cenotic analysis of the species *Viola hymettia* in the southern area of Romania.

MATERIALS AND METHODS

Study area

The thematic area is located between Tâmburești and Sadova, in the southern part of Oltenia, southeast of Dolj County, in an area with sandy soil, also called "Sahara of Oltenia". The relief of the thematic area consists of plains and meadows covered by sand dunes (Figure 1).

The total area of sands in southern Oltenia exceeds 140,000 ha, being represented by strips of different widths along the Danube and on the left bank of the Jiu. Sandy soils in southern Oltenia have different origins and occupy the largest area in the country. Thus, in the studied territory the soils belong to the Luvisols class, with coarse sandy, sandy-loamy or sandy-loamy texture.



Figure 1. Map of the researched territory (source: Google Earth, modified)

From a climatic point of view, according to the Köppen climate classification, based on the natural distribution of plants native to an area, on annual and specific temperatures, on the precipitation regime, on local fluctuations and on seasonality, the psamosoils area in southern Oltenia is included in the Cfax climatic province, having a pronounced temperate continental character, with a slight Mediterranean influence, characterized by a pronounced dryness in the months of July - September and a surplus of precipitation in the months of May and June. This plain area has a climate with high annual thermal averages

(11.67ºC), being included in the area with the highest values in the country, but with low precipitation (556.77 mm/year) and frequent droughts. In southwestern Romania, the air temperature has increased progressively, the increase becoming more pronounced after 1995. Many monthly and daily temperature records have been broken in all months of the year. Summers have become frequently hot with long intervals of heat and sweltering heat, accentuating the drought. The hottest area of Oltenia is the Oltenia Plain and especially Dolj County (Marinică et al., 2024). In terms of the evolution of precipitation recorded in the area, these are variable depending on the year and unevenly distributed, being insufficient for plant consumption, especially in the summer months.

For the identification of the taxa, we have used the *Romanian Flora*, *vol. III*, and *Flora Europaea*, *vol. I*. Regarding the nomenclature, we have adopted the classified list solutions which are considered correct, according to the *International Code of Botanic Nomenclature*.

For coenological studies, we would use the working method of the Central European phytocenological school.

For the identification of the plant communities and the coenotaxonomic classification, synthesis works of Păun & Popescu, 1983, 1985, Coldea, 1991, Sanda et al., 1998, Sanda, 2002, as well as Oberdorfer, 1992, Mucina et al., 2016, Mucina, 1997, Rodwell, 2002 were used.

For the cenological studies, samples were taken in which the surface, altitude, degree of vegetation cover were noted. For each species within the plant community, we provide the abundance-dominance values, as well as their constancy within the sampling. Due to the variability of vegetation and dominant species, all samples were included in a single synthetic table. Surveys were carried out in each locality, the surface of each survey being 25 m^2.

In order to identify the habitats, we looked into: *Natura 2000 in Romania habitat fact sheets* (2008); *Habitats from Romania* (Doniţă et al., 2005); *Manual interpretation of Natura 2000 habitats in Romania* (Gafta & Mountford - coord., 2008).

RESULTS AND DISCUSSIONS

Chorological studies:

Viola hymettia is widespread in the southern part of Oltenia, on the sands on the left bank of the Jiu River, and grows in uncultivated places, on the sand dunes in the area, but also in the inter-dune areas, on the edge of the pea crops, vineyards, vegetable gardens, melons as well as on the edge of the acacia groves, in the forest patches or at the edge of the meadow forest from Bratovoiești. The species forms populations with a number of individuals ranging between 4-17/m^2 in the localities of Teasc, Tâmburești, Rojiște, Dobrești, Piscul Sadovei, Dolj County.

Phytosociological studies

On the sandy soils of Oltenia, the specific biotic conditions allow the establishment of special plant communities. Likewise, the natural habitats built by these plant communities have a specific structure and shelter a special fauna.

As a result of the research carried out, it was found that on the sandy soils from southern Oltenia, *Viola hymettia* is one of the edifying species of the plant community: *Violaeto hymettiae-Cynodontetum* Cîrţu 1973. Studies in this research were carried out in the localities of Tâmburești, Teasc, Rojiște, Dobrești, Piscul Sadovei, Sadova.

The plant community *Violaeto hymettiae-Cynodontetum* Cîrţu 1973 does not occupy very large areas, but develops well in the rainiest springs.

This plant community species presents a special importance both from a conservative and pedogenetic point of view, through the species in the floristic structure. The conservative role of this plant community is given by the presence in the floristic structure of other rare, endemic, vulnerable species, characteristic of the desertified lands of the "Sahara of Oltenia": *Centaurea arenaria* ssp. *borysthenica, Achilea ochroleuca Ehrh. (A. kitaibliana* Soó*), Bassia laniflora* (S.G. Gmelin) A. J. Scott (*Kochia laniflora* (S.G. Gmelin) Borbas)*, Jasione montana* L.*, Viola kitaibeliana* Schultes, *Polygonum arenarium* Waldst. et Kit, *Silene borysthenica* (Gruner) Walters., (*S. otites* (L.) Wib ssp. *parviflora*

(Ehrh.) Hayek), *Tragopogon floccosus* Waldst. et Kit., *Mollugo cerviana* (L.) Ser., *Ceratocarpus arenarius* L., *Potentilla arenaria* Borkh. (*P. cinerea* auct. non. Chaix.), *Corispermum nitidum* Kit. in Schultes., *Silene borysthenica* (Gruner) Walters., (*S. otites* (L.) Wib ssp. *parviflora* (Ehrh.) Hayek), *Euphorbia seguieriana* Necker (*E. gerardiana* Jacq.).

The plant community has a vegetation coverage of between 80-90%. In the floristic structure of the phytocenoses, the predominant bioforms are represented by therophyte species. Taking into account the forest elements, the species that dominate in these phytocenoses built by *Viola hymettia* are the Eurasian and cosmopolitan ones. In some phytocenoses, *Conyza canadensis* as an invasive plant has a high abundance-dominance, influencing the conservation status of the plant community. From a syndynamic point of view, it can evolve towards the following plant communities: *Mollugo cervianae-olteniae* Borza 61, *Plantagetum indicae* Păun 1964, *Setario-Digitarietum* 1942 em. Soó 1943, *Digitario-Portulacetum* (Timár 1953) Bodrogh 1955, especially in dry years, with excessive temperatures characteristic in recent years in southern Oltenia.

Table 1. *Violaeto hymettiae-Cynodontetum* Cîrţu 1973 plant community

Number of relevée	**1**	**2**	**3**	**4**	**5**	**K**
Area (m^2)	**25**	**25**	**25**	**25**	**25**	**2**
Altitude (m.s.m)	**65**	**67**	**76**	**74**	**61**	
Coverage (%)	**90**	**80**	**80**	**90**	**90**	
Char. Ass.						
Viola hymettia	1	1-2	+-1	+-1	+	V
Cynodon dactylon	4-5	4	4	4-5	4-5	V
Festuco-Mollugion						
Achillea ochroleuca	+	+	+	+	+	V
Tragus racemosus	+	+	-	-	+	III
Alyssum desertorum	+	+	+	+	+	V
Anthemis ruthenica	+	+	+	+	+	V
Chondrilla juncea	+	+	+	+	+	V
Tragopogon floccosus	+	-	+	-	-	II
Jasione montana	+	+	+	+	+	V
Ceratocarpus arenarius	+	-	-	+	+	III
Potentilla arenaria	+	+	+	+	+	V
Centaurea arenaria ssp. *borysthenica*	-	+	-	+	+	III
Corispermum nitidum	+	+	+	+	+	V
Polygonum arenarium	+	+	+	+	+	V
Silene borystenica	+	+	+	+	+	V
Festucetalia vaginatae						
Festuca vaginata	-	-	+	-	+	II
Euphorbia seguierana	+	+	+	+	+	V
Erysimum diffusum	+	+	+	+	+	V
Viola arvensis	+	+	+	+	+	V
Allium rotundum						
Festucetalia et Festuco-Brometea (incl. Festucetea vaginatae)						
Kochia laniflora	+	+	+	+	+	V
Holosteum umbellatum	+	+	+	+	+	V
Veronica verna	1	1	+	1	+-1	V
Arenaria serpyllifolia	+	+	+	+	+	V
Bassia laniflora	+	-	+	1	+-1	IV
Plamtago indica	-	-	+	-	+	II
Tribulus terrestris	+-1	+-1	1	1	1	V
Helichrysum arenarium	+	+	+	+	+	V
Apera spica-venti	+	+	+	+	+	V
Linum hirsutum	+	+	+	+	+	V
Poa bulbosa ssp. *vivipara*	+-1	1	1	+-1	+-1	V
Chondrilla juncea	+	+	+	+	+	V
Centaurea diffusa	+	+	+	+	+	V
Anthemis tinctoria	+	+	+	+	+	V
Linaria genistifolia	+	-	-	+	-	II

Potentilla argentea	+	+	+	+	+	V
Anchusa officinalis	+	+	+	+	+	V
Bromus hordeaceus	+-1	1	+	1	+	V
Dianthus armeria	-	-	+	+	-	II
Eryngium planum	+	+	+	+	+	V
Stachys germanica	+	+	+	+	+	V
Xeranthemum annuum	-	+	-	+	+	IV
Picris hieracioides	+	+	+	+	+	V
Salvia verticillata	+	+	+	+	+	V
Achillea setacea	+	+	+	+	+	V
Medicago lupulina	+	+	+	+	+	V
Myosotis stricta	+	+	-	-	+	III
Erodium cicutarium	+	+	+	+	+	V
Dichanthium ischaemum	+	+-1	+	+-1	+	V
Variae Syntaxa	+	+	+	+	+	V
Veronica persica	+	+	+	+	+	V
Torilis arvensis	+	-	+	-	-	II
Sagina procumbens	+	+	+	+	+	V
Gagea lutea	+	-	-	-	+	III
Viola tricolor	-	-	-	+	+	II
Veronica polita	+	+	+	+	+	V
Lactuca serriola	+	+	+	+	+	V
Sonchus arvensis	+	+	+	+	+	V
Convolvulus arvensis	-	+	+	-	-	II
Scleranthus perennis	1	+-1	+	+	+	V
Cerastium glomeratum	+	+	+	+	+	V
Conyza canadensis	2	2	1	+	+	V
Stachys recta	-	-	-	+	+	II
Cynoglossum officinale	+	-	+	-	+	III
Lappula squarrosa	+	-	+	+	+	IV

Place and data of relevées: 1 - 20.06.2023, Tâmburești; 2 - 15.05.2023, Teasc; 3 - 4.05.2024, Rojiște; 4 - 28.05.2024, Dobrești; 5 - 12.05.2024, Piscul Sadovei.

Viola hymettia species was also identified within other plant communities specific to sandy lands in the researched area, between the localities of Tâmburești and Sadova: *Mollugo cervianae-olteniae* Borza 61, *Plantaginetum indicae* Buia et al., 1960; Păun 1964, *Brometum tectorum* (Kern. 1863) Soó (1939) 1957, *Setario-Digitarietum* 1942 em. Soó 1943, *Digitario-Portulacetum* (Timár 1953) Bodrogh 1955, *Vicio-Polygonetum arenarii* Timar (1953) 1957, *Tribulo-Tragetum* Soó et Timat 1964, *Poetum (bulbosae) – Cynodontetum dactyloni* Res., Spîrchez et Csüros 1967, *Festucetum vaginatae oltenicum* Păun and Popescu 1972, 1983, 1985 and *Bromo sterilis-robinietum* (Pócs 1954) Soó 1964.

The species takes part of the floristic composition of plant communities that edify natural habitats of community or priority importance:

1. **Habitat 2110 Embryonic shifting dunes; Ro habitat type code: R1602, R1605**, vegetation adapted to ecological special conditions: high temperatures during the summer, xerophite and xerotherm key species due to powerful insolation, more in July and August, the poor in humus soil and lack of humidity (Gafta & Mountford, coord., 2008; Niculescu et al., 2014).

2. **Habitat 6120* Xeric sand calcareous grasslands; Ro habitat type code: R3502, R6402, R6403** - plant communities which are made on sandy soil with hydric lack from the summer season Here are some rare species such as: *Tragopogon floccosus, Silene conica, Viola kitaibeliana, Viola hymettia, Centaurea arenaria* (Gafta & Mountford, coord., 2008; Niculescu et al., 2014).

3. **Habitat 6260* Pannonic sand-steppes; Ro habitat type code: R6405 -** caracterized by continental sandy vegetation and represented by the followings: *Plantaginetum arenariae* (Buia et al., 1956, Popescu & Sanda 1987; *Brometum tectorum* Bojko 1934; *Potentillo arenariae-Festucetum pseudodalmaticae* (Domin, 1933) Majovsky 1954; *Bassio laniflorae-Brometum tectorum* (Soo 1938) Borhidi 1996 (Gafta & Mountford, coord., 2008, Niculescu et al., 2014).

CONCLUSIONS

Viola hymettia is a valuable species for the Oltenia region, having a pedogenetic but also conservative role, being an edifying species of a plant community rarely found in Romania, specific to sandy soils: *Mollugo cervianae-olteniae* Borza 61, *Plantagetum indicae* Păun 1964, *Vicio-Polygonetum arenarii* Timar (1953) 1957, *Festucetum vaginatae oltenicum* Păun and Popescu 1973.

We believe that phytocenotic studies on this vascular species characteristic of sandy lands in southern Oltenia are particularly important given the current climate changes and the aridification and even desertification character of this area.

Although *Viola hymettia* is not considered a threatened species in Romania, we believe that in Oltenia this species occurs on restricted areas, populations have a relatively small number of individuals, and the protection of natural habitats is essential for maintaining its populations. Human activities, to which current climate change is added, may negatively affect the distribution and conservation status of this species.

REFERENCES

Baniță, P.M. (1981). *Cultura plantelor pe nisipuri*, Ed. Scrisul Românesc, Craiova.

Borza, A.L. (1958). Contribuţii la flora şi vegetaţia din răsăritul României, *Contrib. bot. Univ. Cluj*: 127-158

Borza, A.L. (1963). Fitocenoze specifice pentru ţara noastră (I), *Acta Botanica Horti Bucurestiensis*, f. 2: 779-784.

Borza, A.L. (1966). Cercetări asupra florei şi vegetaţiei din Câmpia Română. (I). *Contrib. Bot. Univ. Cluj*: 141-162.

Borza, A.L. (1968). Cercetări asupra florei şi vegetaţiei din Câmpia Română. (II). *Contrib. Bot. Univ. Cluj*: 149-183.

Buia, Al., Păun, M. (1964). Flora şi vegetaţia nisipurilor, în "Nisipurile Olteniei şi valorificarea lor", *Bul. Şt. Inst. Agron. Craiova*, 7, supl.: 97-136.

Buia, Al., Păun, M., Maloş, C. & Olaru, M. (1960). Contribuţii noi pentru flora Olteniei. *Lucr. Şti. Inst. Agron. Craiova*, 4: 121-130.

Braun-Blanquet, J. (1932) Plantsociology. In The Study of Plant Communities (Transl. G. D. Fullerand H. S. Conrad); McGraw-Hill: New York, NY, USA; p. 539.

Chiriță, C. (1937). Nisipurile de la Hanu Conachi din punct de vedere naturalist şi forestier, *An. ICEF*, seria I, III: 3-125.

Ciocârlan, V. (2009). Flora ilustrată a României, Pteridophyta et Cormophyta. Ed. Ceres, Bucureşti.

Ciocârlan, V. (2000). *Flora ilustrată a României, Pteridophyta et Spermatophyta. ed. a 2a*, Bucureşti, Edit. Ceres: p. 1138.

Cîrţu, D. (1973). Vegetaţia terenurilor nisipoase din interfluviul Jiu-Desnăţui, Dolj, *An. şt. Univ. Craiova*: 36-43.

Coldea, G. (1991). Prodrome des associations végétales des Carpates du Sud-Est (Carpates Roumaines) [Prodrome of plant associations of South East Carpathians (Romanian Carpathians]. Documents Phytosociologiques, N.S., Camerino; 13, p. 317-539.

Dihoru, Gh. (1987). *Festuca beckeri* în flora României, St. cerc. biol., Seria biol. veget., t. 39, 1: 3-20.

Donita, N., Popescu, A., Comanescu Pauca, M., Mihailescu, S., Biris Iovu, A. (2005). Habitatele din Romania. Ed. Tehnică Silvică Bucureşti.

Enculescu, P. (1924). Zonele de vegetaţie lemnoasă din România în raport cu condiţiunile orohidrografice, climatice, de sol şi subsol. Mem. Inst. Geol. t. I, Bucureşti, p. 338.

Gafta, D., Mountford, J.O. (Eds) (2008). *Manual de interpretare a habitatelor Natura 2000 din România*. Editura Risoprint, Cluj-Napoca.

https://climate.ec.europa.eu/climate-change/consequences-climate-change_ro (accessed 17.01.2025).

IUCN Red List 2017–2020 Report https://nc.iucnredlist.org/redlist/resources/files/1630480997-Iucn_Red_List_Quadrennial_Report_2017-2020.pdf (accessed 26.02.2025).

Irimia I., Bartok, A. (2017). Threatened tracheophytes from Romania in the herbarium collection of "Alexandru Ioan Cuza" University of Iași. *Analele Ştiinţifice ale Univeritătii "A.I. Cuza" Iași*, s. II a, *Biologie vegetală*, 63, 1-2: 25-53.

Marinică, I., Vătămanu, V.I., Marinică, A.F. (2024). *Variabilitatea temperaturii aerului în Oltenia*, Ed. Universitaria, Craiova, p. 10.

Mititelu, D., Moţiu, Tamara, Barabaş, N. (1973). Vegetaţia rezervaţiei de nisipuri de la Hanu Conachi, Stud. Comunic. Muz. Şt. Nat. Bacău: 359-376.

Mititelu, D., Sârbu, I., Pătraşc Adriana, Gociu Zoe, Oprea, A. (1993). Flora şi vegetaţia jud. Galaţi, *Bul. Grăd. Bot. Univ. Iaşi*, t. 4: 69-101.

Mititelu, D., Ştefan, N. (1988). Two new plant associations, *Rev. Roum. Biol.-Biol. végét.*, t. 33, 2: 71-74.

Mucina, L., Bültmann, H., Dierßen, K., Theurillat, J.P., Raus, T., Tichý, L. (2016). Vegetation of Europe: hierarchical floristic classification system of vascular plant, bryophyte, lichen, and algal communities. In: *Applied Vegetation Science*, 19 (Suppl. 1), 3–264, Doi: 10.1111/avsc.12257.

Mucina, L. (1997). Conspectus of classes of european vegetation, *Folia Geobot. Phytotax*., 32: 117-172.

Niculescu, M., Grecu, F., Popescu, C. (2014). The corology, ecology and phytosociology of the sands plant communities and natural habitats in the sandy area from Dăbuleni, Dolj county - Romania. *Ecology and Environmental Protection*, 2(5):697-702. 14th

International Multidisciplinary Scientific GeoConference, SGEM.
Oberdorfer, E. (1992). SüddeutschePflanzen – gesellschaften, Teil IV: Wälder und Gebüsche 2, Stark berabeileteAuflageTexband, Gustav Fischer Verlang Jena, New York.
Oprea, A.D. (1997). Contribuţii cenotaxonomice din Câmpia Tecuciului, *Bul. Grăd. Bot. Univ. Iaşi*, t. 6: 397-404.
Oprea, A.D. (1997). Contribuţii floristice din Câmpia Tecuciului, *Bul. Grăd. Bot. Univ. Iaşi*, t. 6: 405-407.
Oprea, A.D. (1998). *Festuca beckeri* (Hack.) Trautv. in the southern part of Moldavia, *An. Şt. Univ. "Al. I. Cuza Iaşi*, t. XLIV, a II a, *Biol. Veget*., 109-112.
Păun, M., Georgescu, L., Fulga, Gh. (1971). Importante puncte floristice și de vegetație în cuprinsul Olteniei. Stud. și Cercet. C.C.E.S. Dolj: 67-84.
Păun, M. & Popescu, Gh. (1972). La vegetation des sables de la corbure du Danube (Olteniae). *Acta Botanica Horti Bucurestiensis*. Edit. Universitaria. București: 569-587.
Păun, M., Popescu Gh. (1983). Flora și vegetația terenurilor nisipoase din Oltenia. *Analele Universității din Craiova*, vol. XIII (XXIII): 25-29. Craiova.
Păun, M., Popescu Gh. (1985). Aspecte din flora și vegetația psamofilă a Olteniei. *Analele Univ. Craiova*, vol. XVI(XXVI): 39-43. Craiova.
Pop, L., Matei, I., Chichea, I. (1977). *Agrofitotehnia pe solurile nisipoase*, Editura Ceres, București.
Popescu, A., Sanda, V. (1987). Structura vegetaţiei din clasa *Festucetea vaginatae* Soó 68 de pe teritoriul României, *Culeg. de stud. şi artic. de biol., Grăd. Bot. Univ. Iaşi*, **3:** 225-235.
Popescu, A., Sanda, V., Doltu, M.I. (1980). Conspectul asociaţiilor vegetale de pe nisipurile din România, St. Com. Șt. Nat., Muz. Brukenthal, Sibiu, 24: 147-314.
Prodan, I. (1925). Flora nisipurilor din România sub raportul fixării și ameliorării. *Bul. Agr. supl., Bucureşti*, t. III: 1-92.
Răduțoiu, D. (2020). Ornamental plants species from spontaneous flora in Oltenia region, Romania. *Scientific Papers. Series B, Horticulture*. Vol. LXIV:1, 602-607.
Răvăruţ, M. (1949-1950). Contribuţiune la flora şi vegetaţia nisipurilor de la Matca-Ivești-Hanu Conachi, jud. Tecuci, *An. Acad. R. P. R., s. Şt. Geol., Geogr. şi Biol.*, Ser. A, t. 2: 747-767.
Rodwell, J.S., Schaminée, J.H.J., Mucina, L., Pignatti, S., Dring, Moss J.D. (2002). The diversity of European vegetation – An overview of phyto-sociological alliances and their relationships to EUNIS habitats. National Reference Centre for Agriculture, Nature and Fisheries [report no. EC-LNV 2002(054)], Wageningen.
Sanda, V. (2002). *Vademecum ceno-structural privind covorul vegetal din România*, Edit. Vergiliu, București, p. 331.
Sanda, V., Popescu, A., Barabaş, N. (1998). *Cenotaxonomia şi caracterizarea grupărilor vegetale din România. Stud. şi Com. Biol. Veg. Bacău*: Edit. „I. Borcea". p. 342.
Sârbu, I. (1984). Vegetaţia arenarie din bazinul Chinejii (jud. Galaţi), *Acta Botanica Horti Bucurestiensis*: 211-214.
Săvulescu, T. (Ed) (1952-1976). *Flora R.P Române. R.S România,* 1-XIII. București. Edit. Acad. R.P. Române (R.S. România), București.
Simeanu, C.G., Măceșeanu, D. M., Răduțoiu, D. (2019). The actual state of the psamophilic flora and vegetation in the Oltenia region (Romania). Muzeul Olteniei Craiova. Oltenia. *Studii şi comunicări. Ştiinţele Naturii*. Tom. 35, No. 1, p. 57-64.
Scoppola, A., & Magrini, S. (2019). Comparative palynology and seed morphology in annual pansies (Viola sect. Melanium, Violaceae): implications for species delimitation. Plant Biosystems - An *International Journal Dealing with All Aspects of Plant Biology*, 153(6), 883–899. https://doi.org/10.1080/11263504.2019.1610113.
Tufescu, V. (1966). *Modelarea naturală a reliefului şi eroziunea accelerate*. Ed. Academiei RSR, Bucureşti.
Tutin, T.G., Heywood, V.H. (coord.) (1964-1980). *Flora Europaea*, t. I-V, 1st Edition, Cambridge University Press.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

THE ROLE OF RESIDENTIAL GREEN SPACES IN ENHANCING WELL-BEING, BIODIVERSITY, AND SUSTAINABILITY: A CASE STUDY OF IAŞI, ROMANIA

Ana-Maria-Roxana ISTRATE, Clara-Teodora BĂLAN, Mirela COJOCARIU

"Ion Ionescu de la Brad" Iaşi University of Life Sciences, 3 Mihail Sadoveanu Alley, Iaşi, Romania

Corresponding author email: mirela.cojocariu@iuls.ro

Abstract

Recent studies have painted a compelling picture of the beneficial impact of rigorously designed open spaces on quality of life and urban social cohesion. These spaces significantly influence community closeness, urban viability, safety, sustainability, and vitality. Inter-community interactions embedded in open spaces' planning and design process are vital in shaping and valuing them, influencing social interaction's level of use, value, and intensity. This study focuses on the impact of residential green spaces on the resident's well-being, urban biodiversity, and sustainability. Based on international examples, case studies, and rigorous local context analysis, we proposed a landscape design for the Tatarasi district in Iasi, Romania. The proposed landscape design offers multiple functional zones, such as recreational areas or community gardens, using sustainability principles and urban biodiversity. The results can guide urban planners and officials to create regions that benefit residents' well-being and foster a greener environment. This study employs a multi-method approach, integrating spatial analysis, biodiversity assessment, and case study comparisons to develop an adaptive landscape intervention.

Key words*: biodiversity, sustainability, urban design, well-being, landscape architecture.*

INTRODUCTION

The design of urban green spaces plays a pivotal role in enhancing the well-being of residents, with its benefits stemming from three primary factors: spatial characteristics, biodiversity and naturalness, and community involvement. These elements collectively influence mental and physical health, foster social connections, and contribute to overall life satisfaction in urban environments. This study extends beyond theoretical discourse by situating its investigation within the residential green spaces of the Tatarasi district in Iasi, Romania, aiming to propose actionable landscape interventions that align with contemporary urban sustainability principles.

The spatial characteristics of green spaces significantly affect residents' mental well-being. Research demonstrates that a more significant number and larger total area of public green spaces are associated with improved mental health outcomes, suggesting a dose-response relationship where increased green space availability leads to better mental well-being (Pascu et al., 2021; Wood et al., 2017). Moreover, partially open spaces with a high degree of naturalness offer restorative benefits, surpassing green spaces dominated by hard surfaces (Cojocariu et al., 2023; Linghan et al., 2022). Even small nature features, such as balconies or roof gardens, positively contribute to well-being, albeit to a lesser extent than larger green areas (Allard-Poesi et al., 2022).

Biodiversity and naturalness within green spaces further enhance their impact on mental and physical health. Spaces rich in biodiversity, including natural elements like trees and water, have been linked to improved mental health and stronger social connections (Reyes-Riveros et al., 2021). Less domesticated natural environments, such as forests and open fields, promote psychological well-being by encouraging calm and reflective activities while improving physical health through exposure to natural elements (Allard-Poesi et al., 2022). These natural features also support environmental restoration, positively affecting physiological and psychological health (Linghan et al., 2022).

Community involvement in the planning, design, and maintenance of green spaces adds another layer of benefit. Engaging local

communities in these processes has been shown to improve psychological well-being and enhance life satisfaction among residents. Furthermore, increased satisfaction with personal safety and a stronger sense of community belonging, bolstered by community participation, mediate the relationship between environmental quality and well-being. Integrating local stakeholders into the planning stages fosters a sense of ownership and connection to green spaces, amplifying their positive effects on urban communities (van den Bogerd et al., 2021).

Despite the well-documented benefits of urban green spaces, many cities, including those in Eastern Europe, face significant challenges in maintaining, revitalizing, and equitably distributing green infrastructure. The Tatarasi district in Iasi exemplifies these challenges, where existing green spaces suffer from fragmentation, underutilization, and inadequate biodiversity. Addressing these deficiencies requires a comprehensive approach incorporating spatial equity, ecological integrity, and community participation. This research investigates how best practices in green space design from India, China, and Canada can be adapted to improve urban well-being and sustainability in the local context. By synthesizing international insights with rigorous local analysis, this study aims to develop an evidence-based landscape plan that enhances ecological and social resilience in Tatarasi.

Building on these theoretical insights, this study applies these principles to the Tatarasi district in Iasi, Romania. By examining the spatial characteristics, biodiversity, and community engagement within the district's green spaces, this research proposes targeted interventions that align with global best practices while addressing local challenges.

MATERIALS AND METHODS

Site Description

The site analyzed in this research is located in the southern part of Iasi, on the Central-Eastern side. According to the measurements in the Green Register of Iași County, for the objective Green Space, Al. Gradinari, Tatarasi neighborhood, the site's total area is approximately 17,185 m^2.

Figure 1 shows the objective's zoning, with Chimiei Boulevard and Bahlui River representing the spatial delimitations on the South side, Vasile Lupu Street on the West side, and Gradinari Street in the North. Near the site, there is green space, which Tatarasi Park represents.

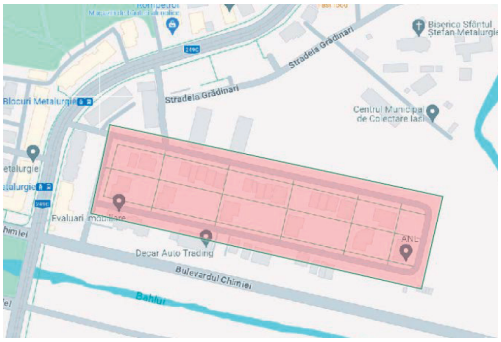


Figure 1. Urban placement of the site within Iasi

Table 1 presents the distribution of space in the studied site. The site, spanning 17,185 m^2, integrates various functional areas. The dominant greenspace, covering 12,232 m^2 (71.18%), is crucial in biodiversity conservation. Complementing this, the floristic amenity, extending over 227 m^2 (1.32%), enhances the landscape with decorative plant species, adding aesthetic and ecological value. A playground of 1,471 m^2 (8.56%) fosters recreation and social interaction, strengthening community engagement. Meanwhile, the infrastructure, consisting of roads, sidewalks, and driveways, occupies 3,232 m^2 (18.80%), ensuring seamless connectivity and accessibility throughout the site.

Table 1. Distribution of spaces on the study site

Sym.	Category	Surface m^2	Percentage %
FL	Floral landscaping	227	1.32
BU	Buildings	23	0.14
PL	Playground	1,471	8.56
RO	Roads, sidewalks, driveways	3,232	18.80
GS	Green space	12,232	71.18
Total land		17,185	100
Of which green area (FL+GS)		12,459	72.50

Research Approach

This study adopts a multi-method approach combining site analysis, international case study comparisons, and sustainability-driven design

principles (Booth & Hiss, 2011; Istrate et al., 2023). The research integrates geospatial analysis, biodiversity assessment, and participatory urban planning methodologies to propose a comprehensive landscape intervention.

Selection of Design Elements

The proposed intervention design drew insights from international precedents:

- Godrej Rivergreens from India (Figure 2) is a model for biodiversity integration and ecological resilience, demonstrating the effectiveness of native plant species in enhancing local ecosystems (StudioPOD, 2023).

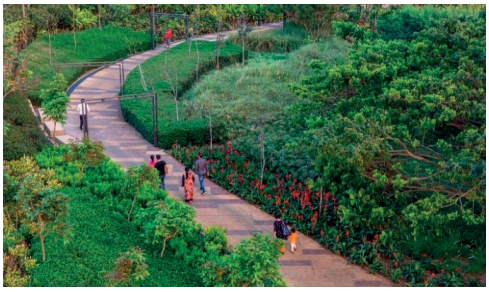


Figure 2. Godrej Rivergreens (StudioPOD, 2023)

- Oriental One from China (Figure 3) offers inspiration for multifunctional space design, focusing on the harmonious integration of green spaces into high-density urban environments (Gillespies, 2023).



Figure 3. Oriental One (Gillespies, 2023)

- Ketcheson Park from Canada (Figure 4) is a reference for sustainable infrastructure and stormwater management, showcasing advanced water retention and green infrastructure solutions (PWL, 2021).



Figure 4. Ketcheson Park (PWL, 2021)

These case studies were selected based on their relevance to the challenges faced in Tatarasi. Godrej Rivergreens exemplifies biodiversity conservation in an urban setting, Oriental One demonstrates the successful integration of green spaces in high-density environments, and Ketcheson Park highlights innovative approaches to stormwater management and sustainable infrastructure. The proposed interventions will enhance ecological and social resilience in the study area by adopting these strategies.

RESULTS AND DISCUSSIONS

Current Green Space Analysis

The existing landscape of the residential green spaces in the Gradinari area of Tatarasi, Iasi, presents a combination of functional and dysfunctional elements that impact residents' well-being, urban biodiversity, and sustainability. The site assessment highlights the accessibility, infrastructure conditions, vegetation composition, and existing amenities within the green spaces.

Accessibility and Pathways

The residential area is accessible from three main directions: from the south via Chimiei Blvd, from the west via Vasile Lupu Street, and through an internal connection via Gradinari Street. As seen in Figure 5, these access points provide good connectivity, yet specific pedestrian pathways require maintenance due to overgrown vegetation encroaching on walking spaces. The predominant paving material for pedestrian alleys is concrete, ensuring durability; however, localized sections show deterioration, necessitating cleaning, and minor repairs.



Figure 5. Accessibility and pathways

Sports Facilities

A notable feature within the green space is a 450 m^2 sports field. However, the facility is in a state of advanced degradation, with visible wear on the playing surface and perimeter infrastructure (Figure 6). This degradation negatively impacts its usability and reduces the green space's aesthetic quality. The lack of proper maintenance and rehabilitation limits its potential as a recreational and community engagement space.



Figure 6. Sports facilities

Public Furniture and Play Equipment

The existing urban furniture, consisting of benches and children's play equipment, exhibits various degrees of deterioration (Figure 7).



Figure 7. Public furniture and play equipment

The materials, mainly wood and metal, have suffered damage due to environmental exposure and prolonged use. Moreover, the furniture is not integrated harmoniously within the green space, affecting functionality and visual appeal. The absence of designated seating areas or shaded resting zones further reduces user comfort.

Vegetation and Biodiversity

The site's vegetation includes a diverse mix of tree, shrub, and flower species, contributing to ecological richness. The green space hosts over 30 species of trees and shrubs, such as *Acer platanoides* (Norway maple), *Fraxinus excelsior* (common ash), *Tilia tomentosa* (silver linden), and *Prunus armeniaca* (apricot). Most plant species exhibit good viability, yet the landscape appears unkempt due to insufficient maintenance practices. The distribution of vegetation is somewhat disorganized, reflecting a lack of structured landscape planning. Overgrown plants and excessive tree density in certain areas contribute to an unbalanced visual composition and reduce the perceived openness of the space (Figure 8). The presence of allergenic species, particularly the high number of linden trees, raises concerns regarding potential health impacts for residents sensitive to pollen.



Figure 8. Vegetation distribution

Identified Issues and Required Interventions

Several dysfunctions have been observed that require targeted interventions to enhance the green space's usability and sustainability:

- Overgrown vegetation on pedestrian pathways, making circulation difficult and requiring regular maintenance.
- Degradation of benches and playground equipment, demanding refurbishment or replacement to improve functionality and aesthetics.
- The poor condition of the sports field diminishes recreational opportunities and requires surface restoration.

- Inadequate lighting, which compromises security in the area during night-time hours.
- Lack of structured vegetation management, leading to an untidy appearance and reduced biodiversity value.

Addressing these issues through a comprehensive landscape design strategy will enhance the green space's overall sustainability, biodiversity, and well-being benefits. Proposed interventions should integrate maintenance plans, structured planting schemes, and improvements to recreational infrastructure to foster a more functional and visually appealing environment for residents.

While the proposed interventions offer substantial benefits, their implementation may face several challenges. Limited maintenance funding, potential resistance from local stakeholders, and the need for long-term community engagement could hinder sustainability. To address these challenges, collaboration with local authorities, securing external funding for green infrastructure projects, and establishing community stewardship programs will be essential.

Proposed Interventions

Functional Zoning for Inclusive Public Use

Based on Lefebvre's (Lefebvre, 1991) spatial theory, the proposed interventions transition the green space from a fragmented, underutilized area (perceived space) to a well-integrated and socially engaging environment (conceived space). The design includes functional zoning, as seen in Figure 10, to maximize the use of green spaces for all residents.

- Relaxation and Socialization Zones

Several spaces will be dedicated to relaxation and social interaction, featuring comfortable seating arrangements, shaded spots, and diverse vegetation to create a tranquil atmosphere. These areas will serve as gathering points where people can socialize, read, or enjoy the natural surroundings. The carefully designed layout will ensure these spaces are easily accessible and interlinked through well-maintained pathways (Figure 9).



Figure 9. Proposed relaxation area

Small rest areas will be developed for residents who prefer quieter, more secluded spots. These spaces will be surrounded by lush vegetation, providing privacy and immersion in nature. Benches and shaded structures will be integrated to enhance comfort and usability, encouraging people to spend more time outdoors.

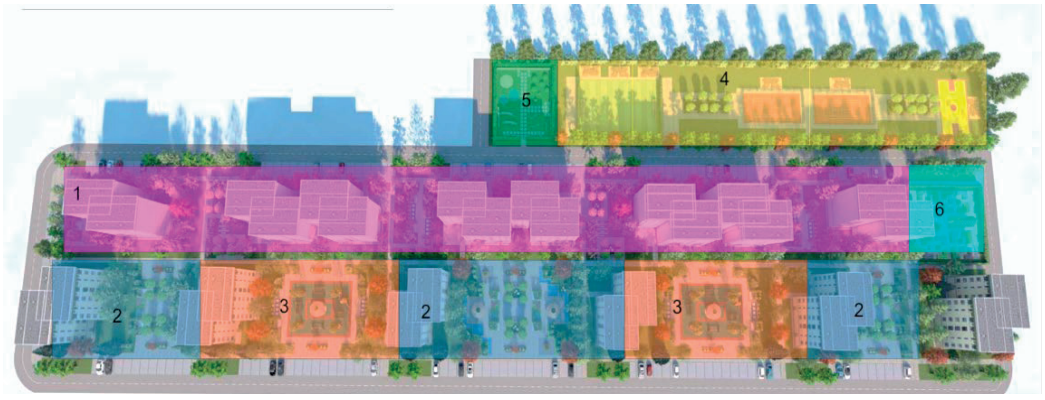


Figure 10. Proposed functional zoning:

1 - Small relaxation areas; 2 - Large relaxation areas; 3 – Playgrounds;
4 - Sports facilities; 5 - Dog park; 6 - Community garden

- Dedicated Play Areas

The playgrounds will undergo significant improvements, incorporating child-friendly, durable equipment designed to foster physical activity and creative play (Vladimir & Cojocariu, 2024). The materials will be safe, weather-resistant, and environmentally friendly, ensuring a sustainable long-term solution. The playgrounds will be positioned strategically within the green space, allowing caregivers to have clear sightlines while seated in nearby relaxation zones (Figure 11).



Figure 11. Proposed playground

- Community Garden

The proposal includes creating a community garden where residents can participate in urban gardening initiatives (Figure 12). This space will encourage sustainable practices, provide fresh produce, and foster a deeper connection with nature. A community garden will also strengthen social ties among neighbors as residents collaborate on shared gardening projects and workshops (Grecu et al., 2023; Hangan et al., 2023).



Figure 12. Proposed community garden

- Sports Facilities Rehabilitation

The existing sports field will be restored with upgraded surfaces, seating, and perimeter improvements to encourage active recreation (Figure 13).



Figure 13. Proposed sports facilities

- Pet-friendly Area

A dedicated pet-friendly zone will allow pet owners to enjoy outdoor time with their companions without interfering with other recreational activities. The space will include designated walking trails, agility equipment, and shaded seating, ensuring both pets and owners have a pleasant experience. Proper waste disposal facilities will be installed to maintain cleanliness and hygiene (Figure 14).



Figure 14. Proposed dog park

Vegetation Management and Biodiversity Enhancement

Inspired by Godrej Rivergreens (India), which prioritizes ecological resilience, the intervention will emphasize the importance of a well-maintained and diverse plant ecosystem. The project will introduce structured vegetation management strategies to enhance the site's ecological value while ensuring a visually appealing and sustainable landscape.

Pruning and restructuring efforts will improve tree health, increase sunlight penetration, and enhance plant vitality. Existing trees will be maintained, with special attention given to species contributing to urban cooling and air

purification. The inclusion of flowering plants such as *Syringa vulgaris* (common lilac), *Hydrangea macrophylla*, and *Spiraea japonica* 'Anthony Waterer' will not only enhance the visual appeal but also support pollinator populations by attracting bees and butterflies.

To improve natural cooling and create a comfortable microclimate, strategically placed trees like *Acer platanoides* (Norway maple), *Fraxinus excelsior* (common ash), and *Tilia tomentosa* (silver linden) will provide ample shade throughout the space. These species are well-adapted to urban environments and will contribute to reducing the heat island effect, making the green space more pleasant during warmer months.

The project also includes improvements to ground cover using resilient, moisture-retaining species such as *Carex oshimensis* 'Evergold' and *Pennisetum alopecuroides* 'Little Bunny'. These plants stabilize the soil, reduce erosion, and improve water absorption. Their drought-resistant properties will ensure the landscape remains lush and green with minimal irrigation requirements.

Multifunctional Green Spaces

Incorporating information from Oriental One (China), the intervention prioritizes creating multifunctional spaces that cater to diverse activities and user needs. The project envisions open, adaptable green areas that serve different purposes, allowing residents to use the spaces according to their preferences.

Small seating areas will be introduced to foster cultural engagement and creativity. These spaces will provide a venue for outdoor performances, poetry readings, educational talks, and community meetings. Designed with comfortable seating and natural shading, these areas will encourage spontaneous social interactions and cultural events that enhance the neighborhood's vibrancy (Figure 15).



Figure 15. Small seating areas

Another key feature will be thematic gardens, which will highlight local biodiversity and educate visitors about sustainable landscaping practices (Figure 16). Informational signage will be placed throughout these gardens, offering insights into the ecological role of various plant species and their benefits for urban ecosystems.

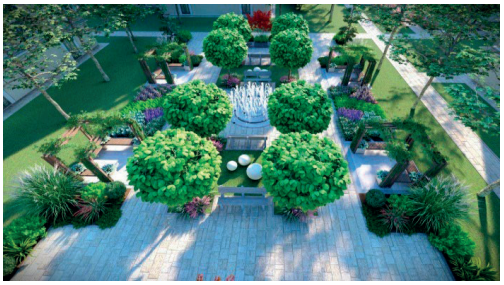


Figure 16. Thematic gardens

Strategically placed shaded seating areas will enhance user comfort, particularly near playgrounds and active zones (Figure 17). Parents and caregivers will have access to comfortable spots to supervise children while enjoying the benefits of green space. The seamless integration of these elements will create a holistic environment that serves the community's diverse needs.

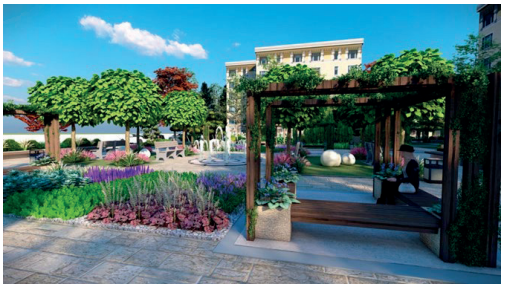


Figure 17. Shaded seating areas

Community Engagement and Social Inclusion

Taking inspiration from Ketcheson Park (Canada), the proposal highlights the significance of community-driven green spaces, ensuring that residents play an active role in shaping and maintaining their urban environment. This participatory approach fosters a sense of ownership and pride, making the green space a more cherished part of the neighborhood.

Residents will be encouraged to participate in urban gardening initiatives through designated plots to cultivate flowers, herbs, and vegetables. These gardening spaces will serve as educational hubs where workshops on sustainable agriculture, composting, and native plant species can be conducted. The initiative will strengthen community relationships while promoting environmental awareness.

To ensure meaningful community participation, structured engagement initiatives such as workshops, neighborhood meetings, and participatory design sessions will be organized. These platforms will allow residents to voice their preferences, contribute ideas for seasonal plantings, and participate in maintenance programs. Additionally, partnerships with local schools and environmental organizations can help integrate educational programs that promote sustainable practices within the community.

To accommodate diverse user groups, outdoor seating areas will be designed to encourage intergenerational interaction. Features like storytelling corners and shaded benches will create welcoming spaces for younger and older residents. These elements will enhance social inclusion and provide opportunities for meaningful exchanges across generations.

To maintain the space's vibrancy, flexible-use areas will be designated for pop-up art exhibitions and cultural festivals. These spaces will adapt to changing community needs, ensuring the green space remains relevant and actively used throughout the year. Public involvement will also extend to participatory landscaping initiatives, where residents can contribute ideas for seasonal plantings and temporary design installations.

Improved accessibility features, including smooth pathways, wheelchair-friendly seating, and clear signage, will ensure that the green space welcomes individuals of all abilities. By prioritizing inclusivity, the design aims to create a space that truly belongs to everyone in the community.

CONCLUSIONS

This study highlights the essential role of residential green spaces in improving well-being, biodiversity, and sustainability, focusing on the Tatarasi district in Iasi, Romania. Through spatial analysis, biodiversity assessment, and case study comparisons, the research demonstrates how well-planned green spaces enhance urban life and ecological resilience.

Key findings reveal that functional zoning, sustainable vegetation management, and community involvement are crucial for maximizing green space benefits. The proposed design - incorporating relaxation areas, playgrounds, a community garden, and sports facilities - creates an inclusive and multifunctional environment. Drawing from international best practices, this intervention balances social and ecological needs, fostering urban sustainability.

Green space planning directly contributes to mental and physical health, biodiversity conservation, and environmental resilience. Community engagement strengthens social cohesion and long-term maintenance, ensuring lasting benefits. These insights offer a practical framework for urban planners and policymakers to create greener, healthier, and more sustainable urban environments.

REFERENCES

Allard-Poesi, F., Matos, L., Massu, J. (2022). Not all types of nature have an equal effect on urban residents' well-being: A structural equation model approach. *Health & Place*, *74*, 102759. https://doi.org/10.1016/j.healthplace.2022.102759.

Booth, N. K., Hiss, J. E. (2011). *Residential landscape architecture: Design process for the private residence*. Prentice Hall.

Cojocariu, M., Bogrug, A., Dascălu, D. M., Istrate, A.-M.-R., Grecu, C. (2023). Introducing architectural-landscape elements in urban squares for higher quality of life. *Scientific Papers, Series B, Horticulture*, *XVII*(1), 348–355.

Gillespies. (2023). Oriental One by Gillespies. *Landezine*. Retrieved January 22, 2025, from https://landezine.com/oriental-one-by-gillespies/.

Grecu, C., Cojocariu, M., Balan, C.-T., Dascalu, D.-M. (2023). Using the principles of therapeutic gardens in the proposed redevelopment of the green space of the "Prof. Dr. Nicolae Oblu" Hospital in Iasi. *Scientific Papers, Series B, Horticulture*, *LXVII*(2), 440–446.

Hangan, A.-M. R., Cojocariu, M., Dascalu, D. M., Chelariu, E. L., Stoleru, V. (2023). Ornamental vegetable garden design possibilities using modular raised beds. *Journal of Applied Life Sciences and Environment*, *56*(1), 1–14. https://doi.org/10.46909/alse-561081.

Istrate, A.-M.-R., Cojocariu, M., Grecu, C., Stoleru, V. (2023). Landscape design process of a private events venue garden in Iasi County, Romania. *Scientific Papers, Series B, Horticulture*, *LXVII*(1), 404–412.

Lefebvre, H. (1991). *The production of space*. John Wiley and Sons Ltd.

Linghan, L., Qu, H., Ma, Y., Wang, K., Qu, H. (2022). Restorative benefits of urban green space: Physiological, psychological restoration and eye movement analysis. *Journal of Environmental Management*, *301*, 113930. https://doi.org/10.1016/j.jenvman.2021.113930.

Pascu, R., Zlati, C., Calance, Al., Bernardis, R., Dodu, D. (2021). Methods of rehabilitation of a degraded area in Orăştie. *Scientific Papers. Series B. Horticulture*, *LXV*(1), 684–693.

PWL, P. L. A. (2021). Ketcheson Neighbourhood Park. *Landezine*. Retrieved January 22, 2025, from https://landezine.com/ketcheson-neighbourhood-park/.

Reyes-Riveros, R., Altamirano, A., De la Barrera, F., Rozas, D., Vieli, L., Meli, P. (2021). Linking public urban green spaces and human well-being: A systematic review. *Urban Forestry & Urban Greening*, *61*, 127105. https://doi.org/10.1016/j.ufug.2021.127105.

StudioPOD. (2023). Godrej Rivergreens by StudioPOD. *Landezine*. Retrieved January 22, 2025, from https://landezine.com/godrej-rivergreens-by-studiopod/.

van den Bogerd, N., Elliott, L., White, M., Mishra, H., Bell, S., Porter, M., Sydenham, Z., Garrett, J., Fleming, L. (2021). Urban blue space renovation and local resident and visitor well-being: A case study from Plymouth, UK. *Landscape and Urban Planning*, *215*, 104232. https://doi.org/10.1016/j.landurbplan.2021.104232.

Vladimir, I., Cojocariu, M. (2024). The use of natural materials in the design of urban playgrounds: A sustainable approach important for the community. *Scientific Papers, Series B, Horticulture*, *LXVIII*(1), 684–689.

Wood, L., Hooper, P., Foster, S., Bull, F. (2017). Public green spaces and positive mental health—Investigating the relationship between access, quantity and types of parks and mental wellbeing. *Health & Place*, *48*, 63–71. https://doi.org/10.1016/j.healthplace.2017.09.002.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

DENDROBIUM ANTENNATUM LINDL. MICROPROPAGATED UNDER THE INFLUENCE OF VARIOUS CONCENTRATIONS OF FERBANAT L®

Endre KENTELKY[1], Zsolt SZEKELY-VARGA[1], Csanád Tas SZABÓ[1], Andrea TILLYNÉ-MÁNDY[2]

[1]Department of Horticulture, Faculty of Technical and Human Sciences, Sapientia Hungarian University of Transylvania, 2 Calea Sighişoara, Târgu-Mureş, Romania
[2]Department of Floriculture and Dendrology, Hungarian University of Agriculture and Life Science (MATE), 1118 Budapest, Hungary

Corresponding author emails: zsolt.szekely-varga@ms.sapientia.ro, tillyne.mandy.andrea@uni-mate.hu

Abstract

Orchids are among the most sought-after cut and potted plants in the 21st century. Dendrobium antennatum Lindl. is widely used as potted plant or as a cut flower. Due to this aspect, breeders need new propagation methods. In the present study, green antelope orchids were micropropagated using axillary buds at different concentrations of Ferbanat L® medium (0.1, 0.5, 1, and 5%) to assess its positive or negative effects regarding the plant's growth and development compared to control. The plants' survival rate was the highest when 5% Ferbanat L® was added to the medium. In the case of the chlorophyll a + b and total carotenoids no significant changes were observed, in the case of the peroxidase activity significant changes were observed by the different treatments. From the study, it can be concluded that control and 5% Ferbanat L® results were almost similar, however the treated plants (5%) recorded a higher survival rate, as well as the healthiest and most size-optimal plants. The results of the present research could positively improve the propagation of the D. antennatum.

Key words*: biostimulators, green antelope orchid, growth medium, propagation, tissue culture.*

INTRODUCTION

Cultivating ornamental plants is one of the developing, most profitable, diverse and fast-changing sector of plant production (Volckaert, 2010; Datta, 2022; Chandel et al., 2022; Gabellini and Scaramuzzi, 2022). Ornamental plants include diverse group of whole plants or parts of plants, usually produced for decorative reasons (Adebayo et al., 2020). According to data provided by the world's largest flower exchanges, Royal Flora Holland, 11.7 billion products were traded in 2021, an increase of 3% in comparison with the previous year (Royal FloraHolland Annual Report, 2021). Decorative plant producers have implemented contemporary cultivation technologies; nonetheless (Salachna, 2022), many future improvements remain to be made since the output of decorative plants is increasing and consumer expectations are constantly changing (Volckaert, 2010; Proietti et al., 2022).

The Orchidaceae is among the angiosperms' largest family, with their origin da-ting back to 120 million years ago (Lam et al., 2015). *Dendrobium* Sw. genus belongs to the family Orchidaceae, it is a pseudobulbous epiphyte and can be found primarily in the wet tropical biome. It is native in Papuasia and North Queensland (POWO, 2023), with 1545 accepted species (WFO, 2023), which are commonly used as a traditional tonic herb or food (Lam et al., 2015; Xu et al., 2013; Hinsley et al., 2018; Cheng et al., 2019). Among orchids, *Dendrobium* is one of the most well-known and significant blooming ornamental plants in the world (Mirani et al., 2017; Herastuti and Ek, 2020).

Dendrobium antennatum Lindl. commonly known as green antelope orchid is an ornamental potted plant, used also as cut flowers, with the flowering period occurring between March and December (Utami and Hariyanto, 2016). According to Nugroho et al. (2019), the green ante-lope orchid is an endangered species due to the conversion of forests; hence, conservation measures are required to preserve the plant in its natural habitat. Moreover, the *D. antennatum* is listed as endangered under the Nature Conservation Act 1992 (Queensland), Schedule 1 of the Endangered Species Protection Act

1992 (Cwlth), and Environment Protection and Biodiversity Conservation Act 1999 (Cwlth) (EPBC Act) (19. Approved Conservation Advice, 1999). For its conservation, another method could be the propagation of *D. antennatum* in higher quantities, to be restored in the wilderness. As previously mentioned, these or-chid species are highly admired for commercial purposes, thus the propagated plants could also satisfy the flower market.

The *D. antennatum* can be propagated generatively and vegetatively. The plants in their native regions can easily produce fruits with an abundance of seeds, unfortunately only a small quantity of viable seeds is able to germinate (Nugroho et al., 2019). According to Herawati et al. (2020), orchids can reproduce less than 1% in natural environment. A main reason for this could be the small size of the seeds and the lack of endosperm, and as well the insufficiency of suitable mycorrhization. Therefore, new methods are needed to produce a high quantity of, healthy and size-optimal plants. Plant tissue culture allows growing plants from organs, tissues or cells in controlled incubated conditions. Moreover, this method supplies the necessary energy, nutrients and water for the plants (Phillips and Garda, 2019) and also produce clean, disease and virus-free new plants. In addition, tissue culture is an important propagation method in plant breeding (Custódio et al., 2022), the most highly exploited version of it being micropropagation (Kulus and Miler, 2021). Plant development can be manipulated by adding natural or synthetic plant growth regulators, which can have a positive influence on the growth and resistance of the newly propagated plants. Micropropagation is an intensely used propagation technique for orchid conservation (Tikendra et al., 2019). The new alternative strategy involves using plant growth regulators and phytohormones in agricultural practices to boost plants' immune-protective abilities against various viruses and pests as well as their ability to better adapt to challenging environmental conditions (Phillips and Garda, 2019).

D. antennatum can have up to 130 cm pseudobulbs, a cluster holds up to 9-21 fragrant, long-lasting flowers, about 6 cm long, with white or greenish sepals, narrow and twisted petals, with purple-veined lips. The cluster inflorescence is mostly erect, rarely longer than the leaves.

The composition of the medium in one of the most important factors in micropropagation (Tuwo et al., 2021). Some previous studies found that micropropagated orchids need nutrients and additional complex compounds to increase the macro and micro-nutrients content (Moraes et al., 2020). In addition, several studies reported the applicability of commercial or organic fertilizers to reduce the propagation costs, and induce faster callus formation (Moraes et al., 2020; Suryani and Sari, 2019; De Stefano et al., 2022; Sasmita et al., 2022). Tissue cultured orchid media can also be supplemented with banana flour, coconut water (Vilcherrez-Atoche et al., 2020), tomato or bean sprout extract (Dwiyani et al., 2022). Furthermore, it is reported that cytokinin 6-benzyladenine (BA) and auxin naphthaleneacetic acid (NAA) influenced the root initiation of *in vitro* propagated *Cymbidium aloifolium* seeds (Potshangbam and Nirmala, 2011).

In recent years, organic fertilizer has emerged as a beneficial substitute for reducing environmental damage. Zayed and Saber, (2020), reported that organic fertilizers increased the growth parameters and chlorophyll content of micropropagated date palm.

Biostimulants can be described as small amounts of organic and inorganic matter, which promote the plants growth and development (Kisvarga et al., 2022). Biostimulator microorganisms are important alternatives to promote plant production (Nephali et al., 2020; Sanó et al., 2022). Previous studies demonstrated that biostimulants positively influence the growth and development by micropropagated orchids (Gontijo et al., 2018; Paris et al., 2019; Bhattacharyya et al., 2021). Furthermore, it is also mentioned that biostimulants can influence the plants in a positive way in their adaptation to *ex vitro* conditions (Karpushina et al., 2021).

The aim of the present study was to investigate the effect of Ferbanat L® biostimulator on micropropagated *D. antennatum* Lindl. In the experiment the influence of Ferbanat L® was analyzed on the plant's growth, development parameters, on its chlorophyll and carotenoids concentration, and peroxidase enzyme activity.

We aimed to determine the appropriate concentration to get healthy, size-optimal and the largest quantity of new green antelope orchid plants, in order to decrease the loss of natural populations and to promote species conservation. The results of the present study are reported here for the first time.

MATERIALS AND METHODS

The experiment was conducted at the Hungarian University of Agriculture and Life Science, Department of Floriculture and Dendrology.

The axillary buds were obtained from the orchid collection of the ELTE Botanical Garden, Budapest. *D. antennatum* protocorms, were micropropagated from axillary buds, transferred to the media in a sterile chamber using sterile equipment. Axillary buds were surface sterilized with 70% ethanol for 1 min, 6 g/L Na-dihydroizocyanurate for 10 min and washed 3 times in sterile distilled water. The inocula were multiplied on 0.5 mg/L BA containing Knudson (KC) (Knudson, 1922) medium and were maintained on KC medium without plant hormones. The plants used for the experiment were maintained for two years. During the experiment the temperature was 22 ± 2°C, light 16/8 h lighting with traditional fluorescent lamp, 2000 lux light intensity.

D. antennatum protocorms were introduced to the media. These contained different concentrations of Ferbanat L® (0% (as control), 0.1%, 0.5%, 1%, 5%). For each treatment 30 individual plants were selected as biological replicas.

The preparation of the medium was a modified Vacin and Went medium (Vacin and Went, 1949) began with the accurate measurement of the required additives 500 mg/L $(NH_4)_2SO_4$, 200 mg/L $Ca_3(PO_4)$, 250 mg/L $MgSO_4*7H_2O$, 250 mg/L KH_2PO_4, 525 mg/L KNO_3, 25mg/L Na-Fe-EDTA and 75 mg/L $MnSO_4$). These additives were completed with 20 g/L sucrose, 5.5 g/L Plant Agar (Duchefa). pH was adjusted to 5.7. The solution containing the required amount of culture medium was mixed with distilled water, and then all the stock solutions were pipetted into individual beakers, followed by the addition of sucrose, agar and Ferbanat L® at different concentrations levels (0.1%, 0.5%, 1%, 5%). All of this was eventually diluted to the desired volume. During the autoclaving process, a temperature of 121°C and a pressure of 1.2 bar were applied during 30 minutes.

Ferbanat L®, produced originally by the Turkish Ekosistem company, was allowed in Hungary, "Bistep conditioner" (Ördögh et al., 2019; Yaseen and Takácsné Hájos, 2021), which is a liquid humic extract made of bio-humus basis. It contains micro- and macro-elements, natural growth hormones, humic material, soil antibiotics, fito-vitamins, amino acids and useful soil microorganisms (Ferbanat Labs, 2023).

After six months, once the plants' vegetative components had fully matured, the size of the plants, root length, the number of leaves, roots, and ramifications were assessed. Furthermore, the plants survival rate was recorded at the end of the experiment. Plant length measurement was carried out under sterile conditions. During the experiment the plants were transplanted two times as this species (like many orchids) grows slowly.

The Chlorophyll *a* + *b* and total carotenoids were measured from 120-150 mg of fresh material according to the modified method of Arnon and Cooper (1949). The material was ground to become a cooled mortar with ice-cold 80% (v/v) acetone and a small amount of pure quartz sand. The sample was completed for a final volume of 10 ml with 80% acetone. The prepared samples were centrifuged at 1350 rpm for 5 min at a temperature of 4 °C. The absorbance of the supernatant was determined at 663 and 664 nm for chlorophyll *a* + *b* and 480 nm for carotenoids with Thermo Scientific Genesys 10 vis (Thermo Fisher Scientific Inc., Finland) spectrophotometer.

Chlorophylls *a* + *b* and carotenoid concentrations were calculated using the following equations:

$$\text{Chlorophyll } (a + b)\ \mu g/g \text{ fresh material} = (20.2 \times A644 + 8.02 \times A663) \times V/w$$

$$\text{Carotenoid } \mu g/g \text{ fresh material} = 5.01 \times A480 \times V/w$$

Where: V = volume of tissue extract (ml); w = fresh weight of tissue (g); A_{1min} = absorbance, the light absorption at the wavelength of the blind. A means absorbance on the given wavelength. A_{1min} shows the change of absorbance during 1 min. As the enzyme acts eliminates H_2O_2, the color of orthodianizidine changes. The instrument measures the absorbance every 10th second and calculates the

absorbance change during this period (1 min), against the blank.

For peroxidase enzyme activity 300-500 mg of fresh material was measured with analytical balance, and ground with 1200 µl K-phosphate buffer (pH: 6.5) using clean quartz sand to an ice-cold mortar. After the material was centrifuged at 1350 rpm for 20 min at a temperature of 4°C, 1700 µl of sodium acetate buffer (pH 4.5), 30 µl H_2O_2 (1000x dilution (30%) of concentrated H_2O_2) and 20 µl ortho-dianisidine (10 mg/ml) were added to 10 µl of sample (Shannon et al., 1966). The absorbance of the supernatant containing the solution was determined at 460 nm, ten times/min and the absorbance changes were calculated by the Thermo Scientific Genesys 10 vis spectrophotometer (Thermo Fisher Scientific Inc., Finland).

The enzyme activities were calculated using the following equations:

$$\text{enzyme activity (U/g)} = \Delta A_{1min} * \text{dilution}/\varepsilon$$

Where: ΔA_{1min} = measured value; ε = molar extinction coefficient; $\varepsilon_{ortho\text{-}dianisidine}$=11.3

ΔA_{1min} means the absorbance change of the solution within 1 min.

The significance of the differences between the treatments was tested by applying one-way ANOVA, at a confidence level of 95%. When the ANOVA null hypothesis was rejected, Tukey's post hoc test was carried out to establish the statistically significant differences at $p < 0.05$. Heatmap and dendrograms were generated using the Euclidean distance based on Ward's algorithm for clustering. The analyses were performed using the Paleontological Statistics (PAST) software (Oslo, Norway) (Hammer, 2001).

RESULTS AND DISCUSSIONS

From the obtained data, one the one hand statistically significant differences were observed at the 0.1 and 0.5% treatments compared to the control, but 1 and 5% con-centration treatments gave results similar to the control. On the other hand, 0.1 and 0.5% treatments had a detrimental effect on the micropropagated green antelope orchids, even preventing them from growing (Figure 1). Control, 1 and 5% concentration treatments produced a higher growth, with ~ 5 mm compared to the other two treatments (0.1 and 0.5%).

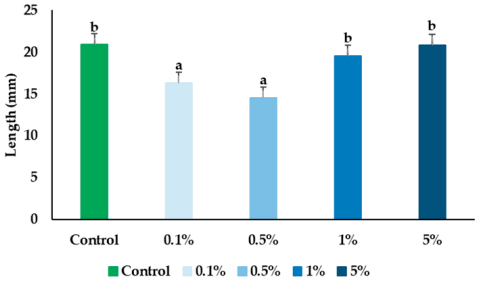


Figure 1. Effect of the Ferbanat L® on the length parameters of micropropagated *Dendrobium antennatum*. Plantlets under control conditions, and in the presence of the indicated concentration of Ferbanat L® (0.1, 0.5, 1 and 5%). Bars represent the means ± SE (n = 30). Different letters indicate significant differences between treatments ($p < 0.05$)

Under our experimental conditions, the root of the green antelope orchid was significantly influenced under the Ferbanat L® treatments (Figure 2). Root length recorded statistically significant differences under the treatments (Figure 3a). Under Ferbanat L® treatment, *D. antennatum* axillary buds presented significantly decreased root length compared to control.

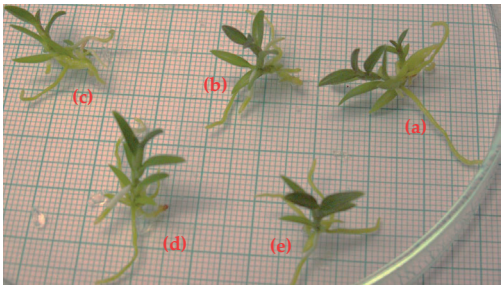


Figure 2. Root formation of *D. antennatum* under the effect of the Ferbanat L®. Control (a), 0.1% (b), 0.5% (c), 1% (d), and 5% (e)

Statistically significant differences were recorded when comparing the control to the buds treated with each of the four concentrations. However, the highest decreases were observed by the axillary buds treated with 0.5% of Ferbanat L®, where ~ 14 mm differences were measured. Lower differences were reported between control and the other three treatments. The longest roots were determined by the control where 15.62 mm was the average length, followed by 5% with 11.12 mm. A smaller inhibition was observed at the 1% concentration where the average recorded length was 8.59

mm. Furthermore, a greater inhibition of average root length was reported at 0.1% (5.2 mm) followed by 0.5%, in this case only 1.86 mm was the average root length.

Similar changes were detected concerning the number of roots, as in the case of root length (Figure 3b). The 0.1, 0.5 and 5% treatments significantly decreased the root number compared to control. The 0.5% concentration treatment resulted in almost five times less roots formed compared to untreated *D. antennatum* axillary buds. However, in the case of the 1% concentration no statistically significant differences were determined in the number of roots compared to control. The greatest number of roots was reported under control conditions with an average of 5.1, which was followed by the 1 % with an average of 4.2, by 5 % with 3.73, and by 0.1% concentration level with 2.46 roots counted. The lowest number of roots was determined at 0.5 % concentration treatment, where only 0.5 were counted.

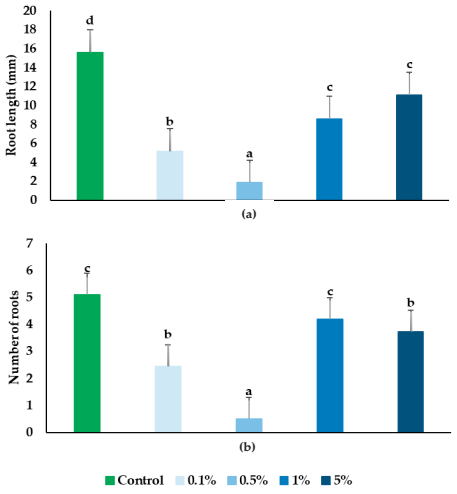


Figure 3. Effect of the Ferbanat L® on root length (a) and increment in root number (b) parameters of micropropagated *D. antennatum*. Plants under control conditions, and in the presence of the indicated concentrations of Ferbanat L® (0.1, 0.5, 1 and 5%). Bars represent the means ± SE (n = 30). Different letters indicate significant differences between treatments ($p < 0.05$)

Under our experimental conditions, no statistically significant differences were observed in leaves number appearance between the micropropagated *D. antennatum* axillary buds when subjected different to treatments (Figure 4a). The average number of leaves was between 9 and 12.

Regarding the number of ramifications, a few minor changes were recorded, however, no statistically significant differences were found (Figure 4b). The smallest number of ramifycations was observed at the 1% concentration treatment (0.66), followed by the 5%, 0.5%, and control. The highest number was determined at the 0.1% concentration of Ferbanat L® where in this case an average of 1.93 was recorded.

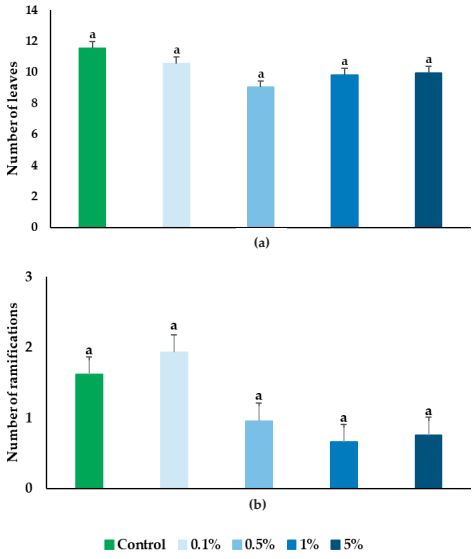


Figure 4. Effect of the Ferbanat L® on the number of leaves (a) and increment of ramifications (b) parameters of micropropagated *D. antennatum*. Plants under control conditions, and in the presence of the indicated concentration of Ferbanat L® (0.1, 0.5, 1 and 5%). Bars represent the means ± SE (n = 30). Different letters indicate significant differences between treatments ($p < 0.05$)

The effect of Ferbanat L® is also confirmed by our results based on the presence of necrosis on plants parts (Figure 5). During the experiment, any plants that displayed necrosis on any of their organs were documented. It should be pointed out that all the axillary buds subjected to the 5% concentration survived the experiment (all 30 axillary buds). The treatment with 5% concentration was followed by the 1% concentration (63% of plants survived) and buds under control conditions (53% of plants survived). The highest plant loss was recorded

at the treatment with 0.1 and 0.5% concentration of Ferbanat L®, in both cases only 30% of the axillary buds survived the propagation experiment (Table 1).

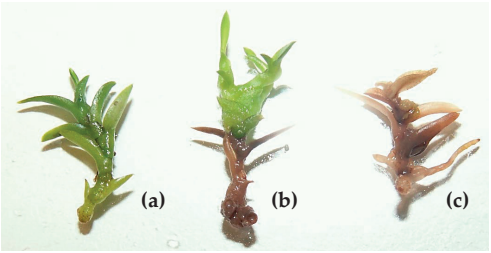


Figure 5. Plant health stage: healthy plant (a), starting of necrosis (b), necrosed (c). Plant health stage: healthy plant (a), starting of necrosis (b), necrosed (c)

Table 1. Effect of the Ferbanat L® on the plant survival rate of micropropagated *D. antennatum*. Plants under control conditions, and in the presence of the indicated concentration of Ferbanat L® (axillary buds without treatment were considered as control, 0.1, 0.5, 1 and 5%)

Plant survival rate (%)				
Treatments applied				
Control	0.1%	0.5%	1%	5%
53	30	30	63	100

Photosynthetic pigments content of leaf - chlorophylls *a* + *b* (Table 1) and carotenoids (Table 2) - were determined in the selected *D. antennatum* plants, following the applied treatments.

Table 2. Photosynthetic pigment concentrations under the effect of the Ferbanat L®. Leaf content of chlorophyll *a* + *b* and total carotenoids in micropropagated *D. antennatum*. Plants under control conditions, and in the presence of the indicated concentration of Ferbanat L® (0.1, 0.5, 1 and 5%)

Photosynthetic pigment concentrations (Chlorophyll (*a*+*b*) µg/g)				
Treatments applied				
Control	0.1%	0.5%	1%	5%
0.26 ± 0.02 a	0.26 ± 0.03 a	0.25 ± 0.02 a	0.25 ± 0.04 a	0.30 ± 0.03 a
Total carotenoids (µg/g)				
Treatments applied				
Control	0.1%	0.5%	1%	5%
0.06 ± 0.01 a	0.06 ± 0.01 a	0.06 ± 0.01 a	0.06 ± 0.01 a	0.07 ± 0.01 a

On the one hand, under the effect of 0.1, 0.5, and 1% Ferbanat L® the average values of chlorophyll were reduced, but the differences compared to control were not statistically significant. Furthermore, no statistically significant differences detected. Total carotenoid contents were relatively low, with minimal variation between the various treatments. No statistically significant differences were reported between the control and the treated plants.

In this study the peroxidase enzyme activity was determined from fresh leaf material (Figure 6). The enzyme activity changed in plants subjected to the Ferbanat L® treatment. No statistically significant differences were recorded by 0.1 and 0.5% concentration compared to control. Statistically significant differences were reported by treatments with 1 and 5% concentration when compared to control. With these treatments (1 and 5%) enzyme activity was reduced by half compared to control. The greatest decrease was determined by the 5% Ferbanat L® treatment with 0.762 U/g.

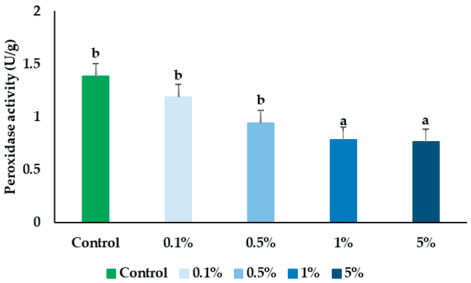


Figure 6. Leaf content peroxidase activity under the effect of the Ferbanat L® of micropropagated *D. an-tennatum*. Plants under control conditions, and in the presence of the indicated concentration of Ferbanat L® (0.1, 0.5, 1 and 5%). Bars represent the means ± SE (n = 30). Different letters indicate significant differences between treatments ($p < 0.05$)

The growth of the plants showed a strong positive correlation with root length (r=0.93), number of roots (r=0.93), plant survival rate (%) (r=0.79), and was moderately associated with number of leaves (r=0.57), chlorophyll content (r=0.52) and total carotenoids (r=0.43). Conversely, negative significant difference was recorded with number of ramifications (r=−0.19) and no association with peroxidase activity (U/g) (r=−0.01). Root length (mm) showed strong positive association with number of roots (r=0.93) and number of leaves (r=0.78), significant positive association with plant survival rate (%) (r=0.57), chlorophyll (r=0.38), peroxidase activity (r=0.32) and total carotenoids (r=0.27), but no significant association was noted with total carotenoids

(r=0.27) and number of ramifications (r=0.08). Number of roots was found to have highly significant correlation with number of leaves (r=0.74) and moderate association with plant survival rate (r=0.55), chlorophyll (r=0.25), and peroxidase activity (r=0.21), though a weak association was recorded with total carotenoids (r=0.16) and number of ramifications (r=0.03). Number of leaves showed negative and weak association with total carotenoids (r=-0.13), positive and weak association with chlorophyll (r=0.08) and plant survival rate (r=0.03), though positive and significant association peroxidase activity (r=0.78) and number of ramifications (r=0.67). Number of ramifications showed negative and moderate significant association with plant survival rate (r=-0.57), and total carotenoids (r=-0.42), and negative significant difference was recorded with chlorophyll content (r=-0.20), whereas a strong and positive association was found with peroxidase activity (r=0.89). Plant survival rate (%) showed negative and significant association with peroxidase activity (r=-0.53), whereas a strong positive correlation was found with total carotenoids (r=0.86) and chlorophyll contents (r=0.82). The chlorophyll content was strongly and positively associated with total carotenoids (r=0.97), negative and intermediate significant association was recorded with peroxidase activity (r=-0.31), whereas the total carotenoids showed negative and significant association with peroxidase activity (r=-0.51).

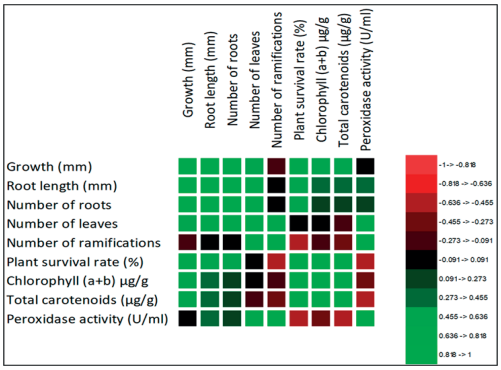


Figure 7. Correlation cluster heatmap illustrating the relationship among the evaluated traits of the micro-propagated *D. antennatum* revealed by XLSTAT. Note: Positive and negative correlations between the evaluated characteristics are displayed in red and green. A darker hue represents a strong correlation, whereas a lighter hue represents a weak correlation among the traits

Table 3. Correlation matrix (Pearson)

Variables	Growth (mm)	Root length (mm)	No. of roots	No. of leaves	**No. of ramifications**	Plant survival rate (%)	Chlorophyll (*a+b*) µg/g	Total carotenoids	Peroxidase activity (U/ml)
Growth (mm)	1	0.935	0.938	0.572	**-0.192**	0.794	0.525	0.473	-0.014
Root length (mm)	0.935	1	0.935	0.780	**0.082**	0.575	0.388	0.278	0.324
No. of roots	0.938	0.935	1	0.742	**0.033**	0.553	0.258	0.167	0.211
No. of leaves	0.572	0.780	0.742	1	**0.676**	0.036	0.080	-0.133	0.785
No. of ramifications	-0.192	0.082	0.033	0.676	1	-0.575	-0.205	-0.426	0.891
Plant survival rate (%)	0.794	0.575	0.553	0.036	**-0.575**	1	0.820	0.866	-0.532
Chlorophyll (a+b) µg/g	0.525	0.388	0.258	0.080	**-0.205**	0.820	1	0.970	-0.316
Total carotenoids (µg/g)	0.473	0.278	0.167	-0.133	**-0.426**	0.866	0.970	1	-0.521
Peroxidase activity (U/g)	-0.014	0.324	0.211	0.785	**0.891**	-0.532	-0.316	-0.521	1

The results of the present study indicate that an appropriate percentage of Ferbanat L® can have a positive effect on *D. antennatum* micropropagation. Biostimulators can be used to increase the growth, the development and the survival rate of tissue cultured plants (Sanó et al., 2022; Vargas-Hernandez et al., 2017; Fei et al., 2019; Brazienė et al., 2019; Paradiković et al., 2019;

Ogunsanya et al., 2022). Moreover, biostimulant application could have a positive effect in stressful growth conditions (Parađiković et al., 2019), stimulate several physiological and molecular processes that lead to improved nutrient uptake and utilization efficiency (Rouphael and Colla, 2018; Meddich, 2022). Also, the medium in which the plants are tissue cultured is an important fact for achieve a higher success of *in vitro* propagation (Tharapan and Obsuwan, 2016). Nugroho et al. (2019) mentioned that the key fact of a successful micropropagation depends on the culture medium, the composition of the nutrients and the content of growth regulators. For instance, in a previous study it was reported that supplementing the Murashige and Skoog semi-solid medium with α-naphthalene acetic acid, 2,4-dichlorophenoxy acetic acid and 6-benzylaminopurine increased the development of the *Dendrobium longicornu* axillary buds, into plantlets (Dohling et al., 2012). Moreover, it was also reported that adding 1.5 mg/L BAP (benzylaminopurine) to the same Murashige and Skoog medium leads to a higher plantlet viability (Mullin et al., 2022). Regarding the plants survival rate the possibility of *in vitro* contamination is one of the main issues in micropropagation (Poobathy et al., 2019).

From the data obtained it can be concluded that different concentration levels in-fluence the growth of the subjected plants, in a positive way (1 and 5%), however by these two treatments no significant differences were observed when compared to control in the case of growth (in spite of the fact by this treatment the highest survival rates were recorded). Moreover, when the concentration level of the biostimulator was lower (0.1 and 0.5%) the plant growth was inhibited. Moraes et al., (2020) mentioned that commercial fertilizer supplemented with potato and banana pulp recorded better plant growth results by orchid micropropagation when compared with the organic fertilizer.

Roots growth and development are vital for any plant. The goal of micropropagation is to generate plants with the most diverse root systems possible, which will allow them to adapt more effectively to environmental changes or stressors. The induction of adventitious roots is a crucial step during plant propagation (Koroch et al., 2002; Villafuerte et al., 2022). Regarding the roots length of the micropropagated green antelope orchids it can be clearly observed that unfortunately the roots of treated plants were inhibited, under effect of the Ferbanat L®. The root length of untreated plants increased significantly compared with the treated plants. In a previous study it was determined that another type of biostimulator decreased the root and shoot values of *Hosta* 'Gold drop' (Ördögh et al., 2019). Considering the number or roots, here again the root formation was inhibited compared to control, at 0.1, 0.5, and 5%. No statistically significant differences were determined between control and 1% treatment. The greatest inhibition of the roots number was detected at the 0.5% concentration level of biostimulator. Contrary to our findings De Stefano et al., (2022) reported that, the organic supplements added to the growth medium showed greater values of plant length and number of roots compared with control. In a different study, the highest root number in *Dendrobium densiflorum* plants was obtained at full-strength Murashige and Skoog medium supplemented with 1.5 mg/L IBA (Pant et al., 2022). In yet another study it was reported that Murashige and Skoog medium supplemented with 1.5 mg/L indole-3-butyric acid produced the highest number of roots at *Pyracantha angustifolia* (Latunra et al., 2021).

Determining the number of leaves is also an important factor in assessing plant growth. Under our experimental conditions the number of leaves appearance was not influenced in a statistically significant manner, with only small variations between various treatments. Similarly, to previous findings, the biostimulator had an impact on the number of ramifycations, although no statistically significant variations were found. An experiment showed that liquid organic fertilizer had a positive effect on the number of shoots and number of leaves at *Vanda tricolor* Lindl. var. suavis protocorm (Nowakowska et al., 2022). Pant et al. (2022) revealed that, the greatest number of shoot formation at *Dendrobium densiflorum* protocorms was determined at a full-strength MS medium supplemented with 15% coconut water. Perhaps one of the most important results of the present experiment is be the plant survival rate. According to Nowakowska et al. (2022), although the tissue culture method is a hugely

popular, the growth conditions of *in vitro* propagation are the cause of some species' extremely low plant survival rates (i.e., medium containing high humidity, large quantities of nutrients and sugar which can be easily obtained by plants). Shoot necrosis is described as a physiological condition brought on by culture conditions and resulting in the loss of the shoot tip of *in vitro* plants (Teixeira da Silva et al., 2020). From the obtained data it can be clearly determined that, the greatest survival rate was recorded at a 5% concentration level of Ferbanat L®. With other concentrations, only 30% of the plants survived the trial with no necrosis observed, with the largest losses observed at 0.1% and 0.5%. Instead in the case of control and of the plants treated with 1% of biostimulant more than 50% of the plants survived. From these results, it can be concluded that using Ferbanat L® at a concentration of 5% helps in the alleviation of environmental stress factors. Healthy plants can be generated that are expected to perform better under any acclimation.

According to Clapa et al. (2022) the photosynthetic pigments content for *in vitro* cultivation seems to be more affected by the specificity of the plant than by the growing medium. Regarding, the photosynthetic pigments concentration (chlorophyll *a* + *b* and total carotenoids), no statistically significant differences were determined in the present experiment. Carotenoids are a group of natural tetraterpenoid pigments which are naturally occurring and are synthesized by bacteria, algae, fungi, and plants (Nisar et al., 2015; Sun et al., 2018). It was reported that carbon organic fertilizer improved the chlorophyll content of micropropagated date palm (Zayed and Saber, 2020). In a study by Shekhawat et al. (2022) it was determined that plants developed *in vitro* using liquid medium obtained higher content of chlorophylls and carotenoids. In a study conducted by El-Naggar et al., (2023) the chlorophyll content of blue *Hyacinthus orientalis* was increased when benzyl adenine, or kinetin was added to the medium. Photosynthetic pigments content of micropropagated *Cassia alata* plantlets was significantly higher in *ex vitro* conditions compared with *in vitro* conditions (Ahmed and Anis, 2014). In this matter, in comparison to in vitro circumstances, it was found that the pigment content greatly increased when the *Calathea* 'Maui Queen' plants were transferred in *ex vitro* conditions (Van Huylenbroeck et al., 2000).

According to the present experiment the peroxidase enzyme activity decreased at 1 and 5% concentration levels of biostimulator. It can be concluded that Ferbanat L® helps plants in reducing the negative effect of various environmental stressors, especially at the mentioned concentrations. A previously conducted study revealed that the peroxidase enzyme activity at *in vitro* cultivated *Hosta* 'Gold Drop' plants was reported the lowest if Ferbanat L® was added to the growth medium (Ördögh et al., 2019).

CONCLUSIONS

To our knowledge, this study presents the first report on the investigation of Ferbanat L® used on the micropropagation of *D. antennatum* orchid.

The present experiment provides data on the comparison of different concentration levels of Ferbanat L® used as biostimulant and their influence on growth and development parameters. Moreover, photosynthetic pigment concentration, and peroxidase enzyme activity levels of micropropagated *D. antennatum* plantlets were also determined. According to the obtained results, it can be concluded that by most of the examined factors no statistically significant differences were determined between the control and the treated plants. Root number and length of non-treated plants presented significantly larger quantities compared to the treated plants; however, the highest survival rate, the healthiest and most size-optimal plants were observed by the 5% Ferbanat L® treatment. Based on our experiment, Ferbanat L® is more important in preventing stress caused by micropropagation than in increasing the morphological parameters. Overall, it can be concluded that the proper dosage of Ferbanat L® can improve the development, growth, and survival rate of the examined plants, although more research is required.

ACKNOWLEDGEMENTS

The authors would like to express their special thanks Mihaiela Cornea-Cipcigan for the help

with this research. Moreover, we would like to express our gratitude to Kázmér Kovács.

REFERENCES

Adebayo, I.A., Pam, V.K., Arsad, H., Samian, M.R. (2020). The Global Floriculture Industry: Status and Future Prospects, *The Global Floriculture Industry*, 1st ed.; Apple Academic Press: Florida, USA, pp.1–14.

Ahmed, M.R., Anis, M. (2014). Changes in activity of antioxidant enzymes and photosynthetic machinery during acclimatization of micropropagated *Cassia alata* L. plantlets. *In Vitro Cell. Dev. Biol.*, 50, 601–609, doi:https://doi.org/10.1007/s11627-014-9609-1.

Approved Conservation Advice (s266B of the Environment Protection and Biodiversity Conservation Act 1999) Approved Conservation Advice for *Dendrobium antennatum* (Antelope Orchid). Retrieved 09 January 2023, from https://www.environment.gov.au/biodiversity/threatened/species/pubs/78702-conservation-advice.pdf.

Arnon, D.I. (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiol*., 24, 3–5, doi:10.1104/pp.24.1.1.

Bhattacharyya, P., Lalthafamkimi, L., Van Staden, J. (2021). Insights into the Biostimulatory Effects of Chitosan in Propagation of Orchid Bioresources. *In Biostimulants for Crops from Seed Germination to Plant Development; Academic Press*: New York, NY, USA, pp. 197–210. ISBN 9780128230480.

Brazienė, Z., Paltanavičius, V., Aleknavičienė, A. (2019). The influence of bioorganic preparations on plant productivity and soil quality. *Mechanization in agriculture & Conserving of the resources*, 65, 146–149.

Chandel, A., Thakur, M., Singh, G., Dogra, R., Bajad, A., Soni, V., Bhargava, B. (2022). Flower regulation in floriculture: an agronomic concept and commercial use. *Plant Growth Regul.*, 1–26., doi: https://doi.org/10.1007/s00344-022-10688-0.

Cheng, J., Dang, P.P., Zhao, Z., Yuan, L.C., Zhou, Z.H., Wolf, D., Luo, Y.B. (2019). An assessment of the Chinese medicinal *Dendrobium* industry: Supply, demand and sustainability. *J. Ethnopharmacol*., 229, 81–88, doi:https://doi.org/10.1016/j.jep.2018.09.001.

Clapa, D., Nemeș, S.A., Ranga, F., Hârța, M., Vodnar, D.C., Călinoiu, L.F. (2022). Micropropagation of *Vaccinium corymbosum* L.: An Alternative Procedure for the Production of Secondary Metabolites. *Horticulturae*, 8, 480, doi: https://doi.org/10.3390/horticulturae8060480.

Custódio, L., Charles, G., Magné, C., Barba-Espín, G., Piqueras, A., Hernández, J.A., Ben Hamed, K., Castañeda-Loaiza, V., Fernandes, E., Rodrigues, M.J. (2022). Application of *In Vitro* Plant Tissue Culture Techniques to Halophyte Species: A Review. *Plants*, 12, 126, doi:https://doi.org/10.3390/plants12010126.

Datta, S.K. (2022). Breeding of ornamentals: Success and technological status. *Nucleus*, 65, 107–128, doi: https://doi.org/10.1007/s13237-021-00368-x.

De Stefano, D., Costa, B.N.S., Downing, J., Fallahi, E., Khoddamzadeh, A.A. (2022). *In-vitro* micropropagation and acclimatization of an endangered native orchid using organic supplements. *Am. J. Plant Sci*., 13, 380–393, doi:10.4236/ajps.2022.133023.

Dohling, S., Kumaria, S., Tandon, P. (2012). Multiple shoot induction from axillary bud cultures of the medicinal orchid, *Dendrobium longicornu*. *AoB Plants*, pls032, doi: https://doi.org/10.1093/aobpla/pls032.

Dwiyani, R., Fitriani, Y., Mercuriani, I.S. (2022). The Alternative Media Supporting the Protocorm and Plantlet Growth of the Indonesian Black Orchid (*Coelogyne pandurata* Lindl.) Grown *In Vitro*. *J. Sustain. Agric*, 37, 152–160, doi: http://dx.doi.org/10.20961/carakatani.v37i1.55956.

El-Naggar, H.M., Shehata, A.M., Moubarak, M., Osman, A.R. (2023). Optimization of Morphogenesis and *In Vitro* Production of Five *Hyacinthus orientalis* Cultivars. *Horticulturae*, 9, 176, doi: https://doi.org/10.3390/horticulturae9020176.

Fei, H., Crouse, M., Papadopoulos, Y.A., Vessey, J.K. (2019). Improving biomass yield of giant *Miscanthus* by application of beneficial soil microbes and a plant biostimulant. *Can. J. Plant Sci.*, 100, 29–39, doi: https://doi.org/10.1139/cjps-2019-0012.

Ferbanat Labs. Retrieved 18 January 2023, from https://www.ferbanat-labs.com/en/.

Gabellini, S., Scaramuzzi, S. (2022). Evolving consumption trends, marketing strategies, and governance settings in ornamental horticulture: A grey literature review. *Horticulturae*, 8, 234, doi: https://doi.org/10.3390/horticulturae8030234.

Gontijo, J.B., Andrade, G.V.S., Baldotto, M.A., Baldotto, L.E.B. (2018). Bioprospecting and Selection of Growth-Promoting Bacteria for *Cymbidium* sp. orchids. *Sci. Agric.*, 75, 368–374, doi: https://doi.org/10.1590/1678-992X-2017-0117.

Hammer, Ø., Harper, D.A., Ryan, P.D. (2001). Past: Paleontological statistics software package for education and data analysis. *Palaeontol. Electron.*, 4, 1–9.

Herastuti, H., EK, S.H. (2020). Effect of Fertilizer Frequency on Growth Varieties of *Dendrobium* Orchid. *In Proceeding of LPPM UPN "Veteran" Yogyakarta Conference Series 2020–Engineering and Science Series*, Yogyakarta, Indonesia, October 2020. doi:https://doi.org/10.31098/ess.v1i1.116.

Herawati, R., Ganefianti, D.W., Romeida, A. (2020). Addition of Coconut Water and Banana Extract on MS Media to Stimulate PLB (Protocorm Like Bodies) Regeneration of *Dendrobiumgatton sunray*. *In International Seminar on Promoting Local Resources for Sustainable Agriculture and Development* (ISPLRSAD). Atlantis Press. doi: 10.2991/absr.k.210609.040.

Hinsley, A., De Boer, H.J., Fay, M.F., Gale, S.W., Gardiner, L.M., Gunasekara, R.S., Kumar, P., Masters, S., Metusala, D., Roberts, D.L., Veldman, S. (2018). A review of the trade in orchids and its implications for conservation. *Bot. J. Linn.*, 186, 435–455, doi:https://doi.org/10.1093/botlinnean/box083.

Karpushina, M., Winter, M., Yaroshenko, O. (2021). Efficiency of using biostimulants Agrinos 1 and Agrinos 2 in adaptation of microplants of garden strawberry to *ex vitro* conditions. *In BIO Web of Conferences*, 10 September 2021. doi: https://doi.org/10.1051/bioconf/20213403006.

Kaviani, B., Deltalab, B., Kulus, D., Tymoszuk, A., Bagheri, H., Azarinejad, T. (2022). *In Vitro* Propagation of *Pyracantha angustifolia* (Franch.) CK Schneid. *Horticulturae*, 8, 964, doi:https://doi.org/10.3390/horticulturae8100964.

Kisvarga, S., Farkas, D., Boronkay, G., Neményi, A., Orlóci, L. (2022). Effects of biostimulants in horticulture, with emphasis on ornamental plant production. *Agronomy*, 12, 1043, doi: https://doi.org/10.3390/agronomy12051043.

Knudson, L. (1992). Nonsymbiotic germination of orchid seeds. *Bot. Gaz.*, 73, 1–25, doi: https://doi.org/10.1086/332956.

Koroch, A., Juliani, H.R., Kapteyn, J., Simon, J.E. (2002). *In vitro* regeneration of *Echinacea purpurea* from leaf explants. *PCTOC*, 69, 79–83, doi: https://doi.org/10.1023/A:1015042032091.

Kulus, D., Miler, N. (2021). Application of plant extracts in micropropagation and cryopreservation of bleeding heart: An ornamental-medicinal plant species. *Agriculture*, 11, 542, doi:https://doi.org/10.3390/agriculture11060542.

Lam, Y., Ng, T.B., Yao, R.M., Shi, J., Xu, K., Sze, S.C.W., Zhang, K.Y. (2015). Evaluation of chemical constituents and important mechanism of pharmacological biology in *Dendrobium* plants. *eCAM* 2015, doi:https://doi.org/10.1155/2015/841752.

Latunra, A.I., Tuwo, M., Rezky, N. (2021). *In vitro* propagation of *Vanda tricolor* Lindl. var. suavis protocorm on media containing liquid organic fertilizer as a substitute for MS media. *In IOP Conference Series: Earth and Environmental Science*, Makassar, Indonesia, 4–5 August 2021, doi:10.1088/1755-1315/886/1/012006.

Meddich, A. (2022). Biostimulants for Resilient Agriculture—Improving Plant Tolerance to Abiotic Stress: A Concise Review. *Gesunde Pflanzen*, 1–19, doi:https://doi.org/10.1007/s10343-022-00784-2.

Mirani, A.A., Abul-Soad, A.A., Markhand, G.S. (2017). Effect of different substrates on survival and growth of transplanted orchids (*Dendrobium nobile* cv.) into net house. *Int. J. Hortic. Sci.*, 5, 310–317.

Moraes, M.C., Camolesi, M.R., Palmieri, D.A., Bertão, M.R. (2020). Commercial fertilizers and organic additives in orchid micropropagation. *Plant Cell Cult. Micropropag.*, 16, 16–e162, doi: https://doi.org/10.46526/pccm.2020.v16.162.

Mullin, A., Costa, B.N.S., Downing, J., Khoddamzadeh, A.A. (2022). Conservation Horticulture: *In Vitro* Micropropagation and Acclimatization of Selected Florida Native Orchids. *HortScience*, 57, 1159–1166, doi:https://doi.org/10.21273/HORTSCI16672-22.

Nephali, L., Piater, L.A., Dubery, I.A., Patterson, V., Huyser, J., Burgess, K., Tugizimana, F. (2020). Biostimulants for plant growth and mitigation of abiotic stresses: A metabolomics perspective. *Metabolites*, 10, 505, doi: https://doi.org/10.3390/metabo10120505.

Nisar, N., Li, L., Lu, S., Khin, N.C., Pogson, B.J. (2015). Carotenoid metabolism in plants. *Mol. Plant*, 8, 68–82, doi:https://doi.org/10.1016/j.molp.2014.12.007.

Nowakowska, K., Marciniak, P., Pacholczak, A. (2022). A protocol for efficient micropropagation of rare orchid *Vanda brunnea* Rchb. f. *S. Afr. J. Bot.*, 150, 233–239, doi: https://doi.org/10.1016/j.sajb.2022.07.023.

Nugroho, J.D., Arobaya, A.Y.S., Tanur, E.A. (2019). Propagation of *Dendrobium antennatum* Lindl via seed culture *in vitro* using simple medium: fertilizer and complex organic based medium. *HAYATI Journal of Biosciences*, 26, 133-133, doi: https://doi.org/10.4308/hjb.26.3.133.

Ogunsanya, H.Y., Motti, P., Li, J., Trinh, H.K., Xu, L., Bernaert, N., Van Droogenbroeck, B., Murvanidze, N., Werbrouck, S.P., Mangelinckx, S., Ramirez, A. (2022). Belgian endive-derived biostimulants promote shoot and root growth in vitro. *Sci. Rep.*, 12, 8792, doi:https://doi.org/10.1038/s41598-022-12815-z.

Ördögh, M., Beregi, Z., Mándy, A.T. (2019). The effect of different biostimulators on morphological and biochemical parameters of micropropagated *Hosta*'Gold Drop'. *Int. J. Hortic. Sci.*, 25, 22–29, doi:https://doi.org/10.31421/IJHS/25/1-2./2357.

Pant, B., Chand, K., Paudel, M.R., Joshi, P.R., Thapa, B.B., Park, S.Y., Shakya, S., Thakuri, L.S., Rajbahak, S., Sah, A.K., Baniya, M.K. (2022). Micropropagation, antioxidant and anticancer activity of pineapple orchid: *Dendrobium densiflorum* Lindl. *J. Plant Biochem. Biotechnol*., 31, 1–11, doi:https://doi.org/10.1007/s13562-021-00692-y.

Parađiković, N., Teklić, T., Zeljković, S., Lisjak, M., Špoljarević, M. (2019). Biostimulants research in some horticultural plant species—A review. *Food Energy Secur.*, 8, e00162, doi: https://doi.org/10.1002/fes3.162.

Paris, L., García-Caparrós, P., Llanderal, A., Silva, J.T., Reca, J., Lao, M. (2019). Plant Regeneration from Nodal Segments and Protocorm-Like Bodies (PLBs) Derived from *Cattleya maxima* J. Lindley in Response to Chitosan and Coconut Water. *Propag. Ornam. Plants*, 19, 18–23.

Phillips, G.C., Garda, M. (2019). Plant tissue culture media and practices: an overview. *In Vitro Cell. Dev. Biol.-Plant*, 55, 242–257, doi: https://doi.org/10.1007/s11627-019-09983-5.

Poobathy, R., Zakaria, R., Murugaiyah, V., Subramaniam, S. (2019). Surface sterilization and micropropagation of *Ludisia discolor*. *Biocatal. Agric. Biotechnol*., 22, 101380, doi: https://doi.org/10.1016/j.bcab.2019.101380.

Potshangbam, N., Nirmala, C. (2011). *In vitro* rapid propagation of *Cymbidium aloifolium* (L.) Sw.: a medicinally important orchid via seed culture. *J. Biol. Sci.*, 11, 254–260, doi:10.3923/jbs.2011.254.260.

POWO 2023. "Plants of the World Online. Facilitated by the Royal Botanic Gardens, Kew. Retrieved 23 January 2023, from www.plantsoftheworldonline.org

Proietti, S., Scariot, V., De Pascale, S., Paradiso, R. (2022). Flowering mechanisms and environmental

stimuli for flower transition: Bases for production scheduling in greenhouse floriculture. *Plants*, 11, 432, doi:https://doi.org/10.3390/plants11030432.

Rouphael, Y., Colla, G. (2018). Synergistic biostimulatory action: Designing the next generation of plant biostimulants for sustainable agriculture. *Front. Plant Sci.*, 9, 1655, doi: https://doi.org/10.3389/fpls.2018.01655.

Royal FloraHolland Annual Report 2021. Retrieved 20 January 2023, from www.floraholland.com.

Salachna, P. (2022). Trends in ornamental plant production. *Horticulturae*, 8, 413, doi:https://doi.org/10.3390/horticulturae8050413.

Sanó, L., de Oliveira, L.L.B., Leão, M.D.M., Dos Santos, J.E.D.Á., de Medeiros, S.C., Schneider, F., de Sousa, A.B.O., Taniguchi, C.A.K., Muniz, C.R., Grangeiro, T.B., da Silva, C.D.F.B. (2022). *Trichoderma longibrachiatum* as a biostimulant of micropropagated banana seedlings under acclimatization. *Plant Physiol. Biochem.*, 190, 184–192, doi: https://doi.org/10.1016/j.plaphy.2022.09.008.

Sasmita, H.D., Dewanti, P., Alfian, F.N. (2022). Somatic Embryogenesis of *Dendrobium lasianthera* X *Dendrobium antennatum* with the Addition of BA and NAA. *J. Agron. Indones*., 50, 201–207, doi:https://doi.org/10.24831/jai.v50i2.39715.

Shannon, L.M., Kay, E., Lew J.Y. (1966). Peroxidase isozymes from horseradish roots. I. Isolation and physical properties. *J. Biol. Chem.*, 241, 2166–2172, doi:https://doi.org/10.1016/S0021-9258(18)96680-9.

Shekhawat, M.S., Latha, R., Priyadharshini, S., Manokari, M. (2022). Improved micropropagation, morphometric traits and photosynthetic pigments content using liquid culture system in *Spathoglottis plicata* Blume. *Vegetos*, 35, 9–18, doi:https://doi.org/10.1007/s42535-021-00303-0.

Sun, T., Yuan, H., Cao, H., Yazdani, M., Tadmor, Y., Li, L. (2018). Carotenoid metabolism in plants: the role of plastids. *Mol. Plant*, 11, 58–74, doi:https://doi.org/10.1016/j.molp.2017.09.010.

Suryani, R., Sari, M.N. (2019). The use of various kinds of plant medium and provision of liquid organic fertilizer in acclimatization stage on the growth of plantlet orchid (*Phalaenopsis amabilisi*) from network culture. *JAAST*, 3, 105–114, doi:10.32530/jaast.v3i1.63.

Teixeira da Silva, J.A., Nezami-Alanagh, E., Barreal, M.E., Kher, M.M., Wicaksono, A., Gulyás, A., Hidvégi, N., Magyar-Tábori, K., Mendler-Drienyovszki, N., Márton, L., Landín, M. (2020). Shoot tip necrosis of *in vitro* plant cultures: a reappraisal of possible causes and solutions. *Planta*, 252, 1–35, doi:https://doi.org/10.1007/s00425-020-03449-4.

Tharapan, S., Obsuwan, K. (2016). Effect of cultured medium on the growth of *Dendrobium antennatum*× *Dendrobium bigibbum* axillary buds. In Agricultural Innovation for Global Value Chain, *Proceedings of 54th Kasetsart University Annual Conference, Kasetsart University*, Thailand, 2–5 February 2016.

Tikendra, L., Koijam, A.S., Nongdam, P. (2019). Molecular markers based genetic fidelity assessment of micropropagated *Dendrobium chrysotoxum* Lindl. *Meta Gene*, 20, 100562, doi: https://doi.org/10.1016/j.mgene.2019.100562.

Tuwo, M., Latunra, A.I., Ana, E.T. (2021). Micropropagation of *Vanda tricolor* Lindl. var. suavis with various concentrations of organic growth supplements. *In IOP Conference Series: Earth and Environmental Science*, Makassar, Indonesia. 4–5 August 2021. doi:10.1088/1755-1315/886/1/012004.

Utami, E.S.W., Hariyanto, S. (2016). The effect of organic nutrient and growth regulators on seed germination, embryo and shoots development of *Dendrobium antennatum* Lindl. orchid by *in vitro*. *Biosaintifik*, 8, 165–171, doi:https://doi.org/10.15294/biosaintifika.v8i2.5165.

Vacin, E.F., Went, F.W. (1949). Some pH changes in nutrient solutions. *Bot. Gaz.*, 110, 605–613, doi:https://doi.org/10.1086/335561.

Van Huylenbroeck, J.M., Piqueras, A., Debergh, P.C. (2000). The evolution of photosynthetic capacity and the antioxidant enzymatic system during acclimatization of micropropagated *Calathea* plants. *Plant Sci.*, 155, 59–66, doi: https://doi.org/10.1016/S0168-9452(00)00201-6.

Vargas-Hernandez, M., Macias-Bobadilla, I., Guevara-Gonzalez, R.G., Romero-Gomez, S.D.J., Rico-Garcia, E., Ocampo-Velazquez, R.V., Alvarez-Arquieta, L.D.L., Torres-Pacheco, I. (2017). Plant hormesis management with biostimulants of biotic origin in agriculture. *Front. Plant Sci.*, 8, 1762, doi:https://doi.org/10.3389/fpls.2017.01762.

Vilcherrez-Atoche, J.A., Rojas-Idrogo, C., Delgado-Paredes, G.E. (2020). Micropropagation of *Cattleya maxima* J. Lindley in Culture Medium with Banana Flour and Coconut Water. *IJPAES*, 10, 179–193.

Villafuerte, D.E., Angeles, E., Bayog, A., Duka, R., Menoza, N.L., Sanchez, M.A., De Jesus, R. (2022). Root organogenesis induction in *Epipremnum aureum* stem cuttings with plant biostimulants and synthetic rooting hormone. *bioRxiv*, 2022–07.

Volckaert, E., Gobin, B. (2010). Ornamental plants and floriculture. In Soils, Plant Growth and Crop Production; Verheye, W.H., Ed.; *EOLSS Publications*: Paris, France.

WFO (2023). *Dendrobium* Sw. Retrieved 24 January 2023, from http://www.worldfloraonline.org/taxon/wfo-4000011060.

Xu, J., Han, Q.B., Li, S.L., Chen, X.J., Wang, X.N., Zhao, Z.Z., Chen, H.B. (2013). Chemistry, bioactivity and quality control of *Dendrobium*, a commonly used tonic herb in traditional Chinese medicine. *Phytochem. Rev.*, 12, 341–367, doi:https://doi.org/10.1007/s11101-013-9310-8.

Yaseen, A.A., Takácsné Hájos, M. (2021). Effect of biostimulants on some bioactive compounds and nitrate level in lettuce (*Lactuca sativa* L.) grown under unheated plastic tunnel. *Iraqi J. Agric. Sci.*, 52, 1318–1325.

Zayed, Z.E., Saber, T.Y. (2020). Effect of carbon organic fertilizer (humic matter) on the growth of micropropagated date palm cv. Sewi during establishing stage in the open field. *Curr. Sci. Int.*, 9, 407–417, doi:10.36632/csi/2020.9.3.35.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

DESIGNING A PRIVATE GARDEN WITH AN ORNAMENTAL AND UTILITARIAN CHARACTER

Manuela MANDA, Carmen NICU, Ruxandra BĂLUȚĂ, Andreea TRIF

University of Craiova, 13 A.I. Cuza Street, Craiova, Romania

Corresponding author email: alexa25nicu@yahoo.com

Abstract

Currently, private gardens tend to have a highly functional character due to the fast-paced lifestyle of our days, the reduction of garden space, as well as the need for consuming healthier foods. The ornamental-utilitarian garden represents an intelligent and balanced way of using the green space around the house, providing both beauty and functionality. It is an concept that maximizes the garden`s full potential to meet the needs and preferences of the owners. Although this approach is relevant in Europe, in Romania, there is a need for the development of this concept. Currently, utilitarian gardens are mostly positioned in the backyard, isolated, without exploring the multiple possibilities of incorporating utilitarian plants into ornamental garden designs. In this work, besides researching the ornamental-utilitarian garden concept, we aim to design a private garden, adhering to the principles underlying this approach to landscaping.

Key words*: garden, ornamental, utilitarian, concept, design.*

INTRODUCTION

The concept of ornamental-utilitarian gardens is currently relevant in the field of landscaping. Pettenati (2017) considers that between food and landscape there is a complex relationship; the landscape is, on the one hand, a context of physical and symbolic reference in which food-related choices and actions take place, both individually and collectively, on the other hand, a useful reference of values through which one can create links between urban food policies, consumer choices and urban and territorial planning.

These gardens offer multiple benefits to the environment, people, and ecosystems (Galluzzi et al., 2010; Bigirimana et al., 2012; Taylor and Lovell, 2014; Beumer and Martens, 2015; Galea et al., 2018). As green pockets that are either aesthetic or productive utilitarian gardens, urban domestic gardens represent a considerable portion of remaining green spaces in the city that interact with nature (Al-Kofahi et al., 2019). Used for aesthetic value as well as consumption, this form of urban agriculture promotes a healthy lifestyle and sustainable practices at multiple levels; participation in the edible landscape can provide physical activity, social cohesion, and the opportunity to develop a stronger emotional connection and relationship to food (Çelik, 2017). This approach encourages landowners and gardeners to use available space for food purposes, promoting the creation of greener and more functional spaces in urban and rural environments.

The main purpose of foodscaping is to create beautiful and functional spaces that provide a source of fresh food, such as vegetables, herbs, fruits, and medicinal plants. The ornamental characteristics of these horticultural species are manifested through various elements: the plant itself, degree of branching, leaf color, flower color, and fruit color (Manda & Nicu, 2023; Nicu & Manda, 2024; Mihai, 2012). Although medicinal and aromatic plants are used in pharmaceutical preparations and folk remedies, they also possess aesthetic qualities in ornamental and landscaping contexts (Abdelnaby, 2020). By integrating utilitarian plants into existing or new landscaping designs, people can enjoy organically grown produce. Foodscaping emphasizes sustainable cultivation, the use of local resources, and waste reduction, contributing to the promotion of a healthier lifestyle and the protection of the environment.

The Château de Villandry Gardens in France, located in the Loire Valley, serves as a source of inspiration (Larkcom, 2017) and a classic example of ornamental-utility gardens,

representing a perfect synthesis between functionality and aesthetics. They are among the most renowned gardens globally due to their complex design and continue to inspire landscapers and gardening enthusiasts worldwide.

The history of late Renaissance gardens in Transylvania is primarily about economic sustainability (Fekete & Sárospataki, 2023). As Fekete & Sárospataki (2022) mention, these had a mixed character, merging the concepts of vegetable and ornamental gardens (vegetable-flower gardens and even orchards together).

Currently, utilitarian gardens can be incorporated into various landscaping styles, paving the way for concepts such as ornamental-utilitarian gardens, foodscape, edible landscaping, or edible gardens. Foodscaping can range from relatively small kitchen gardens in backyard spaces to larger urban or suburban landscapes that include extensive areas dedicated to food cultivation. Depending on the location and method of arrangement, these gardens can be cultivated in the ground, in containers, on rooftops or terraces, or as vertical gardens.

Although this approach is prevalent in Europe, there is a need for the development of this concept in Romania as well. Currently, vegetable gardens are often positioned separately in the backyard, isolated, without fully exploring the multiple possibilities of integrating utilitarian plants into ornamental garden designs. In this work, besides researching the ornamental-utilitarian garden concept, we aim to design a private garden, adhering to the principles underlying this approach to landscaping.

MATERIALS AND METHODS

In this paper, our aim was to extensively research the concept of ornamental-utility gardens and design a private garden while adhering to the principles underlying this approach to landscaping. The landscaping solution was developed for an individual garden of 261 square meters located in the city of Craiova. The building is oriented towards the northwest, facing the main street, and divides the yard into two different-sized gardens.

The total land area is 387 square meters, out of which 103 square meters are occupied by the house, 13 square meters by an annex used for storage, and the remaining 274 square meters are designated for the garden (including lawn, plants, and pathways) (Figure 1).

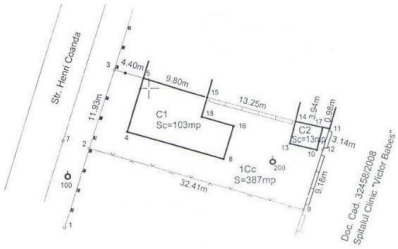


Figure 1. Land Cadastre

The initial state of the space to be landscaped is a typical garden, arranged with ornamental plants constituting over 80% of the area and 20% of the area dedicated to edible plants. Some vegetation elements are arranged linearly (such as grapevines and raspberries) or in a zigzag pattern (roses), while most ornamental plants are scattered freely. The garden's appearance is slightly unkempt due to the abundance of plants or their placement in certain areas.

The design process began with an on-site visit, during which measurements were taken, an initial sketch was created, and the project objectives and theme were defined. After obtaining the necessary field data and documentation, such as land cadastre or topographic surveys (RLV), the site plan was developed to scale. For the realization of this landscaping proposal, the Realtime Landscaping Architct 2016 software by Idea Spectrum was utilized, allowing both plan and perspective views of the layout (Figure 2).



Figure 2. Site Plan

The preparation of the site plan began with delineating the space, translating essential elements onto the plan, including the house with the placement of cardinal points and windows, other constructions, the entrance area to the yard, stairs, concrete or paved platforms, pathways and fountains. In the subsequent stage, the existing vegetation and other hardscape elements such as benches, conduits, or poles were depicted on the plan. With all the necessary spatial information, including details about climate, soil, client requirements, and preferences, the landscaping proposal was then developed.

RESULTS AND DISCUSSIONS

In order to create a design solution, alongside the preferences of the owners, the existing situation and the landscaping possibilities offered by the respective space are taken into account. The redesign concepts adopted are often based on the lines already defined by the existing vegetation or infrastructure, as is the case here. Built in 1956 during the communist regime, the architecture of the house is simple and neutral, spanning only one level. This simplicity necessitates that the only way to infuse an aesthetic personality into this property is through the landscaping of the garden area. For the landscaping solution, the choice was made for a garden with an ornamental-utility character. Geometric elements, such as the layout of pathways and green spaces delimited into several rectangles that are hard to modify, have been preserved. Regarding vegetation, a freer arrangement was opted for.

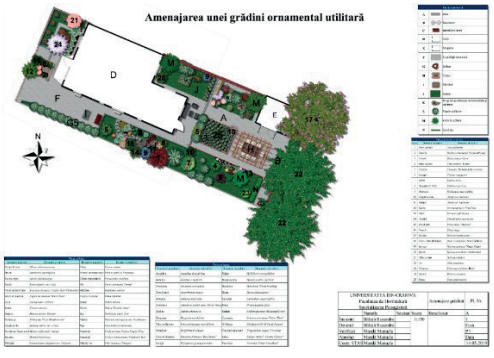


Figure 3. Landscaping Plan

Most of the ornamental plants already present in the garden (*Wisteria, Hydrangea, Chaenomeles japonica*) were retained in the project and complemented by other species with ornamental and food value arranged in borders, floral patches, mixed borders, container cultures, and for decorating pergolas.

Taking into account the owner's requirements, in the lateral area of the garden, a decision was made to create an atmosphere of an oriental garden by combining traditional species with decorative elements from Japanese culture, which complement the composition (Figure 3).

Presentation of the Landscaping Project

In the current garden landscaping, the basic layout has been preserved, including the pathways, delineation of green areas, and a significant portion of the existing plants.

The front garden has been enhanced in the shaded area between the L-shaped pathway and the house with a border of *Hosta* sp. with simple or variegated leaves in white or yellow, along with shade-tolerant grass. In this area, alongside an impressive *Wisteria sinensis* specimen, ferns and shrub species such as *Hydrangea macrophylla, Kerria japonica, Syringa vulgaris*, and roses have been retained.

The bench near the stairs has been removed, making space for plants, thus balancing the composition (Figure 4).



Figure 4. The front garden

The green area on the side of the house adopts an oriental influence through the addition of characteristic elements (a winding cobblestone pathway, a small bridge, a stone bench, a stone lantern, and typical plant elements). A pathway made of natural stone slabs and gravel has been proposed, following a winding route and bordered by various ornamental shrubs and

perennial herbaceous plants (*Cotinus alba, Spirea japonica, Picea abies nidiformis, Astilbe, Hosta plantaginea, Dicentra, Delphinium*).
This pathway leads to a resting area designed with two types of decorative gravel. On the right side, there is a stone bench, a lantern typical of this culture, and in the center, an artesian fountain. Around this area, in the foreground, ornamental species such as P*hyladelphus coronarius, Hydrangea macrophylla, Juniperus virginiana* and *Bergenia crassifolia* have been arranged. In the background, the red leaves of a Japanese maple (*Acer palmatum* Atropurpureum), *Dicentra spectabilis*, and *Ribes alpinum* can be seen. When designing this area, the semi-shaded conditions of the terrain were taken into account, using species with modest light requirements.
The passage to the backyard is made through a Japanese-style bridge, with a gravel pathway at the base, creating the illusion of a bridge over water. On both the right and left sides, there are *Bergenia* and *Hosta plants* (Figure 5).



Figure 5. Backyard - Hardscape and Softscape Elements

The backyard, an extension of the lateral garden, begins with a column of *Hedera helix* that conceals a pole and a row of *Rubus idaeus* along the fence line, which will be complemented with utilitarian and decorative plants such as: *Echinacea*, *Sambucus nigra* 'Eva' (decorative with dark reddish leaves and white flowers), *Chaenomeles japonica*, and *Pyracantha coccinea* (decorative with white flowers and orange berries). In the front, there is an irregular grouping of ornamental and utilitarian herbaceous species: *Artemisia stelleriana, Lactuca sativa* (lettuce), *Hosta plantaginea* (green leaves in various shades), *Anemone coronaria, Antirrhinum majus, Viola; Dicentra, Verbena canadensis, Veronica* 'Giles van Hees' (pink flowers); *Tulipa* (white flowers) and *Achillea kellerei* (yellow flowers).
The back section features a solitary specimen of *Cotinus coggygria*, decorative with purple leaves and pink flowers, and *Vaccinium corymbosum* (blueberry) with bluish-bloomed fruits. *Viburnum carlesii* is on the right, and *Paeonia suffruticosa* is on the left (Figure 6).



Figure 6. Backyard - Herbaceous Plant Group

The vegetable garden, located in the central rectangle behind the house, is almost entirely arranged with decorative edible plants such as *Latuca sativa* var. crispa, *Ocimum basilicum, Brassica oleracea* var. Osaka Red, *B. oleracea* 'Chidori Red', *Raphanus sativus, Beta vulgaris* var. cicla, *Petroselinum crispus, Allium schoenoprasum*, arranged in serpentine rows separated by river stones. As ornamental plants, *Bergenia cordifolia* and *Tropaeolum majus* were added, and in the center, there is a specimen of *Yucca filamentosa* (Figure 7a).
Due to the area being very sunny, a portion of the grapevine was retained around the pergola and complemented with a *Clematis* 'Madame Julia Correvon'. The initial metal structure of the pergola was replaced with one made of wood in light oak tones (Figure 7b).



a. ……..b.

Figure 7. a) Vegetable garden; b) Pergola

The old rose garden was partially replaced by lawn and floral species such as *Tulipa, Armeria maritima, Chrysanthemum, Pratia pedunculata, Sedum, Senecio cineraria, Hosta*, and utility species like *Daucus carota, Salvia, Viola cornuta, Melissa officinalis*. In the middle, there is *Aronia melanocarpa*, and in the corner, a variety of *Prunus persica* was planted. On the grassy area, an ornamental pot was placed containing annual flowering species like *Impatiens* and *Verbena*. The contrast created by the ornamental mulch and grass or plants adds a special charm to the arrangement (Figure 8 a).

The last part of the garden embraces the concept of cultivating vegetable plants and ornamental herbs in containers. The first rectangular container is 3 m long, 0.5 m wide, and 0.3 m high, containing vegetable species *Capsicum annuum* 'Black Pearl' and *Armoracia lapathifolia*, which decorate with their leaves. The second container, square-shaped with a side length of 1.3 m, contains species/varieties with edible flowers: *Viola cornuta* 'Lemon Purple Wing', *Viola wittrockiana* 'Pandoras Box', and *Antirrhinum majus* 'Montego White'.
From the initial vegetation, in this area, a vigorous rose and a *Hydrangea* were retained, and the rest of the garden was complemented on the side with specimens of *Spirea prunifera, Acanthus longifolius, Delphinium elatum, Solidago* 'Little Lemon'. Towards the wall, there is a black-purple leaved elderberry, *Sambucus nigra* 'Eva' (Figure 8b).



a. …….b.

Figure 8. a) Group of herbaceous plants;
b) Container vegetable garden

The use of various types of fruit, vegetables, aromatic, and medicinal plants, as well as the trained grapevine on the pergola, constituted an essential aspect in the landscaping proposal. Despite the limited area of the garden, a selection of fruits was integrated, and vegetable were used in greater numbers, being combined with ornamental plants.
Landscaping using edible plants can be a style of gardening that offers decoration, a space for relaxation, physical activity, learning, and also might contribute to changing attitude towards eating vegetables, especially among children (Istrate et al., 2023).
In theory, almost all vegetable plants can be used in an ornamental-utilitarian garden. However, certain species/cultivars have significant ornamental potential due to the appearance of their leaves, shape, texture, and sometimes due to their flowers or fruits. Moreover, recent trends show a continuous diversification of asortment, with the emergence of new cultivars possessing superior decorative characteristics.

CONCLUSIONS

In the proposed landscaping solution, emphasis was placed on integrating various utilitarian plants alongside ornamental ones, resulting in a garden with aesthetic and functional role. This approach is not new but represents a different perspective on cultivating food plants.
Although this concept is trendy in Europe and beyond, in Romania, vegetable gardens are often situated separately, behind the house, without exploring the multiple possibilities of incorporating edible plants into ornamental gardens.
The assortment of plants used in such landscaping is extensive, as it can include, in addition to ornamental plants, vegetable, medicinal, aromatic plants, fruits and even grapevines.
This integrated approach, not only enriches the garden visually but also gives it a distinctive character, maximizes its use, offering a variety of fresh products in a limited space.

REFERENCES

Arslan M., Yanmaz R. (2009). Use of ornamental vegetables, medicinal and aromatic plants in urban landscape design. *In II International Conference on Landscape and Urban Horticulture* 881, Ankara, 207-211.

Abdelnaby, A. S., Mewead, A. A., Gendy, A. S. H., & Abdelkader, M. A. I. (2020). Survey of medicinal and aromatic plants and determine its landscaping values in home gardens and Malls in Cairo Festival City, Cairo Governorate, Egypt. *Zagazig Journal of Agricultural Research*, 47(5), 1175-1182.

Al-Kofahi, S. D., Gharaibeh, A. A., Bsoul, E. Y., Othman, Y. A., & St. Hilaire, R. (2019). Investigating domestic gardens' densities, spatial distribution and types among city districts. *Urban Ecosystems*, 22, 567-581.

Beumer, C., Martens, P. (2015). BIMBY's first steps: a pilot study on the contribution of residential front-yards in Phoenix and Maastricht to biodiversity, ecosystem services and urban sustainability. *Urban Ecosystems 19*(1), 45–76.

Bigirimana, J., Bogaert, J., De Cannière, C., Bigendako, M. J., & Parmentier, I. (2012). Domestic garden plant diversity in Bujumbura, Burundi: Role of the socio-economical status of the neighborhood and alien species invasion risk. *Landscape and Urban Planning*, *107*(2), 118-126.

Çelik F., 2017, The Importance of Edible Landscape in the Cities. Turkish Journal of Agriculture: *Food Science and Technology*, 5, 118-124.

Fekete, A., & Sárospataki, M. (2022). Baroque Gardens in Transylvania: A Historic Overview. *Land*, *11*(6), 949.

Fekete, A., & Sárospataki, M. (2023). Plant Use in the Late Renaissance Gardens of the 17–18th Century Transylvania. *Plants*, *12*(9), 1798.

Galea F.M., Munteanu, N., Stoleru, V., & Teliban, G. C. (2018). Positive aspects of an ornamental vegetable garden and its effects towards family and community sustainability. *Scientific Papers. Series B, Horticulture.* Vol. LXII, 517-520.

Galluzzi, G., Eyzaguirre, P., Negri, V. (2010) Home gardens: neglected hotspots of agro-biodiversity and cultural diversity. *Biodivers Conserv* 19, 3635–3654.

Istrate, A. M. R., Cojocariu, M., Teliban, G. C., Cojocaru, A., & Stoleru, V. (2023). Quality and Yield of Edible Vegetables from Landscape Design. *Horticulturae*, *9*(6), 615.

Larkcom, J. (2017). *Creative vegetable gardening*. Hachette, UK.

Mihai, C., & Hoza, G. (2012). Researches regarding the productive and ornamental characteristics of vegetable growing in utilitarian gardens. *Scientific Papers, Series B, Horticulture*, 56, 323-328.

Manda, M., & Nicu, C. (2023). Growth and flowering performance evaluation of ten potted Chrysanthemum cultivars. *Scientific Papers. Series B. Horticulture*, *67*(1), 432-437.

Nicu, C., & Manda, M. (2024). Evaluation of some hyacinth cultivars under open field conditions. *Scientific Papers. Series B. Horticulture*, *68*(1), 659-665.

Pettenati, G. (2017). Landscape and Urban Food Planning: Theoretical and Operational Intersections. *Bollettino della Società Geografica Italiana*, 10, 104-116.

Taylor, J.R., Lovell, S.T. (2014). Urban home food gardens in the global north: research traditions and future directions. *Agriculture Human Values, 31*(2), 285–305.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

RESILIENCE OF URBAN TREES TO EXTREME WEATHER IN BUCHAREST, ROMANIA

Cristina Rodica MĂNESCU, Elisabeta DOBRESCU, Ana VIȚA

University of Agronomic Sciences and Veterinary Medicine of Bucharest,
Faculty of Horticulture, 59 Mărăşti Blvd, District 1, Bucharest

Corresponding author email: veradobrescu@gmail.com

Abstract

In the last years, Europe has experienced extreme weather conditions with significant impact on vegetation. Extremely warm summers and poor rainfall in all seasons transformed Bucharest (Romania) into one of the hottest and driest cities in Europe. In this research, more than 190 trees species, hybrids and cultivars present in the green spaces of University of Agronomical Sciences and Veterinary Medicine Bucharest were investigated for their resistance to extreme weather. The results showed that trees from 24% of the existent species, hybrids and cultivars were affected and lost in the last two years. Over 55% of the dead trees were native species. In 2024, when summer temperatures exceeded 35°C for 28 days, all of the lost trees were placed in areas without irrigation. Monitoring tree under extreme weather conditions can provide essential information for resilient plantations and sustain biodiversity in cities.

Key words*: drought, heatwaves, native and non-native species, resistance.*

INTRODUCTION

Urbanization was proved to be close linked to climate change (Chapman et al., 2017; Khosia & Bhardwaj, 2019; Sarvari, 2019; Xu et al., 2021). Yet, it has been found that thermal comfort in cities is getting worse and extreme events are occurring mainly due to climate change and less due to increased urbanization (Argüeso et al., 2014; Oleson et al., 2015; Ren et al., 2022). Green areas play a key role in ensuring thermal comfort in cities. The cooling effect of vegetation is greater during summer than winter (Hamada & Ohta, 2010). Also, the cooling effect depend on the size of green areas (Aram et al., 2019) and their design and composition (Jaganmohan et al., 2016; Wu et al., 2021; Gallay et al., 2023). For this reason, green areas in cities are now critically needed in the fight against climate threats. Appropriate green areas can solve not only thermal comfort of the urban population but also limiting the flood risks (Kim et al., 2016; Zimmermann et al., 2016; Green et al., 2021; Zia et al., 2022), reduce winds velocity (Rafael et al., 2018; Feitosa et al., 2021) or the impact of air pollutants (De la Sota et al., 2019). In this context, cities need more green areas but in recent years, heatwaves and droughts, two major events associated with climate change, have affected more frequently woody species (Marchin et al, 2022; Wang et al., 2023). Tree species are impacted differently by these events. Symptoms can vary from leaf damage to tree death, depending on the species (Teskey et al., 2015; Percival, 2023), age (Lucas-Borja et al, 2021; Haase & Hellwig, 2022), phenophase (Geng et al., 2022) and duration of exposure (Teskey et al., 2015; Kunert et al., 2022). In Central European cities, for species such as *Acer platanoides*, *Tilia cordata* and *Quercus robur*, drought stress caused a significant negative response in terms of trees growth, but not for *Robinia pseudoacacia* and *Platanus* x *acerifolia* (Dervishi et al., 2022; Franceschi et al., 2023). Some species have ability to recover fast after the drought event, such as *Aesculus hippocastanum*, *Quercus nigra*, *Acer campestre* and *Tilia tomentosa* (Stratópoulos et al., 2019; Dervishi et al., 2022).

Extreme high temperatures over a prolonged period often combine with drought to impact trees. Marchin et al. (2022) showed that in urban conditions, heat stress presents a greater risk to tree survival than drought stress. However, in green areas without irrigation, trees death can occur faster when these two stress events are combined (Percival, 2023). A recent study about the future intensity of heat in European cities estimated that the greatest increase is expected in Central Europe, but also in South-East

Europe, in cities such as Bucharest and Sofia (Smid et al., 2019). Bucharest is also known as a city located in a region frequently affected by drought (Mihailescu et al., 2009; Grigorescu et al., 2021). In episodes of severe drought, the law imposes water preservation and green areas are the first deprived of water.

For these reasons, studies about impact of extreme weather on tree species are very important for the future green spaces. The aim of this study was to identify resistant and sensitive species to heatwaves and drought, for future more resilient plantations and to sustain biodiversity in cities.

MATERIALS AND METHODS

The studies were conducted between 2022 and 2024 at the University of Agronomic Sciences and Veterinary Medicine of Bucharest (USAMV), Romania (44°24'N, 26°05'E). Climate of Bucharest is temperate continental with hot and dry summers, followed by cold and humid winters. In 2023 and 2024, Bucharest was listed among the hottest and driest cities of Europe and with the warmest summer on record (NMA, 2025), exceeding 39ºC for a week in July 2024 (Figure 2). Therefore, in order to assess the impact of extreme weather on the tree species, data recorded in 2022 were taken as reference for comparison.

Campus university where the investigations were carried out is located in the northern part of the city and cover an area of 38 ha (Figure 1).



Figure 1. Map of the USAMV campus

Various categories of green areas of campus, such as Dendrological Park, Botanical Garden, those around buildings, green parking lots and circulation paths, include over 4700 specimens of ornamental trees and shrubs. At the beginning of 2022, a total of 2891 trees from 115 species, 73 cultivars and 6 hybrids grew in the campus' green areas. This diversity of trees from 27 botanical families has been maintained without irrigation in most of the area, except for those covered with lawn. For the present study, trees in these areas were excluded.

During study, the dead trees were recorded annually at the end of vegetation period in terms of species, dimensions (height and DBH at 1 m) and the area in which they grew.

Chi-squared tests were applied to compare native vs. non-native species and conifers vs. broadleaves affected by extreme weather. Also, differences between conifer and broadleaf cultivars were examined with Chi-squared test. Data on the origin and type of dead trees species (conifers or broadleaves) in the reference year 2022 and the extreme weather years, 2023 and 2024, were statistically analysed using one-way ANOVA test and significance of the difference among means was estimated with LSD (Least Significant Difference) Post Hoc Test at 5% level of significance. Data on the size (height and dbh) of dead trees in the reference year 2022 and the extreme weather years 2023 and 2024, were examined by F-test at $p<0.05$.

RESULTS AND DISCUSSIONS

The extreme weather has had an important impact on trees. During the three years of observations, more than 30% of species, 10% of cultivars and 30% of hybrids were affected by heatwaves and drought (Table 1).

Table 1. Dead trees by plant taxonomy and number

	Total no. in campus	Dead trees			Total of dead trees
		Year 2022	Year 2023	Year 2024	
Species	115	7	23	26	
No of trees	2221	13	62	86	161
Cultivars	73	0	4	5	
No of trees	397	0	4	9	13
Hybrids	6	0	1	2	
No of trees	272	0	1	11	12

Compared to the reference year 2022, in which no extreme weather events were recorded, it was observed a tripling of the number of species, from 7 species, to a total of 36 species affected by extreme weather in 2023 and 2024. Annual losses were observed in four species: *Acer platanoides*, *Juglans regia*, *Prunus cerasifera* and *Tilia platyphyllos*, including in the reference year 2022 (Table 2). However, for these species, the number of dead trees also increased in 2023 and 2024.

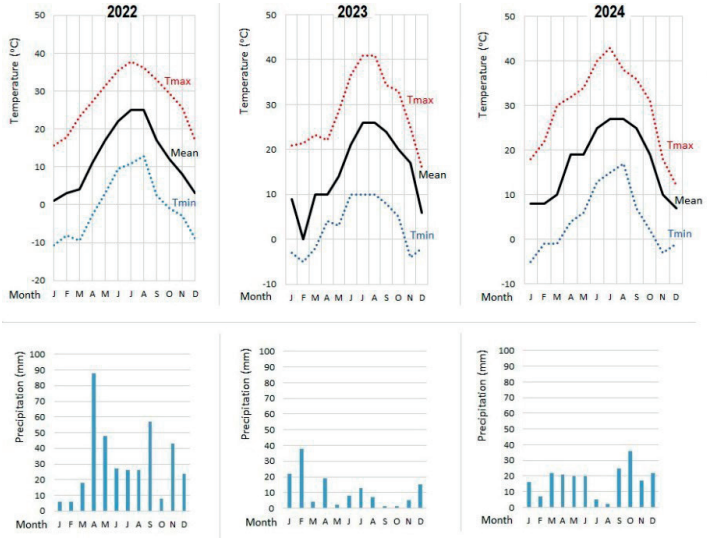


Figure 2. Mean monthly temperatures and precipitations during studied period (2022-2024)

Table 2. Species affected by extreme weather

Scientific name	Percent of dead trees (from total no. in campus)		
	Year 2022	Year 2023	Year 2024
Conifers			
Abies lasiocarpa	-	-	100
Chamaecyparis lawsoniana	-	16.7	50.0
Picea abies	-	7.7	-
Pinus nigra	-	1.2	2.3
Pinus strobus	-	5.3	10.5
Pinus sylvestris	-	11.1	-
Pseudotsuga menziesii	12.5	-	50.0
Taxus baccata	-	-	13.6
Thuja occidentalis	2.7	-	-
Thuja plicata	-	-	6.2
Broadleaves			
Acer campestre	-	6.5	7.8
Acer negundo	-	-	8.3
Acer platanoides	0.6	1.3	5.7
Acer pseudoplatanus	-	5.3	5.2
Acer tataricum	-	-	4.3
Aesculus hippocastanum	-	-	40.0
Ailanthus altissima	-	1.9	16.7
Carpinus betulus	-	-	1.8
Castanea sativa	-	-	25.0
Catalpa speciosa	-	-	20.0
Celtis occidentalis	-	4.3	7.5
Fagus sylvatica	-	-	100
Fraxinus excelsior	-	4.0	3.0
Gleditsia triacanthos	-	2.7	-
Juglans regia	4.6	6.4	6.4
Malus baccata	-	7.7	-
Morus alba	-	-	8.3
Phellodendron amurense	-	100	-
Populus nigra	-	5.9	-
Prunus avium	-	8.3	-
Prunus cerasifera	0.9	7.0	2.6
Quercus rubra	0.7	1.3	-
Robinia pseudoacacia	-	-	2.6
Scandosorbus intermedia	-	100	-
Tilia platyphyllos	3.0	1.0	1.0
Tilia cordata	-	100	-
Ulmus minor	-	13.3	6.7

Extreme weather killed all trees of *Abies lasiocarpa*, *Fagus sylvatica*, *Phellodendron amurense*, *Scandosorbus intermedia* and *Tilia cordata* and more than 50% of *Chamaecyparis lawsoniana* and *Pseudotsuga menziesii*. Important tree losses associated with drought and hot temperatures were recorded in other 13 species: 40% in *Aesculus hippocastanum*; 25% in *Castanea sativa*; 20% in *Catalpa speciosa*; 20.0% in *Ulmus minor*; 18.6% in *Ailanthus altissima*; 17.4% in *Juglans regia*; 15.8% in *Pinus strobus*; 14.3% in *Acer campestre*; 13.6% in *Taxus baccata*; 11.8% in *Celtis occidentalis*; 11.1% in *Pinus sylvestris*; 10.5% in *Acer pseudoplatanus* and 10.5% in *Prunus cerasifera*. This also had an important impact on the campus green areas, with a total tree loss of 6.84% in years with extreme weather, compared to 0.57% in the reference year 2022.

The loss of non-native tree species was significantly lower than that of native species (Figure 3) in years with extreme weather (χ^2 = 17.175; p = 0.0003). More than half of these are native to North America (Figure 4).

Most of the lost native species are known in Romania as highly adaptable to various temperatures and precipitations regimes. However, native species may be more vulnerable in urban conditions than in their natural habitat (Roloff et al., 2009; Stratópoulos et al., 2019). On the other hand, some non-native species have proved high plasticity that allows them to adapt to a range of

conditions, other than those in their natural habitat (Sjöman et al., 2016; Kendal et al., 2018; Schlaepfer et al., 2020).

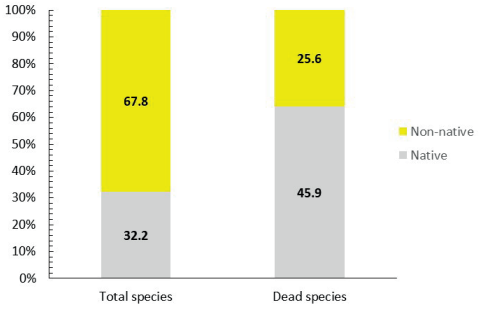


Figure 3. Differences between the loss of native and non-native species from total species

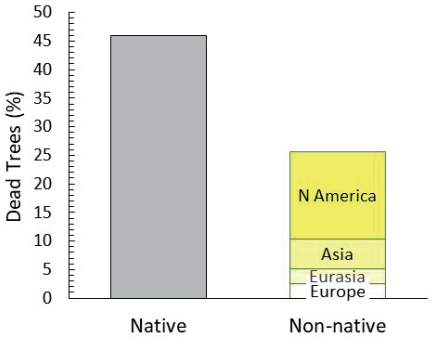


Figure 4. Share of lost species, by their origin

In urban conditions, Esperon-Rodriguez et al. (2021) observed that heatwaves have less impact on native trees species than non-native species. Comparing 806 woody species, Niinemets & Valladares (2006) reported that non-native species have better resistance to drought than native ones. However, trees vulnerability to dieback in extreme weather conditions may be influenced by factors other than genetic resistance of the species, such as: soil type (Karimian et al., 2020; Dervishi et al., 2022; Haase & Hellwig, 2022) or type of plantations (Vogt et al., 2017).

In terms of number of dead trees, significant differences (LSD = 2.33; $p = 0.043$) were observed between native and non-native species lost in extreme weather years, compared to the reference year 2022 (Figure 5). However, there were no significant differences between the two years with extreme weather (2023 and 2024).

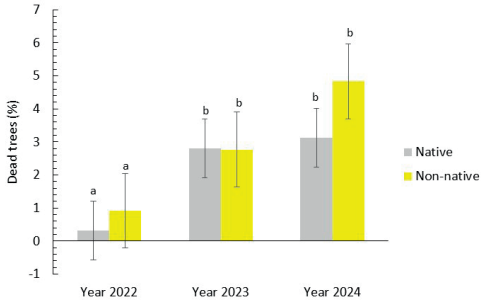


Figure 5. Differences between the loss of native and non-native species in the reference year 2022 and the extreme weather years, 2023 and 2024 (Error bars indicate SE. Data with the same letter are not statistically different at p<.05)

Tree mortality was significantly different (χ^2 = 21.948; p = 0.00001) between conifers and broadleaves species (Figure 6).

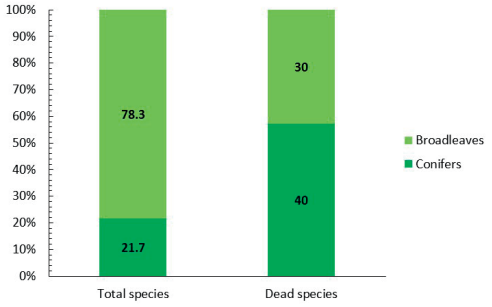


Figure 6. Differences between the loss of conifers and broadleaves species from total species

Also, the number of individuals lost in years with extreme weather was significantly higher (LSD = 9.65; p = 0.020) in conifers species compared to broadleaves ones (Figure 7).

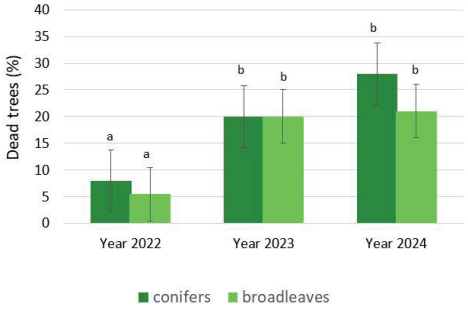


Figure 7. Differences between the loss of conifers and broadleaves species in the reference year 2022 and the extreme weather years, 2023 and 2024 (Error bars indicate SE. Data with the same letter are not statistically different at p<.05)

Some of the cultivars of species were also affected in the years with extreme weather (Table 3).

Table 3. Cultivars and hybrids affected by extreme weather

Scientific name	Percent of dead trees (from total no. in campus)		
	Year 2022	Year 2023	Year 2024
Acer pseudoplatanus 'Erythrocarpum'	-	100	-
Cedrus deodara 'Pendula'	-	-	50
Chamaecyparis lawsoniana 'Ivonne'	-	-	100
Picea pungens 'Argentea'	-	7.2	7.2
Populus nigra 'Italica'	-	-	50
Prunus cerasifera 'Pissardii'	-	3.7	-
Taxus baccata 'Overeyenderi'	-	100	-
Tilia x *europaea*	-	1.7	17.0
Ulmus x *hollandica*	-	-	100

Cultivars such as *Acer pseudoplatanus* 'Erythrocarpum', *Chamaecyparis lawsoniana* 'Ivonne' and *Taxus baccata* 'Overeyenderi' were completely lost. It was remarked that among cultivars of some were vulnerable to drought and heatwaves, while others were not. Consequently, tree cultivars such as: *Cedrus deodara* 'Pendula', *Chamaecyparis lawsoniana* 'Ivonne', *Prunus cerasifera* 'Pissardii' and *Taxus baccata* 'Overeyenderi' suffered losses, while *Cedrus deodara* 'Aurea', *Chamaecyparis lawsoniana* 'Columnaris', *Prunus cerasifera* 'Nigra' and *Taxus baccata* 'Fastigiata', did not.

Also, the cultivar *Picea pungens* 'Argentea' was found susceptible, with a tree mortality of 3,7%, but the other two cultivars, *Picea pungens* 'Glauca', *Picea pungens* 'Hoopsii' or the species *Picea pungens*, were not.

Differences between cultivars resistance have also been reported by other authors. Assessing the drought tolerance of both *Acer saccharum* and *Acer rubrum* cultivars, Sjöman et al. (2015) noted variation among them.

In another research of the drought response of five cultivars of *Spiraea japonica*, Sjöman et al. (2023) observed that cultivar *Spiraea japonica* 'Little Princess' was the most vulnerable and *Spiraea japonica* 'Superstar', the most tolerant, as was the wild-type.

The difference between the mortality of conifers cultivars (6.5%) and broadleaves cultivars (8.9%) was not significant ($\chi^2 = 1.251$; $p = 0.263$). Regarding hybrids, it was observed that the impact of extreme weather affected 30% of them and 4.4% from the total individuals. Trees of *Ulmus* x *hollandica* were completely lost. Vulnerability of this hybrid to prolonged drought was also reported by Nitschke et al. (2017).

Larger trees were more affected by extreme weather. For 59.3% species, dead trees were taller than those alive (Figure 8).

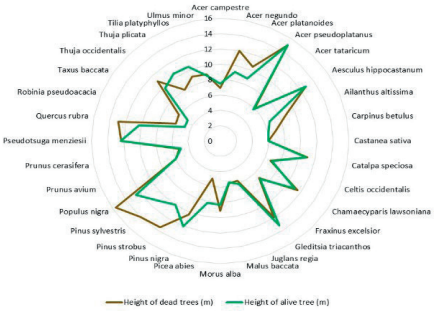


Figure 8. Comparison between mean height of dead and alive trees

Also, for the most of vulnerable species (71.8%), dead trees had a higher dbh than living trees (Figure 9).

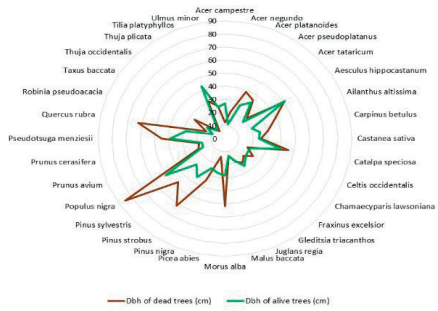


Figure 9. Comparison between mean dbh of dead and alive trees

Most of the individuals lost in extreme weather years were big size trees in some species such as: *Acer platanoides*, *Catalpa speciosa*, *Chamaecyparis lawsoniana*, *Morus alba*, *Pinus strobus*, *Populus nigra*, *Quercus rubra* and *Taxus baccata*. Also, young trees have been lost in some species such as: *Acer campestre*, *Picea abies*, *Tilia platyphyllos* and *Thuja plicata*.

Significant differences were found between the height ($F = 3.327$, $p = 0.008$) and dbh ($F = 2.361$, $p = 0.045$) of dead trees in the reference year 2022, compared to the extreme weather years, 2023 and 2024.

Studying the damages induced by heatwave-compounded drought events on 35 tree species in peri-urban forests of central Europe, Lyu and Saha (2023) showed that crown dieback is size dependent. More vulnerable in dry and hot climates were found small trees, which require more attention. Although the sensitivity of younger trees to drought episodes is higher, they tend to recover faster comparing with older trees (Au et al., 2022). In urban conditions, trees accumulate different stresses, such as improper soil conditions, air pollution or inadequate light conditions (Czaja et al., 2020). Different studies on urban trees have revealed three main factors for their mortality: species, size or age and site characteristics (Hilbert et al., 2019). In our study, drought and heatwave affected tree species of diverse sizes differently, but mainly older trees for most of them. Haase & Hellwig (2022) studying the effects of extreme weather on street trees in Germany, also reported a significant risk of mortality of older trees and newly planted trees.

In 2024, when summer temperatures exceeded 35ºC for 28 days, all individuals of some species placed in areas without irrigation were able to tolerate the hot drought without any damage (Table 4). These represent 47.8% of the total species on campus. Most of them (69.6%) are non-native species, of various sizes/ages.

These species must be investigated more in the future, to understand their ability to cope with extreme weather events

Table 4. Tree species not affected by extreme weather

Scientific name	Origin	Age range (years)
Conifers		
Abies alba	native	10-80
Abies concolor	N America	12-80
Abies nordmanniana	Eurasia	14-80
Hesperocyparis arizonica	N America	10-20
Juniperus virginiana	N America	15-70
Picea pungens	N America	12-60
Pinus ponderosa	N America	10-70
Pinus wallichiana	Asia	10-30
Platycladus orientalis	Asia	12-60
Broadleaves		
Acer buergerianum	Asia	20-40
Acer rubrum	N America	7-30
Acer ginnala	Asia	7-50
Albizia julibrissin	Asia	10-40
Betula pendula	native	8-50
Catalpa bignonioides	N America	10-80
Catalpa ovata	Asia	12-60
Celtis australis	native	10-70
Cercis canadensis	N America	10-50
Cercis siliquastrum	Eurasia	10-50
Corylus colurna	native	12-60
Crataegus monogyna	native	7-50
Diospyros virginiana	N America	10-40
Elaeagnus angustifolia	Asia	14-35
Eucommia ulmoides	Asia	20-50
Ficus carica	Eurasia	5-20
Fraxinus americana	N America	12-60
Fraxinus angustifolia	native	10-60
Fraxinus ornus	native	10-60
Fraxinus pennsylvanica	N America	12-50
Juglans nigra	N America	10-60
Koelreuteria paniculata	Asia	10-40
Liquidambar styraciflua	N America	8-20
Liriodendron tulipifera	N America	10-50
Maclura pomifera	N America	10-50
Magnolia kobus	Asia	7-40
Malus floribunda	Asia	10-50
Malus sylvestris	native	8-60
Morus nigra	Asia	10-60
Parrotia persica	Asia	7-40
Paulownia tomentosa	Asia	7-60
Populus alba	native	10-50
Prunus mahaleb	native	10-35
Prunus padus	native	7-30
Prunus serotina	N America	10-30
Pyrus piraster	native	10-50
Quercus cerris	native	10-80
Quercus coccinea	N America	10-30
Quercus palustris	N America	10-30
Quercus robur	native	10-80
Robinia hispida	N America	7-30
Styyphnolobium japonicum	Asia	10-60
Tilia americana	N America	10-50
Tilia tomentosa	native	7-80
Ulmus laevis	native	10-60
Ulmus pumila	Asia	7-70

CONCLUSIONS

In recent years, episodes of heatwave combined with drought have significantly affected trees in green areas of campus.

Native trees, previously considered highly adaptable to various weather conditions and therefore recommended for massive plantations, have proven vulnerable in current weather conditions.

Non-native species showed a better resistance to extreme weather.

Tree mortality was associated with their larger size in most species, possibly due to their lower ability to recover after drought events compared to younger trees.

The study also highlighted some conifer and broadleaved species that have proven good resilience to extreme weather.

Monitoring tree plantations can provide essential information about species resilience, but future research will need to consider also other factors such as species association, plantations composition, size, density and management, that could improve plants tolerance to extreme weather and longevity of plantations.

REFERENCES

Aram, F., García, E. H., Solgi, E., & Mansournia, S. (2019). Urban green space cooling effect in cities. *Heliyon*, *5*(4).

Argüeso, D., Evans, J. P., Fita, L., & Bormann, K. J. (2014). Temperature response to future urbanization and climate change. *Climate dynamics*, *42*, 2183-2199.

Au, T. F., Maxwell, J. T., Robeson, S. M., Li, J., Siani, S. M., Novick, K. A., ... & Lenoir, J. (2022). Younger trees in the upper canopy are more sensitive but also more resilient to drought. *Nature climate change*, *12*(12), 1168-1174.

Chapman, S., Watson, J. E., Salazar, A., Thatcher, M., & McAlpine, C. A. (2017). The impact of urbanization and climate change on urban temperatures: a systematic review. *Landscape Ecology*, *32*, 1921-1935.

Czaja, M., Kołton, A., & Muras, P. (2020). The complex issue of urban trees - Stress factor accumulation and ecological service possibilities. *Forests*, *11*(9), 932.

De la Sota, C., Ruffato-Ferreira, V. J., Ruiz-García, L., & Alvarez, S. (2019). Urban green infrastructure as a strategy of climate change mitigation. A case study in northern Spain. *Urban Forestry & Urban Greening*, *40*, 145-151.

Dervishi, V., Poschenrieder, W., Rötzer, T., Moser-Reischl, A., & Pretzsch, H. (2022). Effects of climate and drought on stem diameter growth of urban tree species. *Forests*, *13*(5), 641.

Esperon-Rodriguez, M., Power, S. A., Tjoelker, M. G., Marchin, R. M., & Rymer, P. D. (2021). Contrasting heat tolerance of urban trees to extreme temperatures during heatwaves. *Urban Forestry & Urban Greening*, *66*, 127387.

Feitosa, R. C., Wilkinson, S. J., Oliveira, B., & Hacon, S. (2021). Wind and greenery effects in attenuating heat stress: A case study. *Journal of Cleaner Production*, *291*, 125919.

Franceschi, E., Moser-Reischl, A., Honold, M., Rahman, M. A., Pretzsch, H., Pauleit, S., & Rötzer, T. (2023). Urban environment, drought events and climate change strongly affect the growth of common urban tree species in a temperate city. *Urban Forestry & Urban Greening*, *88*, 128083.

Gallay, I., Olah, B., Murtinová, V., & Gallayová, Z. (2023). Quantification of the cooling effect and cooling distance of urban green spaces based on their vegetation structure and size as a basis for management tools for mitigating urban climate. *Sustainability*, *15*(4), 3705.

Geng, X., Fu, Y. H., Piao, S., Hao, F., De Boeck, H. J., Zhang, X., ... & Stenseth, N. C. (2022). Higher temperature sensitivity of flowering than leaf-out alters the time between phenophases across temperate tree species. *Global Ecology and Biogeography*, *31*(5), 901-911.

Green, D., O'Donnell, E., Johnson, M., Slater, L., Thorne, C., Zheng, S., ... & Boothroyd, R. J. (2021). Green infrastructure: The future of urban flood risk management? *Wiley Interdisciplinary Reviews: Water*, *8*(6), e1560.

Grigorescu, I., Mocanu, I., Mitrică, B., Dumitrașcu, M., Dumitrică, C., & Dragotă, C. S. (2021). Socio-economic and environmental vulnerability to heat-related phenomena in Bucharest metropolitan area. *Environmental Research*, *192*, 110268.

Haase, D., & Hellwig, R. (2022). Effects of heat and drought stress on the health status of six urban street tree species in Leipzig, Germany. *Trees, Forests and People*, *8*, 100252.

Hamada, S., & Ohta, T. (2010). Seasonal variations in the cooling effect of urban green areas on surrounding urban areas. *Urban forestry & urban greening*, *9*(1), 15-24.

Hilbert, D. R., Roman, L. A., Koeser, A. K., Vogt, J., & van Doorn, N. S. (2019). Urban tree mortality: A literature review. *Arboriculture & Urban Forestry: 45*(5): 167-200.

Jaganmohan, M., Knapp, S., Buchmann, C. M., & Schwarz, N. (2016). The bigger, the better? The influence of urban green space design on cooling effects for residential areas. *Journal of environmental quality*, *45*(1), 134-145.

Karimian, Z., Farashi, A., Samiei, L., & Alizadeh, M. (2020). Predicting potential sites of nine drought-tolerant native plant species in urban regions. *J Appl Bot Food Qual*, *93*.

Kendal, D., Dobbs, C., Gallagher, R. V., Beaumont, L. J., Baumann, J., Williams, N. S. G., & Livesley, S. J. (2018). A global comparison of the climatic niches of urban and native tree populations. *Global Ecology and Biogeography*, *27*(5), 629-637.

Khosla, R., & Bhardwaj, A. (2019). Urbanization in the time of climate change: Examining the response of Indian cities. *Wiley Interdisciplinary Reviews: Climate Change*, *10*(1), e560.

Kim, H., Lee, D. K., & Sung, S. (2016). Effect of urban green spaces and flooded area type on flooding probability. *Sustainability*, *8*(2), 134.

Kunert, N., & Hajek, P. (2022). Shade-tolerant temperate broad-leaved trees are more sensitive to thermal stress than light-demanding species during a moderate heatwave. *Trees, Forests and People*, *9*, 100282.

Lucas-Borja, M. E., Bose, A. K., Andivia, E., Candel-Pérez, D., Plaza-Álvarez, P. A., & Linares, J. C. (2021). Assessing tree drought resistance and climate-growth relationships under different tree age classes in a Pinus nigra Arn. ssp. salzmannii forest. *Forests*, *12*(9), 1161.

Lvu, H., Saha, S. (2023). Heat and Drought Peril Germany's Peri-Urban Forests, But Drought and Cavitation Tolerance Can Enhance Survival. Available at http://dx.doi.org/10.2139/ssrn.4618929.

Marchin, R. M., Esperon-Rodriguez, M., Tjoelker, M. G., & Ellsworth, D. S. (2022). Crown dieback and mortality of urban trees linked to heatwaves during extreme drought. *Science of the Total Environment*, *850*, 157915.

Mihăilescu, M., Mareş, I., Mareş, C., Hübener, H., & Cubasch, U. (2009). Climate variability of drought indices in Romania. *Rev. Roum. Geophysique*, 52-53.

Niinemets, Ü., & Valladares, F. (2006). Tolerance to shade, drought, and waterlogging of temperate

northern hemisphere trees and shrubs. *Ecological monographs*, *76*(4), 521-547.

Nitschke, C. R., Nichols, S., Allen, K., Dobbs, C., Livesley, S. J., Baker, P. J., & Lynch, Y. (2017). The influence of climate and drought on urban tree growth in southeast Australia and the implications for future growth under climate change. *Landscape and Urban Planning*, *167*, 275-287.

NMA (2025). National Meteorological Administration. Retrieved 2025 January 3 from https://www.meteoromania.ro/clim/caracterizare-lunara/cc_2024_08.html.

Oleson, K. W., Monaghan, A., Wilhelmi, O., Barlage, M., Brunsell, N., Feddema, J., ... & Steinhoff, D. F. (2015). Interactions between urbanization, heat stress, and climate change. *Climatic Change*, *129*, 525-541.

Percival, G. C. (2023). Heat tolerance of urban tree species-a review. *Urban Forestry & Urban Greening*, *86*, 128021.

Rafael, S., Vicente, B., Rodrigues, V., Miranda, A. I., Borrego, C., & Lopes, M. (2018). Impacts of green infrastructures on aerodynamic flow and air quality in Porto's urban area. *Atmospheric Environment*, *190*, 317-330.

Ren, Z., Fu, Y., Dong, Y., Zhang, P., & He, X. (2022). Rapid urbanization and climate change significantly contribute to worsening urban human thermal comfort: A national 183-city, 26-year study in China. *Urban Climate*, *43*, 101154.

Roloff, A., Korn, S., & Gillner, S. (2009). The Climate-Species-Matrix to select tree species for urban habitats considering climate change. *Urban Forestry & Urban Greening*, *8*(4), 295-308.

Sarvari, H. (2019). A survey of relationship between urbanization and climate change for major cities in Iran. *Arabian Journal of Geosciences*, *12*(4), 131.

Schlaepfer, M. A., Guinaudeau, B. P., Martin, P., & Wyler, N. (2020). Quantifying the contributions of native and non-native trees to a city's biodiversity and ecosystem services. *Urban Forestry & Urban Greening*, *56*, 126861.

Sjöman, H., Hirons, A. D., & Bassuk, N. L. (2015). Urban forest resilience through tree selection -Variation in drought tolerance in Acer. *Urban Forestry & Urban Greening*, *14*(4), 858-865.

Sjöman, H., Morgenroth, J., Sjöman, J. D., Sæbø, A., & Kowarik, I. (2016). Diversification of the urban forest - Can we afford to exclude exotic tree species? *Urban Forestry & Urban Greening*, *18*, 237-241.

Sjöman, H., Ignell, S., & Hirons, A. (2023). Selection of shrubs for urban environments - An evaluation of drought tolerance of 120 species and cultivars. *HortScience*, *58*(5), 573-579.

Smid, M., Russo, S., Costa, A. C., Granell, C., & Pebesma, E. (2019). Ranking European capitals by exposure to heat waves and cold waves. *Urban Climate*, *27*, 388-402.

Stratópoulos, L. M. F., Zhang, C., Duthweiler, S., Häberle, K. H., Rötzer, T., Xu, C., & Pauleit, S. (2019). Tree species from two contrasting habitats for use in harsh urban environments respond differently to extreme drought. *International journal of biometeorology*, *63*, 197-208.

Teskey, R., Wertin, T., Bauweraerts, I., Ameye, M., McGuire, M. A., & Steppe, K. (2015). Responses of tree species to heat waves and extreme heat events. *Plant, cell & environment*, *38*(9), 1699-1712.

Vogt, J., Gillner, S., Hofmann, M., Tharang, A., Dettmann, S., Gerstenberg, T., ... & Roloff, A. (2017). Citree: A database supporting tree selection for urban areas in temperate climate. *Landscape and Urban Planning*, *157*, 14-25.

Wang, Y., Xing, C., Gu, Y., Zhou, Y., Song, J., Zhou, Z., ... & Gao, J. (2023). Responses and Post-Recovery of Physiological Traits after Drought–Heatwave Combined Event in 12 Urban Woody Species. *Forests*, *14*(7), 1429.

Wu, C., Li, J., Wang, C., Song, C., Haase, D., Breuste, J., & Finka, M. (2021). Estimating the cooling effect of pocket green space in high density urban areas in Shanghai, China. *Frontiers in Environmental Science*, *9*, 657969.

Xu, D., Gao, J., Lin, W., & Zhou, W. (2021). Differences in the ecological impact of climate change and urbanization. *Urban Climate*, *38*, 100891.

Zia, S., Nasar-u-Minallah, M., Zahra, N., & Hanif, A. (2022). The effect of urban green spaces in reducing urban flooding in Lahore, Pakistan, using geospatial techniques. *Geography, Environment, Sustainability*, *15*(3), 47-55.

Zimmermann, E., Bracalenti, L., Piacentini, R., & Inostroza, L. (2016). Urban flood risk reduction by increasing green areas for adaptation to climate change. *Procedia engineering*, *161*, 2241-2246.

.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

LAVENDER GROWTH AND FLOWERING IN COMPARATIVE PLANTING METHODS

Cristina Rodica MĂNESCU, Elisabeta DOBRESCU, Ilona LICSANDRU

University of Agronomic Sciences and Veterinary Medicine of Bucharest,
Faculty of Horticulture, 59 Mărăşti Blvd, District 1, Bucharest, Romania

Corresponding author email: cristina.manescu@horticultura-bucuresti.ro

Abstract

In urban green spaces, flowering plants are frequently grown in raised beds mainly to improve soil conditions and water regime. Along streets and boulevards, raised beds are often planted with resilient flowering plants that require minimal maintenance, such as lavender. Effects of the planting method on growth and flowering were investigated on young lavender plants grown in raised beds and at ground level. The results revealed that plants cultivated in raised beds were smaller in height and diameter at the end of the growing season. Also, flowering duration, size of floral stems and flower production were significantly lower, comparing to those grown at ground level. At the end of the second year of growth, lavender plants cultivated at ground level were double in size and produced a significantly increased amount of flowers. Hot and dry summers should be considered for flowering bedding plants in urban green spaces.

Key words*: drought, ground-level planting, Lavandula angustifolia `Sevtopolis`, raised beds, urban conditions.*

INTRODUCTION

Flowering plants are essential ornamental components with significant visual impact in urban green spaces. Their richness is generally affected by climate (Egerer et al., 2019; Bretzel et al., 2016), soil (Tresch et al., 2018; Wania et al., 2006), pollution (Skaldina et al., 2024; Fahim et al., 2022) and socioeconomic factors (Bernholt et al., 2009; Lowenstein and Minor, 2016; Jogan et al., 2021). In past decades, various researchers have often observed a higher plant species richness in urban areas compared to rural ones, mainly due to non-native species, more adapted to urbanization problems (Loram et al., 2008; Walker et al., 2009; Aronson et al., 2015; Cadotte et al., 2017; Knapp et al., 2017; Potgieter et al., 2024). Rising temperatures and longer drought episodes during growing season, as effects of climate changes, tend to increase the number of non-native species in European cities, especially of those originating from warmer habitats (Schmidt et al., 2014). This is also the case with lavender (*Lavandula angustifolia* Mill.), which in the recent years has become one of the most popular non-native species used in urban green spaces in Bucharest, Romania.

As a flowering plant, with exceptional characteristics such as flower colour, long blooming period and intense fragrance, *Lavandula* plants are a real attraction for green area visitors. But in Bucharest, lavender is now also present, in green spaces near streets with heavy traffic, attesting its resistance to pollution (Serban et al., 2023). Presence of flowering plants in traffic islands and parking lots has proven that they must be considered for their contribution to urban ecosystem services (Neil & Wu, 2006). However, in urban conditions, the quality of many flowering plants is poor mainly because of soils, especially near different built objectives such as buildings, streets, parking lots etc. and therefore requires improvement of soil conditions (Booze-Daniels et al., 2000; Haselton, 2019). In some cases, it is difficult if not impossible to improve urban soil conditions not only because they are strongly affected, but also built surfaces do not allow easy access to it. For this reason, urban plants are frequently grown in raised beds, using soil, organic amendments or a mixture of substrates (Miernicki et al., 2018). Besides soil conditions, raised beds improve the water regime and weed control, through substrate selection (Cox, 2005; Miernicki et al., 2018) and/or using well adapted plant species, with a high rate of coverage (Matsushima et al., 2017).

Although in the Mediterranean region lavender often grows spontaneously on mountain slopes

at the highest altitudes (Upson & Andrews, 2004), it is perfectly adapted to grow on flat lands, with different soil characteristics. As a flowering plant species, although lavender plants tolerate urban conditions, it is important to identify the factors involved in the flowering process. Flower production is known to be influenced by variety (Mihalascu et al., 2020; Delibaltova et al., 2024), temperature, light (Monaghan et al., 2004), irrigation (Sałata et al., 2020; Akçay et al., 2021) and fertilization (Hassiotis et al., 2014; Minev, 2020; Sarfaraz et al., 2024). Therefore, the aim of present research was to investigate whether the planting method – at ground level or raised beds, has an influence on lavender flowering.

MATERIALS AND METHODS

The comparative experiment was conducted in 2023 and 2024 in Bucharest (44°24'N, 26°05'E), Romania. The city is in the top 10 most-populous cities in the European Union, with a population of 1.7 million residents in an area of 240 km^2 (NIS, 2025). With a built-up area exceeding 70%, Bucharest is characterized by a temperate continental climate marked by some excessive gradations caused by additional heating and heat radiation of the street network, buildings and pollution. In the region, climate has dry and hot summers (air temperature frequently exceed 35ºC) followed by cold winters, with episodes of freezing rain, snowfall and blizzard.

In April 2023, two years old rooted cuttings of *Lavandula angustifolia* 'Sevtopolis', purchased in pots from a local nursery in Sibiu County, were planted using two methods: in raised beds and at ground-level, under field conditions, at the University of Agronomic Sciences and Veterinary Medicine of Bucharest.

The experiment was designed to reproduce the soil conditions that flowering plants experience in a real urban plantation. Therefore, the raised beds (of 40 cm height) were permanent, framed with concrete blocks and filled with the existing soil improved with 30% yard waste compost (made at the same institute from leaves, grass clippings, weeds and woody pruning waste collected from campus park and fields), for a lighter texture. The ground level planting plot was delimitated and the soil was removed (40 cm deep), improved with the same amount of yard waste compost and then refilled. Both planting type plots had the same size of 1m/5m and were arranged next to each other, in full sun and oriented north-south.

Planting was complete in staggered rows at 30 cm apart.

Irrigation was applied weekly in the first year of cultivation. In the second year, the plants from both planting type plots were not irrigated. For observations and measurements, floral stems were harvested gradually, for the second flowering wave), as they reached full stage of flowering.

No fertilizers were applied during the experiment. No cold protection measures were used during the winter. After the first growing season, all plants were pruned at the same size.

The t-test was used to compare the means of the analysed parameters of two types of planting. The statistical hypotheses were tested with $\alpha \leq 0.05$.

RESULTS AND DISCUSSIONS

Planting method of Lavender 'Sevtopolis' plants had significant impact on their growth.



Figure 1. Plants shape two months after planting

Two months after planting, lavender plants in raised beds were taller compared to those at ground level (Figure 1), which were larger in diameter. By the end of first season, growth

dynamics of the plants had changed and those in the raised beds were significantly smaller in height - 20.1 cm ($p<0.0001$) and diameter - 19.4 cm ($p<0.0001$) than those at ground level - 30.5 cm, respectively, 33.7 cm (Table 1).

Table 1. Growth parameters of *Lavandula angustifolia* 'Sevtopolis' planted in raised beds and at ground level

Planting method	Raised beds	Ground level	*P* value
First season			
Plant height (cm)	20.1± 0.93	30.5±1.43	*
Plant diameter (cm)	19.4± 0.09	33.7±0.20	*
Second season			
Plant height (cm)	25.8± 0.15	39.6±0.36	*
Plant diameter (cm)	21.4± 0.23	45.2±0.56	*

Data are presented as Mean ± SE.
NS nonsignificant or * significant differences at $p < 0.05$.

At the end of the first season, the plants grown at ground level have doubled their size compared to those when planting (Figure 2). The plants in the raised beds increased on average their height by 33% and their diameter by 26%.

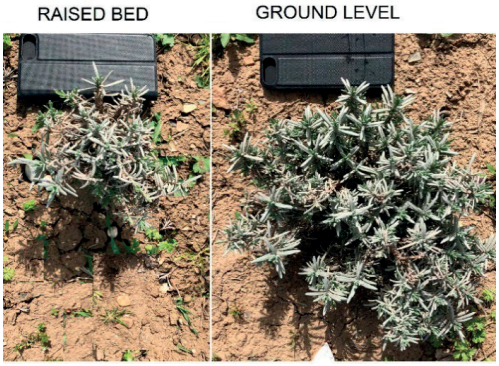


Figure 2. Plants diameter at the end of first season

In the second season, plants grown at the ground level remain significantly larger than those in raised beds. Lavender plants at ground level remained also larger in size in the second growing season. It was remarked that at ground level plants were twice in diameter compared to those in raised beds.

In comparative crops on raised beds versus at ground level, various researchers have shown that growth depends on the species and cultivars. In a field experiment conducted for two consecutive years, Ram et al. (2004) observed that plants of *Pelargonium graveolens* 'Bourbon' grown at ground level had higher height than those in raised bed (78 cm versus 67 cm, in first year and 111 cm versus 99 cm, in the second year). For 25 cultivars of 11 species of flowering plants such as: *Alternanthera dentata*, *Capsicum annuum*, *Catharanthus roseus*, *Dianthus barbatus*, *Gazania rigens*, *Tagetes patula*, *Petunia hybrida*, *Portulaca grandiflora*, *Salvia splendens*, *Spilanthes oleracea* and *Verbena hybrida*, Alsup and Trewatha (2006) found that only 9 of them showed differences in plant height and 12 of them, in plant diameter. In the same research, due to better water and nutrients conditions, 13 cultivars (*Catharanthus roseus* 'Icy Pacifica Pink', *Dianthus barbatus* 'Amazon Neon Cherry', *Petunia hybrida* 'Blue Wave', *Petunia hybrida* 'Clear Waterfall Mix', *Petunia hybrida* 'Easy Wave Pink', *Petunia hybrida* 'Lavender Wave', *Petunia hybrida* 'Madness Magenta', *Petunia hybrida* 'Purple Wave', *Petunia hybrida* 'Stars and Stripes', *Salvia splendens* 'Blue Ribbon', *Tagetes patula* 'Bonanza Harmony', *Tagetes patula* 'Durango Yellow' and *Verbena hybrida* 'Quartz Waterfall Mix') were significantly taller, wider or both, when grown at ground level (Alsup and Trewatha 2006). On the other hand, small shrubs, roses and chrysanthemums are often planted in raised beds for better performance in urban conditions (Chronopoulou & Papafotiou, 2016; Zlesak et al., 2017).

The two planting method used in this study did not influenced bud initiation or start of flowering (Table 2).

Table 2. Phenological stages of plants grown in raised beds and at ground level

Planting method	Phenological stages (days)			
	Planting to bud initiation	Bud initiation to flowering	Flowering duration	Last harvest to dormancy
Raised beds	28.8±3.1	18.2±0.4	58.0±1.9	107.4±2.2
Ground level	31.0±2.6	19.1±0.2	81.6±2.7	71.3±2.8
Significance	NS	NS	*	*

Data are presented as Mean ± SE.
NS nonsignificant or * significant differences at $p < 0.05$

Lavender plants started their flowering at the end of May in both years of observations. The flowering duration of raised beds plants was significantly shorter than of at ground level plants. The average of 23 days' difference between the two planting methods existed

because of the second flowering wave, which occurred only in the plants grown at ground level (Figure 3).

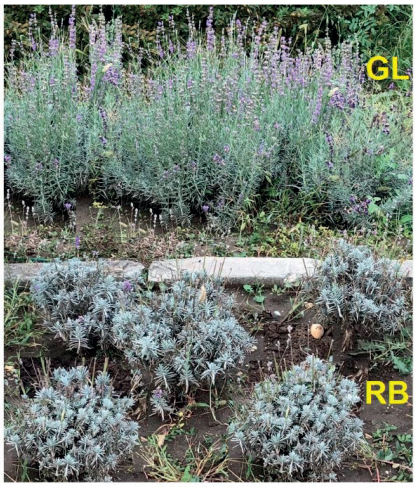


Figure 3. Lavender in raised bed (RB) versus at ground level (GL). Second bloom only at ground level plants

This started in the second half of September and finished at the end of October. Therefore, in both years of observations these plants entered dormancy a month later, in November than those grown in raised beds.

In both experimental seasons, the highest number of floral stems/plant was produced by lavender plants at ground level (Table 3).

Table 3. Flowering parameters of *Lavandula angustifolia* 'Sevtopolis' planted in raised beds and at ground level

Planting method	Number of floral stems/plant	Stems length (cm)	Inflorescence length (cm)
	First season		
Raised beds	15.8±0.49	19.4±0.72	7.0±0.34
Ground level	19.4± 0.47	24.8±0.46	9.1±0.44
Significance	*	*	*
	Second season		
Raised beds	82.8±0.53	12.5±0.68	6.2±0.42
Ground level	172.2± 0.75	12.9±0.69	6.4±0.63
Significance	*	NS	NS

Data are presented as Mean ± SE.
NS nonsignificant or * significant differences at $p < 0.05$.

Due to their greater volume in the second growing season, ground level plants produced more than double the number of flower stems/plant than those in raised beds.

Ground level plants had longer flower stems and inflorescences in the first year of study. In the following year, plants from both planting methods formed qualitatively similar flower stems, with no significant differences in both stem or inflorescence.

In urban plantations, flowering plants are selected based on their capacity to produce as many flowers as possible in certain conditions. The quantity of flowers produced by these plants is essential to achieve the desired visual impact. Therefore, evaluating flower yield was considered necessary for this research. Flower yield in the first flowering wave was higher in lavender plants grown at ground level (Table 4).

Table 4. Flower yield of plants grown in raised beds and at ground level, in the second year

Planting method	Fresh flower yield (kg.ha^{-1})	Dried flower yield (kg.ha^{-1})
	1st flowering wave	
Raised beds	1924.8	520.6
Ground level	5142.8	1522.2
	2nd flowering wave	
Raised beds	-	-
Ground level	2334.4	2041.0

Yield of fresh flower was below the potential of 'Sevtopolis' cultivar for both planting methods. Georgieva et al. (2021) who evaluated this cultivar in Bulgaria during 2015-2017, reported a fresh flowers yield of 5800-7000 kg/ha. Lavender 'Sevtopolis' is a Bulgarian cultivar with excellent adaptability, but flower yield may vary in different climate and soil conditions (Stanev, 2010). Our results were obtained in 2024, when the summer temperatures were critical high and combined with drought. Similar results regarding the decrease of flower production in *Lavandula* cultivars under drought conditions have been observed by other researchers (Zhen and Burnett, 2015).

Flowering occurred in two waves - summer and autumn, only in plants grown at ground level. However, these produced fewer flowers in the second flowering wave than in the first.

Water loss of flowers after drying was almost similar for the two planting methods, but those harvested from ground level plants had lost less water in the autumn harvest compared to the summer. For six *Lavandula angustifolia* cultivars tested in France, water stress induced a decrease in floral biomass (Saunier et al., 2022). This also happened in our study, although

Lavandula angustifolia is a Mediterranean species adapted to warm and dry climate. In urban green spaces with critical soil conditions, drought-resistant shrubs in their natural habitat may require supplemental irrigation (Schroll et al., 2011). Lavender plants grown in the present study were stressed during summer not only by drought, but also by extremely high temperatures. At roses, Shin et al. (2000) reported a 4 times higher dry weight of flowers at 15ºC than at 30ºC. The explanation for this difference was that at high temperatures the carbohydrate consumption by respiration increased or the evapotranspiration rate was higher.

After drying, the flowers kept their intense blue colour for more than 6 months, when harvested during summer. Lavender flowers harvested in the fall did not retain their colour after drying and turned almost grey (Figure 4).

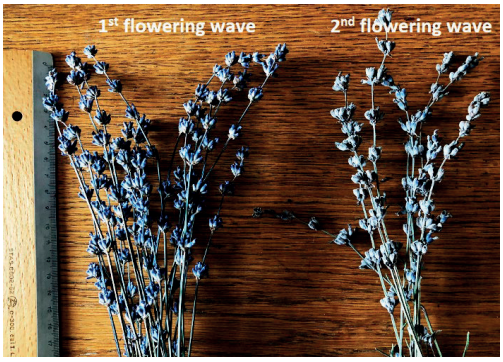


Figure 4. Dried lavender flowers from the 1st and 2nd flowering wave of the ground level plants

Kara et al. (2014) found that drying methods and temperatures have significant influence on the colour of lavandin dry flowers. Minimum level of discoloration occurred at high temperatures, due to a short time of drying process. Our lavender flowers were dried naturally, at room temperature. For this reason, during summer the flowers were dried at a higher temperature than in the autumn and their colour remained more intense.

CONCLUSIONS

In urban areas, raised beds are excellent alternative solutions for poor quality soils. Moreover, raised beds are known to reduce water loss and improve plants water consumption. Even in the most problematic soils near streets, parking lots or densely built-up zones, raised beds provide growing conditions for a wide variety of flowering plants. Results of the present study revealed that lavender manages harder water stress in raised beds than planted at ground level, under hot and dry summer conditions. Plants growth, flowering duration and flower yield were significantly influenced by planting method, being lower in plants grown in raised beds than at ground level. For this reason, hot and dry summers should be considered for flowering bedding plants in urban green spaces, which may need supplemental irrigation for better performance.

REFERENCES

Akçay, S., Dağdelen, N., Tunali, S. P., & Gürbüz, T. (2021). The effect of different irrigation programs on yield and yield parameters of lavender *(Lavandula angustifolia* Mill.) plant. *COMU Journal of Agriculture Faculty*, 9(2), 219-227.

Alsup, C. M., & Trewatha, P. B. (2006). Bagged soil tested as an alternative for growing bedding plants in the landscape. *HortScience 41*, 1272-1275.

Aronson, M. F., Handel, S. N., La Puma, I. P., & Clemants, S. E. (2015). Urbanization promotes non-native woody species and diverse plant assemblages in the New York metropolitan region. *Urban Ecosystems*, *18*, 31-45.

Bernholt, H., Kehlenbeck, K., Gebauer, J., & Buerkert, A. (2009). Plant species richness and diversity in urban and peri-urban gardens of Niamey, Niger. *Agroforestry Systems*, *77*, 159-179.

Booze-Daniels, J. N., Evanylo, G., Daniels, W. L., & Haering, K. (2000). Soil Amendments for Roadside Flower Plantings in Virginia. *Annual Report* CSES Department, Virginia Technology.

Bretzel, F., Vannucchi, F., Romano, D., Malorgio, F., Benvenuti, S., & Pezzarossa, B. (2016). Wildflowers: From conserving biodiversity to urban greening - A review. *Urban forestry & urban greening*, *20*, 428-436.

Cadotte, M. W., Yasui, S. L. E., Livingstone, S., & MacIvor, J. S. (2017). Are urban systems beneficial, detrimental, or indifferent for biological invasion? *Biological invasions*, *19*, 3489-3503.

Chronopoulou, M., & Papafotiou, M. (2016, June). Bedding plants in the landscape of Athens: data on their use and opinions of citizens and municipal employees concerning their use at various green spaces of the city. In *VI International Conference on Landscape and Urban Horticulture 1189*, 59-64.

Cox, C. (2005). Managing weeds in shrub and flower beds. *Journal of Pesticide Reform*, *25*(4).

Delibaltova, V., Manhart, S., Stoychev, I., & Nedyalkov, M. (2024). Comparative research of productive and

qualitative indicators in lavender varieties cultivated in Eastern Bulgaria. *Scientific Papers. Series A. Agronomy*, *67*(1).

Egerer, M. H., Lin, B. B., Threlfall, C. G., & Kendal, D. (2019). Temperature variability influences urban garden plant richness and gardener water use behavior, but not planting decisions. *Science of the Total Environment*, *646*, 111-120.

Fahim, A., Tan, Q., Bhatti, U. A., Nawaz, S. A., & Kaleri, A. H. (2022). Urban Diversity Impact on Plant Species Due to Environmental Conditions. *Polish Journal of Environmental Studies*, *31*(2).

Georgieva, R., Kirchev, H., Delibaltova, V., Chavdarov, P., & Uhr, Z. (2021). Investigation of some agricultural performances of lavender varieties. *Yuzuncu Yil University Journal of Agricultural Sciences*, *31*(1), 170-178.

Haselton, A. M. (2019). Wildflower Growth Responses to Diverse Soil Conditions: Texture, pH, Soil Density, and Compost. *Master's thesis*, North Carolina State University.

Hassiotis, C. N., Ntana, F., Lazari, D. M., Poulios, S., & Vlachonasios, K. E. (2014). Environmental and developmental factors affect essential oil production and quality of Lavandula angustifolia during flowering period. *Industrial crops and products*, *62*, 359-366.

Jogan, N., Küzmič, F., & Šilc, U. (2022). Urban structure and environment impact plant species richness and floristic composition in a Central European city. *Urban Ecosystems*, *25*(1), 149-163.

Kara, N., Baydar, H., & Bayhan, A. K. (2014). Changes in the essential oil content and composition of lavandin (Lavandula x intermedia Emeric ex Loisel.) under the natural and artificial drying conditions. *Akdeniz University Journal of the Faculty of Agriculture*, *27*(2), 113-117.

Knapp, S., Winter, M., & Klotz, S. (2017). Increasing species richness but decreasing phylogenetic richness and divergence over a 320-year period of urbanization. *Journal of Applied Ecology*, *54*(4), 1152-1160.

Loram, A., Thompson, K., Warren, P. H., & Gaston, K. J. (2008). Urban domestic gardens (XII): the richness and composition of the flora in five UK cities. *Journal of Vegetation Science*, *19*(3), 321-330.

Lowenstein, D. M., & Minor, E. S. (2016). Diversity in flowering plants and their characteristics: integrating humans as a driver of urban floral resources. *Urban Ecosystems*, *19*, 1735-1748.

Matsushima, K., Hirama, C., Mitarai, Y. and Koike, Y. (2017). Weed management assessment for public flower beds©. *Acta Horticulture*,1174, 127-132.

Miernicki, E. A., Lovell, S. T., & Wortman, S. E. (2018). Raised beds for vegetable production in urban agriculture. *Urban Agriculture & Regional Food Systems*, *3*(1), 1-10.

Mihalașcu, C., Tudor, V., Bolohan, C., Mihalache, M., & Teodorescu, R. I. (2020). The effect of different fertilization upon the growth and yield of some *Lavandula angustifolia* (Mill.) varieties grown in south east Romania. *Scientific Papers. Series B. Horticulture*, *64*(1), 685-692.

Minev, N. (2020). Effects of foliar fertilization on growth, development and production of flowers and essential oil on lavender *(Lavandula angustifolia* Mill.). *Scientific Papers. Series A. Agronomy*, *63*(1), 415-421.

Monaghan, J. M., Wurr, D. C. E., & Fellows, J. R. (2004). The effects of temperature and lighting on flowering of lavender *(Lavandula angustifolia* 'Hidcote'). *The Journal of Horticultural Science and Biotechnology*, *79*(5), 811-817.

Neil, K., & Wu, J. (2006). Effects of urbanization on plant flowering phenology: a review. *Urban Ecosystems*, *9*, 243-257.

NIS (2025). National Institute of Statistics. Retrieved 2025 January 3 from *https://bucuresti.insse.ro/*.

Potgieter, L. J., Li, D., Baiser, B., Kühn, I., Aronson, M. F., Carboni, M., ... & Cadotte, M. W. (2024). Cities shape the diversity and spread of nonnative species. *Annual Review of Ecology, Evolution, and Systematics*, *55*, 157-180.

Ram, P., Patra, N. K., Kumar, B., & Verma, R. S. (2004). Productivity of rose-scented geranium *(Pelargonium graveolens* L.) under different planting methods in 'Tarai'of Uttaranchal. *Indian Journal of Horticulture*, *61*(3), 256-258.

Sałata, A., Buczkowska, H., & Nurzyńska-Wierdak, R. (2020). Yield, essential oil content, and quality performance of *Lavandula angustifolia* leaves, as affected by supplementary irrigation and drying methods. *Agriculture*, *10*(12), 590.

Sarfaraz, S., Asgharzadeh, A., & Zabihi, H. (2024). Assessing the effects of water stress and bio-organic fertilizers on English and French Lavandula species in different locations. *Italian Journal of Agrometeorology*, (2), 3-22.

Saunier, A., Ormeño, E., Moja, S., Fernandez, C., Robert, E., Dupouyet, S., ... & Bousquet-Mélou, A. (2022). Lavender sensitivity to water stress: Comparison between eleven varieties across two phenological stages. *Industrial Crops and Products*, *177*, 114531.

Schmidt, K. J., Poppendieck, H. H., & Jensen, K. (2014). Effects of urban structure on plant species richness in a large European city. *Urban Ecosystems*, *17*, 427-444.

Schroll, E., Lambrinos, J. G., & Sandrock, D. (2011). An evaluation of plant selections and irrigation requirements for extensive green roofs in the Pacific Northwestern United States. *HortTechnology*, *21*(3), 314-322.

Șerban, E. A., Ștefan, D. S., Peticilă, A., Rău, I., & Boșomoiu, M. (2023). Comparative study of the bioaccumulation of heavy metals in lavender plants. *University Politehnica of Bucharest Scientifical Bulletin, Series B, 85*(4), 145-156.

Shin, H. K., Lieth, J. H., & Kim, S. H. (2000, May). Effects of temperature on leaf area and flower size in rose. In *III International Symposium on Rose Research and Cultivation 547*, 185-191.

Skaldina, O., Nylund, A., & Ramula, S. (2024). Neglected puzzle pieces of urban green infrastructure: richness, cover, and composition of insect-pollinated plants in traffic-related green spaces. *Landscape Ecology*, *39*(4), 80.

Stanev, S. (2010). Evaluation of the stability and adaptability of the Bulgarian lavender (*Lavandula angustifolia* Mill.) sorts yield. *Agricultural Science and Technology, 2(3)*, 121-123.

Tresch, S., Moretti, M., Le Bayon, R. C., Mäder, P., Zanetta, A., Frey, D., & Fliessbach, A. (2018). A gardener's influence on urban soil quality. *Frontiers in Environmental Science*, *6*, 25.

Upson, T.; Andrews, S. (2004). The Genus *Lavandula* (A Botanical Magazine Monograph); *Royal Botanic Gardens*, Kew: Richmond, UK; ISBN 0-88192-642-6.

Walker, J. S., Grimm, N. B., Briggs, J. M., Gries, C., & Dugan, L. (2009). Effects of urbanization on plant species diversity in central Arizona. *Frontiers in Ecology and the Environment*, *7*(9), 465-470.

Wania, A., Kühn, I., & Klotz, S. (2006). Plant richness patterns in agricultural and urban landscapes in Central Germany - spatial gradients of species richness. *Landscape and Urban planning*, *75*(1-2), 97-110.

Zhen, S., & Burnett, S. E. (2015). Effects of substrate volumetric water content on English lavender morphology and photosynthesis. *HortScience*, *50*(6), 909-915.

Zlesak, D. C., Nelson, R., Harp, D., Villarreal, B., Howell, N., Griffin, J., ... & George, S. (2017). Performance of landscape roses grown with minimal input in the north-central, central, and south-central United States. *HortTechnology*, *27*(5), 718-730.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

THE AUTUMN LEAF CHROMATICS OF FRUIT TREE SPECIES AND THEIR AESTHETIC EFFECT

Andreea MELINESCU, Sina COSMULESCU

University of Craiova, Faculty of Horticulture, 13 A. I. Cuza Street, Craiova, Romania

Corresponding author email: melinescu93@gmail.com

Abstract

This study examines the chromatics of fruit trees in autumn and their aesthetic impact on green spaces. Using a Minolta CR400 colorimeter and the CIELab color system, leaf colors from 10 fruit tree species were measured. The analysis revealed significant chromatic variations, with apple, fig, and hazelnut exhibiting higher green tones, while plum displayed the strongest reddish hue. The study emphasizes the importance of integrating fruit trees into urban landscapes, promoting biodiversity, and enhancing aesthetic value. Proper color management, considering interactions with surrounding elements, can create vibrant, functional spaces that counteract the monotony of urban environments, contributing to mental health and social interaction.

Key words*: leaf chromatics, fruit trees, landscape design, autumn color, aesthetic effect.*

INTRODUCTION

The appreciation of a landscape's visual quality involves cataloging and assessing its various visible attributes to guide its planning, design, and management (Palmer and Hoffman, 2001). In a fast-paced era of urban expansion and societal transformation, public and private green spaces offer opportunities for landscapers by designing recreational spaces, but also by experiencing nature by planting trees (Ma et al., 2021). Emphasis is also placed on the utility of green spaces, thus, the polychromatic beauty of fruit trees is also generated by the specific variations of each species that enter the senescence process in the autumn season. Leaf senescence is a developmental process that aims to recycle nutrients of the species in order to reuse them, in the spring season, for the purpose of producing new foliage (Matile, 2000).

Color is a key factor in landscape perception (Luo et al., 2023), with plants playing a crucial role in aesthetics through color, texture, and shape (Daniel, 2001). Studies show that 80% of initial visual attention is drawn to color (Ender et al., 2016), making it the primary characteristic of subjective vision (Zhiming & Yun, 2020; Luo et al., 2023) and a key element in assessing landscape visual quality (Harris et al., 2018). The study of autumn foliage colors is important for grouping fruit trees according to leaf colors, creating contrast in the landscape, but also for combining them with other plants or decorative elements in such a way that the landscape is balanced, harmonious, expressive, and each plant or decorative element is highlighted.

The appearance of fruit trees in different seasons is a visually impressive phenomenon, changing the appearance of the plant and at the same time the aesthetics of the landscape. The aim of this study was to analyze the seasonal variation in leaf colors during autumn and assess its impact on the visual and aesthetic quality of the landscape, exploring how these chromatic changes influence perceptual experiences and the potential applications in landscape design and planning.

MATERIALS AND METHODS

To achieve the study's objective, color measurements were conducted in the laboratory using a Minolta CR400 colorimeter, based on the L*, a*, and b* indicators. This system, known as CIELab, is an international standard for color measurement, established by the Commission Internationale d'Éclairage (CIE) in 1976 (Leon et al., 2006). The luminance component, L*, ranges from 0 (black) to 100 (white), while the chromatic components, a* and b*, range from -120 to 120. The a* parameter represents color variation from green (-a) to red (a), whereas the b* parameter

reflects variation from blue (-b) to yellow (b) (Yam & Papadakis, 2004).

To determine the coloration of leaves during the autumn season, a total of 10 fruit tree species were studied, both from public green spaces and private gardens in the city of Craiova. These species included *Corylus avellana* (hazel), *Prunus armeniaca* (apricot), *Prunus avium* (cherry), *Prunus cerasifera* (cherry plum), *Cydonia oblonga* (quince), *Malus domestica* (apple), *Prunus persica* (peach), *Ficus carica* (fig), *Juglans regia* (walnut) and *Prunus cerasus* (sour cherry). From each species, 20 leaves were collected at the beginning of November.

The leaves were dried in an oven at 60°C for 15 minutes. Each sample was ground into a powder (Figure 1) and subjected to three repetitions to determine the L*, a*, and b* values. To refine color determination, the saturation index (C) and hue angle (H°) were also calculated using the following formulas: C = (a*2 + b*2)½, representing the hypotenuse of a right triangle created by joining the points (0, 0), (a*, b*) and (a*, 0) and H° = arctangent b*/a*, which can be defined as the angle between the hypotenuse and 0° on the a* axis (green-blue/red-violet) (Mcguire, 1992; Cosmulescu et al., 2020). Both the saturation index (C) and the hue angle (H°) relate better to human perception than a* and b* (Lewallen and Marini, 2003). The saturation index (C), or chroma, indicates the degree of deviation from gray or white toward a pure hue and is a measure of brightness. The hue angle (H°) quantifies color as follows: 0° = red-violet, 90° = yellow, 180° = blue-green, and 270° = blue (McGuire, 1992; Lewallen & Marini, 2003; Yang et al., 2007; Cosmulescu et al., 2020).

The statistical analysis of the obtained data, including the mean values and standard deviation, was conducted in Microsoft Excel. A digital color palette was created in Adobe Photoshop to assess the aesthetic effect and the psychological impact of colors on viewers.

RESULTS AND DISCUSSIONS

Human behavior, physical and mental state can be influenced by the colors they perceive, making color a key element in landscape design that enhances nature's aesthetic impact, with fruit tree species contributing differently throughout the seasons, where in autumn, leaves become the primary decorative element.



Figure 1. The samples, in powder form, of the studied fruit species

The color indicators for the 10 studied fruit tree species are found in Table 1 and represent the shades that the leaves acquire at the end of the autumn season.

Analyzing the indices generated by the *a* parameter, we observe both positive and negative values. Apple (-6.41), fig (-4.18), and hazelnut (-0.37) have a higher green tint compared to the other fruit tree species, which have a higher reddish component, with the highest value recorded in cherry plum (6.81).

Only positive values were recorded for the *b* parameter, meaning that the leaves have a significantly more pronounced yellow tint, with the highest value recorded in apricot (23.56), while cherry plum registered the lowest value (7.58), thus highlighting the reddish tint generated by the *a* parameter.

Regarding the brightness parameter (*L*), the values do not exceed the average index, with the highest values recorded in hazelnut (45.88), peach (45.46), and fig (41.16), generating the brightest shades.
Meanwhile, the lowest value was recorded in cherry plum (24.96), giving it a darker color tone.
The saturation index (*C*) showed the highest value in peach (24.93), making it the species with the most intense leaf color, while the lowest value was recorded in cherry plum (10.20).
For the hue angle (*H*°), hazelnut leaves (91.01°) are closest to the yellow color (H°=90°), while cherry plum leaves (48.01°) are closest to red-violet shades (H°=0°). Very subtly, apple leaves (110.59°) take on a bluish-green hue (H°=180°).
The autumn leaf color of most studied fruit tree species, according to the hue angle, falls within the spectrum of red-violet to yellow shades.

Table 1. CIEL **a*b** parameters determined in the leaves of the studied fruit species

Fruit tree species	CIEL*	*a**	*b**	C*ab	H°ab
			Mean±SD		
Hazelnut	45.88±0.24	-0.37±0.22	21.17±0.14	21.17±0.15	91.01±0.61
Apricot	44.19±1.41	4.29±0.13	23.56±0.60	23.96±0.57	79.67±0.58
Cherry	38.27±0.57	4.53±0.06	17.74±0.59	18.32±0.59	75.67±0.30
Cherry plum	24.96±0.77	6.81±0.17	7.58±0.38	10.20±0.21	48.01±2.02
Quince	39.78±0.90	3.92±0.08	20.38±0.37	20.76±0.35	79.11±0.43
Apple	35.40±1.13	-6.41±0.19	17.08±0.62	18.25±0.64	110.59±0.29
Walnut	35.13±0.78	3.99±0.10	16.36±0.37	16.85±0.39	76.30±0.14
Peach	45.46±0.50	2.17±0.03	24.83±0.32	24.93±0.32	84.99±0.12
Fig	41.16±1.01	-4.18±0.08	18.43±0.34	18.91±0.35	102.79±0.06
Sour cherry	38.71±0.43	5.26±0.36	19.06±0.66	19.77±0.66	74.57±1.10

Based on the obtained data, we created a color palette to determine the aesthetic effect that fruit tree species can generate in both public and private green spaces, as well as to assess the influence of colors on the psychological state of viewers. The evolution of leaf coloration in fruit tree species during autumn, throughout the senescence process, is spectacular and visually appealing, thus contributing to the aesthetic impact these species have on the landscape.
Shades of yellow and orange appear due to the unmasking of carotenoids during chlorophyll degradation, brown shades result from the oxidation of phenols associated with dead cells, while red hues emerge from the synthesis of anthocyanins (Anderson & Ryser, 2015). A key advantage of fruit tree species, compared to other landscape elements, is their unique charm derived from the seasonal color changes of each specific plant.
When designing a green space, it is beneficial to follow the guidelines of a color wheel, such as Newton's color disc, to create harmony by using adjacent colors together. Red, blue, and yellow are primary colors, and for achieving color harmony, combinations such as red, violet, and blue; blue, green, and yellow; or red, violet, and orange can be used (Clarke, 2004). Two of these would act as dominant colors, while the third would serve as a focal point.



Figure 2. The color palette generated by CIEL **a*b** parameters determined in the leaves of the fruit trees studied in the autumn season

Color contrast is generated by using colors that are opposite each other on the color wheel. In the case of the autumn coloration of fruit tree species (Figure 2), we can group cherry plum, which has a reddish tint, with apple, which retains a green hue, and any other species displaying yellow to brown shades.

To create a pleasant aesthetic effect in a green space during autumn, we can place hazelnut, apricot, cherry, quince, walnut, peach, or sour cherry in the foreground, as well as apple or fig (Figure 3). In the background, we can choose species with bright red foliage, such as *Cotoneaster*, *Acer rubrum*, *Parthenocissus*, or *Nyssa sylvatica*.

Cherry plum, with its reddish foliage, can be combined with coniferous species or various evergreen shrubs, such as *Juniperus chinensis*, *Cedrus atlantica*, and *Picea pungens*. Regarding the influence of colors on the emotional state of the viewer, yellow is a relaxing color. Studies suggest that prolonged observation of yellow positively affects the neural and circulatory systems (Ender et al., 2016). Psychologically, yellow is the most powerful color.

In landscape design, the correct balance of yellow is essential to suggest optimism, self-confidence, and to boost self-esteem and morale. Otherwise, a green space dominated by yellow, especially in autumn, can cause anxiety, trigger various fears, and lower self-confidence (Kaya & Epps, 2004). It is necessary to maintain a balance of colors in a green space, such as yellow-green, yellow-red, or yellow-blue. The color palette of fruit tree species in autumn is diverse. However, based on this study, shades of brown are predominant, creating a contrast with evergreen species.

Brown is often described as the color of the earth. The death of leaf cells forms dark-colored oxidative products (melanins), and these brown shades result from various phenols, such as tannins. Brown can accelerate human activities (Ender et al., 2016), encouraging outdoor activities such as gardening or agriculture. Light brown shades suggest care, accessibility, and openness to the human psyche. However, too much brown in the landscape can become monotonous and make landscape design appear unsophisticated in autumn. Therefore, it is essential to introduce different species or decorative elements with varied colors around fruit trees with brown foliage to break the monotony.

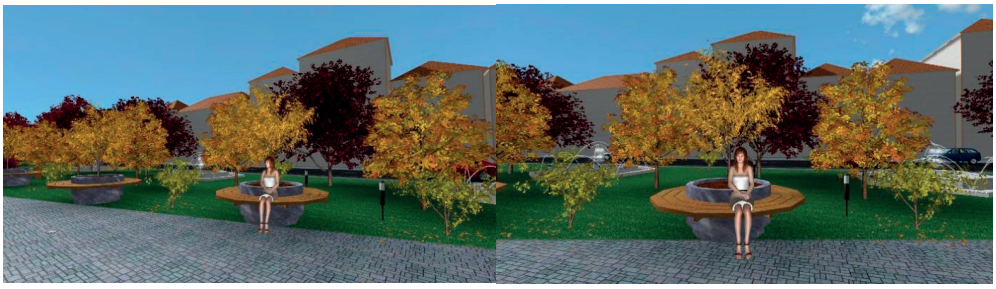


Figure 3. 3D simulation of a green space featuring fruit tree species: pear, peach, cornelian cherry, and cherry plum, in the autumn season

Apple and fig have retained their green hues, though not as bright as in spring, with the fig displaying a yellowish-green shade. Green is a color of balance; it can harmonize the entire design and stands out when placed among warm colors such as red, yellow, and orange. It brings balance and harmony, both in gardens and in the emotional state of the viewer. It is a color that makes people more aware of their surrounding environment and generates a restful feeling. Thus, even in autumn, green foliage should not be absent from the landscape (Figure 4).

Cherry plum was the only fruit tree species with a higher red component, resulting in a reddish-brown foliage shade (Figure 4). Visually, red is a bold color, but in excessive amounts, it can cause agitation, irritation, or nervousness in the viewer, as it overstimulates human attention. Red is a highly energetic color, visually stimulating the human nervous system (Li, 2013).

The color red has the property of appearing closer than it is, making it highly attention-grabbing (Kurt & Osueke, 2014). Warm tones generate a strong sense of movement and enthusiasm, while cool tones provide a feeling of tranquility (Wang, 2021). Thus, most fruit tree species can be placed at the entrance of a public or private green space due to their warm color variations, creating a lively landscape that conveys energy and revitalization. In a green space, warm colors can be used in the foreground to create a sense of closeness, while cool colors can be placed in the background to generate a sense of depth and distance. In urban environments, the variety of leaf colors in fruit tree species enhances the vitality of the space and highlights architectural elements.



Figure 4. 3D simulation of a private garden featuring the cherry plum as the focal point, surrounded by evergreen plants, in the autumn season

All these aspects of color study are necessary to create a balanced landscape design and to integrate fruit tree species into the landscape in a way that highlights all their attributes. In landscape design, it is important to consider that colors interact and relate to one another. Thus, the background and the surrounding area of the fruit tree species selected for gardens or various public and private green spaces are crucial. The background colors of fruit tree species will influence the overall landscape aesthetics, making a color appear lighter or darker, brighter, or duller.

Color harmony can be achieved using a monochromatic color palette (Westland et al., 2007), where the selected colors share the same hue. An example from this study is a combination of apricot, peach, and sour cherry. A complementary color palette (Westland et al., 2007) consists of opposite colors on the hue wheel, such as yellow and blue. A complementary color combination for autumn could include quince, sour cherry, hazelnut, and other species with yellow-brown foliage paired with species featuring silvery-blue tones, such as *Juniperus pf. Pfitzeriana Glauca*, *Chamaecyparis law*, *Columnaris*, or *Festuca glauca*. A complementary palette creates a vibrant design.

A third color palette is the analogous palette, where colors with similar hues, such as yellow and red (Museros et al., 2016), are chosen. These colors appear next to each other on the hue wheel, creating a visually harmonious and comfortable design. A colorful environment stimulates the mind, alters the brain's alpha rhythm, and increases heart rate compared to a gray environment (Küller et al., 2009). This effect on mood also applies to landscapes, highlighting the need for vibrant public and private green spaces, especially in urbanized areas, where gray concrete structures dominate.

In conclusion, the proper use of colors and fruit tree species enhances the functionality of public and private green spaces, particularly in promoting urban horticulture and edible landscapes.

CONCLUSIONS

In the autumn season, the most intense leaf color was recorded in peach, with a saturation index (*C*) of 24.93, while the dullest color was observed in cherry plum, with a saturation index of 10.20. The highest leaf brightness was recorded in hazelnut (45.88), peach (45.46), and fig (41.16).

Analyzing the indices generated by the *a* and *b* parameters, a stronger green tint was observed in the leaves of apple, fig, and hazelnut, while a higher red tint was observed in cherry plum, and the highest yellow tint was recorded in apricot.

A pleasant aesthetic effect can be created by placing fruit tree species with yellow-green leaves in autumn (hazelnut, apricot, cherry, quince, walnut, peach, sour cherry, apple, or fig) in the foreground. In the background, trees with red foliage (Figure 3) can be chosen, while cherry plum, which has reddish leaves in autumn, can be grouped with evergreen plants (Figure 4).

Colors carry various symbolic meanings, and landscape designers must consider how individuals associate colors with certain things or events. A simple example is that most people associate yellow-red hues with the arrival of autumn and a feeling of melancholy due to the lack of sunlight and rainy days. Therefore, garden design should counteract these emotions.

It is crucial for landscape designers to understand and have knowledge of color theory, just as it is important to be familiar with different varieties of fruit tree species, their attributes, and ways to integrate them into the landscape - perhaps even based on the garden style.

Fruit tree species can also be integrated into public green spaces within schools and hospitals or aligned along highways. In all these locations, color plays a crucial role in influencing the mood of students, teachers, patients, doctors, drivers, and others.

In traditional gardens, numerous fruit tree species can be found, often as solitary trees or in alignments, but rarely in combination with other ornamental plants due to a lack of knowledge and specialists in landscape design and green space principles. There is a need for more information and experts to create modern gardens influenced by contemporary trends, such as fusion gardens, a style that allows landscape designers to innovate and use intense colors to create a vibrant atmosphere, or conceptual gardens, which focus more on art than horticulture, with designs defined by patterns or repetitions, achieved by mass planting a single species to emphasize its color throughout the year.

REFERENCES

Anderson, R., & Ryser, P. (2015). Early autumn senescence in red maple (*Acer rubrum* L.) is associated with high leaf anthocyanin content. *Plants*, 4(3), 505-522.

Clarke, E. (2004). Great Gardening with Foliage, *Form and Texture*. David & Charles.

Cosmulescu S., Scrieciu F., Enescu Mazilu I. (2020). Variation of fruit and leaf color in medlar genotypes (*Mespilus germanica* L.) identified in Romania. *Wulfenia* 27 (7), 24-32.

Daniel, T. C. (2001). Whither scenic beauty? Visual landscape quality assessment in the 21st century. *Landscape and urban planning*, 54(1-4), 267-281.

Ender, E., Akdeniz, N. S., & Zencirkiran, M. (2016). Colors and Landscape. *Journal of Agricultural Faculty of Uludag University*, 30, 669-676.

Harris, V., Kendal, D., Hahs, A. K., & Threlfall, C. G. (2018). Green space context and vegetation complexity shape people's preferences for urban public parks and residential gardens. *Landscape research*, 43(1), 150-162.

Kaya, N., & Epps, H. H. (2004). Relationship between color and emotion: A study of college students. *College student journal*, 38(3), 396-405.

Küller, R., Mikellides, B., & Janssens, J. (2009). Color, arousal, and performance - A comparison of three experiments. *Color Research & Application: Endorsed by Inter-Society Color Council*, The Colour Group (Great Britain), Canadian Society for Color, Color Science Association of Japan, Dutch Society for the Study of Color, The Swedish Colour Centre Foundation, Colour Society of Australia, Centre Français de la Couleur, 34(2), 141-152.

Kurt, S., & Osueke, K. K. (2014). The effects of color on the moods of college students. *Sage Open*, 4(1), 2158244014525423.

Leon, K., Mery, D., Pedreschi, F., & Leon, J. (2006). Color measurement in L∗ a∗ b∗ units from RGB digital images. *Food research international*, 39(10), 1084-1091.

Lewallen, K. S., & Marini, R. P. (2003). Relationship between flesh firmness and ground color in peach as influenced by light and canopy position. *Journal of the American Society for Horticultural Science*, 128(2), 163-170.

Li, C. (2013). Color Application in Hospital Landscape Design. *Journal of Landscape Research*, 5(3), 43.

Luo, Y., He, J., Long, Y., Xu, L., Zhang, L., Tang, Z., ... & Xiong, X. (2023). The Relationship between the Color Landscape Characteristics of Autumn Plant Communities and Public Aesthetics in Urban Parks in Changsha, China. *Sustainability*, 15(4), 3119.

Ma, B., Hauer, R. J., Xu, C., & Li, W. (2021). Visualizing evaluation model of human perceptions and characteristic indicators of landscape visual quality in urban green spaces by using nomograms. *Urban Forestry & Urban Greening*, 65, 127314.

Matile, P. (2000). Biochemistry of Indian summer: physiology of autumnal leaf coloration. *Experimental gerontology*, 35(2), 145-158.

Mcguire, R. G. (1992). Reporting of objective color measurements. *HortScience*, 27(12), 1254-1255.

Museros, L., Sanz, I., Falomir, Z., & Gonzalez-Abril, L. (2016, October). A qualitative color harmony theory. *In Artificial Intelligence Research and Development-Proceedings of the 19th International Conference of the Catalan Association for Artificial Intelligence*, Barcelona, Catalonia, Spain (pp. 98-107).

Palmer, J. F., & Hoffman, R. E. (2001). Rating reliability and representation validity in scenic landscape assessments. *Landscape and urban planning*, 54(1-4), 149-161.

Wang, D. (2021). Seasonal color matching method of ornamental plants in urban landscape construction. *Open Geosciences*, 13(1), 594-605.

Westland, S., Laycock, K., Cheung, V., Henry, P., & Mahyar, F. (2007). Colour harmony. *Colour: Design & Creativity*, 1(1), 1-15.

Yam, K. L., & Papadakis, S. E. (2004). A simple digital imaging method for measuring and analyzing color of food surfaces. *Journal of food engineering*, 61(1), 137-142.

Yang, Z., Zheng, Y., Cao, S., Tang, S., Ma, S., & Li, N. A. (2007). Effects of storage temperature on textural properties of Chinese bayberry fruit. *Journal of Texture Studies*, 38(1), 166-177.

Zhiming, T., & Yun, M. (2020). Elements of plant color matching in intelligent landscape design. *In 2020 International Conference on Intelligent Design* (ICID) (pp. 214-218). IEEE.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

TULIPA HUNGARICA BORBÁS – CONSERVATION STATUS, SPECIES DISTRIBUTION AND POTENTIAL THREATS

Luiza Silvia MIHAI, Marilena ONETE, Tiberiu SAHLEAN, Minodora MANU, Simona MIHĂILESCU

Institute of Biology Bucharest of Romanian Academy, 296 Splaiul Independentei, District 6, Bucharest, Romania

Corresponding author email: marilena.onete@gmail.com

Abstract

The yellow gorge tulip (Tulipa hungarica Borb.) is an endemic species of community interest, protected by the Berne Convention and the Romanian laws; it is in Annex 2 of the Directive on the conservation of natural habitats and species of wild fauna and flora and is found only on the territory of Serbia and Romania. In the Romanian literature, the species is described as critically vulnerable. From the point of view of taxonomy, it is perennial, robust, with an ovoid bulb. Among the habitat preferences, the following are mentioned: heliophilic, thermophilic and calciphilous, on moderately moist, fertile, neutral soils. The main pressure on this species refers to collection activities, but the intensity of this pressure is low, probably due to the difficult access of people to the area where the species is found. The main threats we consider climate change with all the general consequences. As limiting factors, we list the fact that there are few individuals, and as conservation measures specialists recommend monitoring populations and preserving seeds in gene banks.

Key words*: conservation measures, endemic, pressure, threat, Tulipa hungarica.*

INTRODUCTION

Threats to biodiversity are very diverse and it is very important for the conservation of biological diversity to know them and find methods of managing them (Cucu et al., 2013). Human activities put great pressure on biodiversity at European and global level. Agriculture is the main pressure affecting species and habitats, followed by the expansion of urban areas, unsustainable management actions on forests, water, soil and air pollution, fragmentation and degradation, respectively the destruction of habitats through various activities, the introduction of invasive species, illegal hunting or intensive fishing actions. Thus, in order to maintain the health of nature, the implementation of well-thought-out and applied conservation strategies is needed (EEA, 2025a). Among other aspects of assessing the conservation status of a species, it is essential to estimate its risk of extinction, thus prioritizing conservation actions. Thus, for habitat management and the development of sustainable development models, it is important to identify and quantify the main types of threats (Cucu et al., 2013). To protect nature and slow down or halt the degradation of ecosystems and species, EU Member States have adopted an ambitious, comprehensive and long-term plan containing specific commitments and actions to restore biodiversity by 2030, called the EU Biodiversity Strategy for 2030 (EC, 2021). At EU level, vascular plants are generally assessed as having a good conservation status (27 % of species), but many are also in a poor conservation status, affected by the main pressures specified above (EEA, 2025a). The conservation status of species under the Habitats Directive refers to the sum of the influences acting on species that may affect their long-term distribution and abundance of their populations across the EU. (HD, 1992). Favourable conservation status refers to: population dynamics indicating that the species could survive in the long term in its natural habitats, the natural range of the species will not be reduced in the foreseeable future, the habitat containing the species is large enough to maintain the populations of the species in the long term. In accordance with Article 17 of the Habitats Directive, each Member State reports on the conservation status of species of

Community interest (listed in Annexes II, IV and V) at national biogeographical level. Four parameters are used to establish the conservation status of a species: distribution area, population, habitat of the species, future prospects (EIONET, 2025). When the species are facing high risk of global extinction, the IUCN Red List Categories and Criteria (IUCN, 2025) created a classification system that might be easily and widely understood: Not Evaluated (NE), Data Deficient (DD), Least Concern (LC), Near Threatened (NT), Vulnerable (VU), Endangered (EN), Critically Endangered (CR), Extinct in the Wild (EW) and Extinct (EX).

Between 2007 and 2024, Romania had 3 reporting periods on the conservation status of species: 2007-2012 (the report was carried out in 2013), 2013-2018 (the report was carried out in 2019) and 2019-2024 (the report will be carried out in 2025).

The purpose of this article is to analyse the conservation status of the species *T. hungarica* Borbás, a rare species present on Annexes II and IV of the Habitats Directive, its distribution and the identification of the pressures and threats acting on it.

MATERIALS AND METHODS

Description of the species

T. hungarica is popularly known as the Cauldron Tulip. It is a perennial plant and has a maximum height of 50 cm (PM-PNPF, 2014). It is part of the Liliaceae family and has several synonyms: Danube tulip (Segal & Alen, 2020), Đerdap tulip (after the Serbian name "Đerdap Gorge" (Palin, 2013) or Banat tulip (Bonifaciu, 1985). It is also known as Hungarian tulip (Goriup, 2008). There are uncertainties in the classification of the taxonomic status of this species: *T. orientalis* var. *urumoffii*, *T. orientalis* Levier, *T. hungarica* var. *urumoffii* (synonymous with *T. urumoffii* Hayek) (Ciocârlan, 2009), *T. hungarica* var. *undulatifolia* (synonymous with *T. urumoffii* Hayek) (Ciocârlan, 2009), *T. hungarica* Borbás subsp. *undulatifolia* (Roman) Roman et Beldie (Sârbu et al., 2013).

The studied species has a robust habitus, ovoid bulb, glabrous stem, light yellow and odourless flowers and flat, brown seeds (Dihoru & Negrean, 2009). The flowers of this species are 4.5-8 cm in size. The perigonal leaflets are almost equal, the outer ones narrower. The stamens have 10-12 mm length filaments and 11-12 mm length yellow anthers. The ovary is elongated, 20-22 mm length, and the stigma is 5 mm wide, about 2 times wider than the ovary. The capsule is elongated elliptical, up to 70 mm length, attenuated at both ends (PM-PNPF, 2014; CNIPTMN, 2025).

Data collection

The desk work focused on publications and reports. The field work consisted of data collection in the framework of two projects coordinated by the Ministry of Environment, Water and Forests as the basis for Romania to report to the European Commission: POS project "Monitoring of the conservation status of species and habitats from Romania in the framework of article 17 of Habitats Directive" (2011-2015) and POIM project "Completing knowledge level of biodiversity through implementing the monitoring system of conservation status of species and habitats from Romania in the framework of article 17 of Habitats Directive 92/43/EEC" (2019-2023). The expertise developed within the POS and POIM projects focused (as required by the European Commission), on assessment of the conservation status of *T. hungarica*, trends, main pressures and threats. All of this was carried out based on monitoring and evaluation methods in accordance with the appropriate guidelines (Mihăilescu et al., 2015b; OM no. 3351 of December 28, 2023).

Distribution maps

The distribution map standard is: ETRS89 grid of 10 x 10 Km, ETRS LAEA 5210 projection. The methods used were mainly based on extrapolation from a limited number of data (e.g. other predictive models or extrapolations using a less complete sample of data on coverage and environmental factors). Observations and data collected during field trips formed the basis of all processing and interpretations, in order to obtain results that are as objective and of scientific value as possible. For vascular plants that are difficult to access in the field and thus difficult to inventory, the 1 x 1 km grid was established at

the European Commission level as the reporting unit for this group of species to which *T. hungarica* also belongs. The population size was calculated by reporting as a range (minimum and maximum value) and/or as a single value (measured or estimated) when only a single value obtained through expert opinion is known. The type of estimates is minimal, where insufficient data can be used for an estimate, but where the population size is known to be above a certain value, or estimates from the reporting period come from field inventories or projects that are likely to have underestimated the true population size.

Plant species monitoring plan

T. hungarica have been monitored in the field in April-May, as long as weather conditions allow, during its optimal development period, especially to observe pressures and threats, as well as other parameters useful in assessing the conservation status. This species belongs to the group of vascular plant species present in areas of siliceous or calcareous rocks and screes in mountainous/subalpine areas. This particular species vegetates in areas of calcareous and steep slopes located of the Danube River banks, benefiting from open places, in full sun during the summer, with shallow soil. *T. hungarica* is a geophyte species, perennial and clonal.

The field studies of the species assessed direct and indirect attributes. The methods for assessing direct attributes are: species identification/recording (for assessing presence and absence); functional individual counts (for estimating population size (number of individuals), mapping (estimating population size/population distribution); visual assessment (estimating regeneration success). The methods for assessing indirect attributes are: Mapping (for estimating niche availability and total coverage); visual assessment (identifying negative indicators as grazing, shading, rock dynamics, extreme climatic events, alien species) (Mihăilescu et al., 2015b; OM no. 3351 of December 28, 2023). Our monitoring studies have been developed during 2 reporting periods (6 years).

Since the habitats of this group of species are very unstable and can be damaged even by the researcher's intervention, non-destructive methods have been used, from a distance, for example, photographing the slopes from the same point and making sketches of the vegetation distribution. Where and if possible, the point for taking shuts is marked with persistent paint (if on land) or use GPS for a specific point (if on land or water) and the same lens with the same photographic magnification have been used. Binoculars have been used by a plant specialist who could identify the plant species.

The species must be in their optimal flowering period, especially when it is necessary to monitor them from a distance.

RESULTS AND DISCUSSIONS

History

The presence of the species was first reported in 1838 in the Cazanele Dunării area (Coste & Faur, 1970).

The name *T. hungarica*" was given in 1882 by the Hungarian botanist Vincze von Borbás (1844-1905).

It was the first tulip to be described from the Balkan region (Egreta, 2017; Wilson, 2023). It was verified by the United States Department of Agriculture and the Agricultural Research Service in 1996 and is an accepted name by the Royal Horticultural Society of the United Kingdom (RHS, 2025). In 2013, phylogenetic relationships within the genus using DNA sequences were used to determine taxonomy and classifications. As a result, *T. hungarica* was placed in the subgenus Tulipa (along with *T. agenensis* Redouté, *T. fosteriana* W. Irving, and *T. greigii* Regel) with the characteristics of bulbous tunics densely lined on the inside with wavy or silky hairs or (nearly) glabrous (Christenhusz et al., 2013).

Ecological requirements

From a biological point of view, the species is geophytic, reproduction is amphimictic, allogamous through entomophily and barochorous and blooming in April (Dihoru & Negrean, 2009). The species is a heliophilous, thermophilic, calcicolous species, growing on moderately moist, fertile, neutral soils (Mihăilescu et al., 2015b; OM no. 3351 of December 28, 2023). The species have optimum development on sunny slopes at 100-250 m altitude tougher with other thermophilic

species: *Centaurea atropurpurea, Echinops bannaticus, Ferula heuffelii, Festuca pallens, Piptatherum holciforme, Ruscus aculeatus, Sedum hispanicum, Sesleria filifolia, Veronica crinita* (Dihoru & Negrean, 2009). The habitat preferred by the species are: open mixed deciduous forests, shrubs and steep rocky limestone slopes (Cucu et al., 2013).

International location notes

It is reported to be widespread in the Balkans (Turrill, 1929; Millaku et al., 2018), Greece, southern Bulgaria (probably native), naturalized in Romania (Booy & Raamsdonk, 1998; Christenhusz et al., 2013; Wilson, 2023; USDA, 2025), including the Western Carpathians (Hurdu et al., 2012). It is also stated to be an endemic species in Serbia (Papp, et al; 2006; Hurdu et al., 2012; Millaku et al., 2018). It is also referred to as the "Rhodope tulip", because one of its synonyms is *T. rhodopea* (Velen.). The epithet "Rhodope" refers to the Rhodope Mountains in Bulgaria, where it is mainly found (Zonneveld, 2009; Harisson, 2012; WFO, 2025).

It is mentioned as being located in the Djerdap National Park, eastern Serbia, on the right bank of the Danube canyon. The park is an internationally protected area, which continues into the Iron Gates Nature Reserve, along the left bank of the Danube (Sabovljević, 2006; Ćalić et al., 2012). One of the famous endemic species of the Djerdap Gorge is *T. hungarica* (Sabovljević, 2006). In Serbia, *T. hungarica* was rare on the northern slopes (those towards the Iron Gates), but has not been recorded in the last ten years. The only evidence of its existence in Serbia is constituted by the specimens preserved in the herbarium (Wilson, 2023).

National conservation status

In Romania, it is reported at Cazanele Mari, Ciucaru Mare, Ciucaru Mic (Brânele Cazanelor), Cazanele Mici (at Bălan). The biogeographical region where it is found is continental (Mihăilescu et al., 2015b). On the red list of higher plants in Romania, the species is mentioned as rare (Oltean et al., 1994; Stevanović, 2011), endemic, isolated, permanent, native (PM-PNPF, 2014; CNIPTMN, 2025), critically vulnerable (Dihoru & Negrean, 2009; Schneider-Binder, 2014) and "critically endangered" (IUCN, 2025). The yellow tulip is a protected species under the Bern Convention of 19 September 1979 and Emergency Ordinance No. 57/2007 on the regime of national areas to be conserved.

It is also listed in the EU Habitats Directive 92/43 EEC, in Annex II and Annex IV (EEA, 2025b). The distribution and range (Figures 1 and 2) remain constant from the reporting year 2013 (first reported to the European Commission for the period: 2007-2012) (Mihăilescu et al., 2015a; EC, 2025). Monitoring of the species carried out in the period 2013-2018 and reported in 2019 led to the conclusion that the species had a favourable conservation status, with an unknown trend. In 2025, Romania's third reporting will be carried out based on the reference list and relevant documents available on the reference portal for reporting under Article 17 of the Habitats Directive. This portal contains relevant documents for the reporting format for the period 2019-2024 (EIONET, 2025). For this reporting, a favourable conservation status with a stable trend is maintained.



Figure 1. Distribution of the species *T. hungarica*



Figure 2. Range of the species *T. hungarica*

Population size

About 15 years ago, the population was quite stable, up to 250 plants (Goriup, 2008), but the plant has multiplied, from one year to the next. The number has reached over 1,000 mature individuals on the southern slopes of the Iron Gates (IUCN, 2025). In Romania, there are approximately 1000-1500 mature individuals of *T. hungarica*, on the southern slopes of the Iron Gates (IUCN, 2012). According to the parameters for assessing the conservation status of the species *T. hungarica* from a population perspective, the population size of the species in the protected natural area is estimated at approximately 8,000-10,000 individuals (PM-PNPF, 2014).

Integrative habitats

The assessment of the integrative species' habitat, shows that the habitat covers approximately 30 ha (PM-PNPF, 2014). It is mentioned as being present only in the Cazane region, in several areas: Ciucarul mic, Ciucarul Mare, the Danube Valley at Cazane near the Veteranilor Cave, Cazanele Mari (between Ciorici and Frasin), Cazanele Mici (at Bălan). *T. hungarica* is a Romanian local endemic, present only in Cazane, being of great interest to the field specialists. It is protected in the Iron Gates Natural Park, but also by its own distribution habitat (growing on almost inaccessible cliffs for humans) (Dihoru & Negrean, 2009).

The Danube River banks on which *T. hungarica* vegetate is declared as a protected area on both the Serbian and Romanian sides. The Iron Gates Natural Park (1156 km^2) is located in southwestern Romania in the counties of Caraș-Severin and Mehedinți (Figure 3).

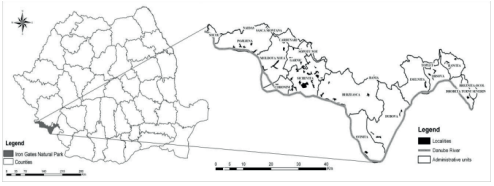


Figure 3. Location of the Iron Gates National Park (After Cucu et al., 2013)

The Iron Gates Natural Park is a transboundary Ramsar site bordering the Republic of Serbia along the course of the Danube River (Cucu et al., 2013) and it can be considered one of the regions with the highest diversity of species and habitats in Romania. *T. hungarica* is one of the many endemic species present on the territory of this natural park (Cucu et al., 2013, Clius & Patroescu, 2014; RAMSAR, 2025).

The Djerdap National Park is the largest in Serbia and covers a total area of 63,786 ha. In this border area, approximately 100 km from the entry point into Romania, the Danube forms the Iron Gates Gorge, the deepest (170 m) and most unique in Europe. This area is the habitat of 1,100 plant species (some unique in the world, such as *T. hungarica*) (Popescu et al., 2022; RAMSAR, 2025).

Pressures and threats

The beauty of the species attracted private collectors (gardeners and crops) thus the main threats is the wild specimen collection mainly because this species is rare in the wild due to its limited distribution (Dihoru & Negrean, 2009; Cucu et al., 2013). Local threats resulting from the Absolute Threats Classification System are: the presence of invasive species, inadequate forest management, some changes in agricultural practices and for some species the collection of plants either for commercial use or for gardening (Cucu et al., 2013). The limiting factors are determined by the small number of individuals, intensive collection and climate changes, which is why it is essential to protect this species and avoid its collection (Mihăilescu et al., 2015). Flowers, leaves and roots of *T. hungarica* individuals can be affected by the parasite *Vankya heufleri* (PPE, 2025).

Blooming beautifully in early April until early May, the sites where the species is present can be visited using boats on the Danube River. Fortunately, the tulips are protected by a steep wall 15 to 20 meters high, so most tourists are content to admire the tulips from a boat and also enjoy the legend that tells the story of these unique flowers (Agerpres, 2016). Because the area is very steep, the plant cannot be picked by flower lovers.

It has been found that floods have caused the extinction of the species in Serbia. Of the 15 plant species that are part of the "Red Book of the Flora of Serbia", three taxa have disappeared from the area of Đerdap National Park (*Veronica bachofenii, Crocus banaticus* and *T. hungarica*). These 3 species were lost due to the flooding of their habitats during the construction of a hydroelectric power plant (Iron Gate I Hydroelectric Power Plant in 1972) (Flora, 2020).

Other threats are: presence of invasive and problematic species, pollution from mixed sources, geological natural events (snow avalanches, land collapse, landslide, floodings, fire) excluding catastrophes and processes induced by human activities or climatic changes), climatic changes (extreme temperatures, long droughts, increase in precipitation flow, changes in location, range and/or habitat quality, desynchronization of biological/ecological processes, etc.).

Conservation measures

In the case of *T. hungarica*, we recommend no intervention as the main management action, monitoring populations and conserving seeds in gene banks. It can also be cultivated in Botanical Gardens (Dihoru & Negrean, 2009). Another possible method of protecting the species *T. hungarica* is the propagation and conservation of plants using *in vitro* cultures. The technique of plant propagation through the culture of anthers and microspores is called *in vitro* androgenesis. This method could be the only way to micro-propagate this rare species, as few plants remain in the wild. In order to induce androgenesis, a few investigations on pollen characters have been made (Calic et al., 2012).

The management plan states that the long-term viability of the species is ensured (PM-PNPF, 2014). The high conservation value of this habitat requires its protection and careful monitoring to minimize damage caused by the evaluator/monitor.

CONCLUSIONS

T. hungarica Borb (Liliaceae) is endemic and endangered plant of the Flora of Europe. The taxonomic status of this species is still rising many taxonomic questions, therefore new techniques should be used for clarifying the taxonomic status, new studies are needed to deepen the morphological and molecular differences of the varieties, subspecies and species associated with *T. hungarica*.

The conservation status of *T. hungarica* species is favourable, and the active conservation measure proposed is species monitoring. The population density and distribution range are stable over two European Commission reporting periods.

The main pressure on the species refers to collection activities, but the intensity of this pressure is low, probably due to the difficult access of people to the area where the species is found.

We propose that micro-propagated plants should be used in gardening and commercial purposes and thus diminishing the collecting pressure on the wild species and their habitats.

ACKNOWLEDGEMENTS

This work was carried out within the projects: RO1567-IBB01/2024 and RO1567-IBB04/2024 within the Department of Taxonomy, Ecology and Nature Conservation of the Bucharest Institute of Biology of the Romanian Academy.

REFERENCES

Bonifaciu, S. (1985). *Romania, Tourist Guide*. 479 p. București. Editura Sport-Turism.

Booy, G. & Van Raamsdonk, L.W. (1998). Variation in the enzyme esterase within and between Tulipa species; usefulness for the analysis of genetic relationships at different taxonomical levels. *Biochemical systematics and ecology*, *26*(2), 199-224.

Ćalić, D., Devrnja, N., Milojević, J., Zdravković-Korać, S., Tubić, L., Djuričković, M.S., & Vinterhalter, B. (2012). Pollen morphology and variability of *Tulipa hungarica* Borb. *African Journal of Biotechnology*, *11*(3), 616-620.

Christenhusz, M. J., Govaerts, R., David, J.C., Hall, T., Borland, K., Roberts, P.S. & Fay, M.F. (2013). Tiptoe through the tulips–cultural history, molecular phylogenetics and classification of *Tulipa* (Liliaceae). *Botanical journal of the Linnean Society*, *172*(3), 280-328.

Ciocârlan, V. (2009). *Flora Ilustrată a României: Pteriodophyta et Spermatophyta*. 1141 p. Editura Ceres.

Clius, M. & Patroescu, M. (2014). An evaluation matrix for ecotourism potential in certain categories of protected areas in Romania, case studies: national parc, nature parc, geopark. *Proceedings of the 14th International Multidisciplinary Scientific Geoconference and EXPO, Albena, Bulgaria*, 17-26.

Coste, I., & Faur, A. (1970). *Cercetări asupra speciei Tulipa hungarica Borb. În România.* Ocrotirea naturii, (2), 203-208.

Cucu, L. A., Niculae, M. I., & Pătroescu, M. (2013). Hierarchical analysis of the threats for species of Community Interest in the Iron Gates Natural Park, Romania. In *Forum geographic*, 12(1): 52-58.

Dihoru, G. & Negrean, G. (2009). *Cartea roşie a plantelor vasculare din România*. Bucureşti, Editura Academiei Române.

Goriup, P. (2008). Natura 2000 in Romania. Species fact list. *Manuscript for the Ministry of Environment and Sustainable Development, Bucureşti https://www.met. hu/eghajlat/magyarorszag_eghajlata/eghajlati_adatsorok/Pecs/leirasok/s zamitasok*.

Harrison, L. (2012). *Latin for gardeners: Over 3,000 plant names explained and explored*. University of Chicago Press.

HD - Habitat Directive, 1992, Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora, https://environment.ec.europa.eu/topics/nature-and-biodiversity/habitats-directive_en

Hurdu, B.I., Puşcaş, M., Turtureanu, P.D., Niketić, M., Vonica, G. & Coldea, G. (2012). A critical evaluation of the Carpathian endemic plant taxa list from the Romanian Carpathians. *Contributii Botanice*, *47*: 39-47.

Mihăilescu, S., Anastasiu, P., Popescu, A., Alexiu, A.F., Negrean, G.A., Bodescu, F., Manole (Aiftimie), A., Ion, R.G., Goia, I.G., Holobiuc, I., Vicol, I., Neblea, M.A., Dobrescu, C., Mogîldea, D.E., Sanda, V., Biţă-Nicolae, C.D., Comănescu, P. (2015b). *Ghidul de monitorizare a speciilor de plante de interes comunitar din România*, Editura Dobrogea, 120p (ISBN: 978-606-565-079-4).

Mihăilescu, S., Strat, D., Cristea, I., Honciuc, V. (2015a). *Raportul sintetic privind starea de conservare a speciilor şi habitatelor de interes comunitar din România*, Editura Dobrogea, 280p (ISBN: 978-606-565-088-6).

Millaku, F. A. D. I. L., Elezaj, I., & Berisha, N. A. I. M. (2018). Sympatric area and ecology of some Tulipa species in the West Balkan Peninsula. *Thaiszia Journal of Botany*, 28(1), 35-47.

Oltean M. (1994). *Lista rosie a plantelor superioare din România*. Studii Sinteze Documentatii Ecologice, 1: 1-52.

Palin M. (2013). *Serbia.* Editura Bradt Travel Guides.

Papp, B., Erzberger, P., & Sabovljević, M. (2006). Contribution to the bryophyte flora of the Djerdap National Park (E Serbia). *Studia botanica hungarica*, *37*, 131-144.

Popescu, F., Trumić, M., Cioabla, A. E., Vujić, B., Stoica, V., Trumić, M., & Trif-Tordai, G. (2022). Analysis of surface water quality and sediments content on Danube Basin in Djerdap-Iron Gate protected areas. *Water*, *14*(19), 2991.

Sabovljević, M. (2006). Contribution to the bryophyte flora of the Djerdap National Park (E Serbia). *Phytologia balcanica*, *12*(1), 51-54.

Sârbu, I., Ştefan, N., & Oprea, A. (2013). *Plante vasculare din România: determinator ilustrat de teren*. Bucureşti. Editura Victor B Victor. 1320 p.

Schneider-Binder, E. (2014). Phytogeographical importance of the mountains along the Danube mountain gap valley and surrounding area. *Transylvanian Review of Systematical and Ecological Research*, *16*(3), 11-28.

Segal, S., & Alen, K. (2020). *Dutch and Flemish flower pieces (2 vols in case): paintings, drawings and prints up to the nineteenth century*. Editura Brill.

Turrill, W. B. (1929). *The plant-life of the Balkan Peninsula: a phytogeographical study* (Vol. 1). Clarendon Press.

Wilson B. (2023). *Tulipa: the taxonomy and evolutionary history of the genus and its impact on conservation priorities in Central Asia* (Doctoral dissertation). 258 p.

Zonneveld, B. J. (2009). The systematic value of nuclear genome size for "all" species of *Tulipa* L. (Liliaceae). *Plant Systematics and Evolution*, *281*(1), 217-245.

Agerpres. (2016). https://www.mmediu.ro/articol/in-cazanele-dunarii-a-inflorit-laleaua-galbena-unica-in-lume/1507 (accessed 20.01.2025).

CNIPTMN - Centrul Național de Informare și Promovare Turistică Moldova Nouă (https://www.cniptmoldovanoua.ro/en/the-boiler-tulip-tulipa-hungarica/) (accessed on 05.03.2025*).*

EC (2021) - 'EU biodiversity strategy to 2020', European Commission (https://ec.europa.eu/environment/nature/biodiversity/strategy_2020/index_en.htm) (accessed on 01 February 2025).

EEA, 2025a- https://www.eea.europa.eu/en/analysis/indicators/conservation-status-of-species-under (accessed January 15, 2025)

EEA, 2025b, *Tulipa hungarica*, Species of community interest listed in the Habitat Directive 92/43 EEC under annex II and under annex IV, https://www.eea.europa.eu/help/glossary/european-species-listed-under-article/tulipa-hungarica (accessed 1 March 2025)

Egreta, 2017, https://complex-egreta.ro/2017/02/01/laleaua-galbena-de-cazane/ (accessed 1 March 2025)

EIONET, 2025, Reporting under Article 17 of the Habitats Directive, https://www.eionet.europa.eu/etcs/etc-be/activities/reporting/article-17 (accessed 20.01.2025)

IUCN, 2025, The IUCN red list of Threatened species, https://www.iucnredlist.org/species/162072/196620267 (accessed on 01 February 2025).

ORDIN nr. 3351 din 28 decembrie 2023 pentru aprobarea Ghidului privind protocoalele și metodologiile unitare de monitorizare a stării de conservare a speciilor de interes comunitar, din cadrul proiectului "Completarea nivelului de cunoaștere a biodiversității prin implementarea sistemului de monitorizare a stării de conservare a

speciilor și habitatelor de interes comunitar din România și raportarea în baza articolului 17 al Directivei Habitate 92/43/CEE", finanțat prin Programul operațional Infrastructura mare 2014-2020.
ORDONANȚĂ DE URGENȚĂ nr. 57 din 20 iunie 2007 privind regimul ariilor naturale protejate, conservarea habitatelor naturale, a florei și faunei sălbatice.
PM-PNPF (2014) - https://www.pnportiledefier.ro/management.html (accessed on 05.03.2025).
PPE (2025). - (https://bladmineerders.nl/host-plants/plantae/spermatopsida/angiosperma/monocots/liliales/liliaceae/tulipa/tulipa-hungarica/) (accessed on 01 February 2025).
RAMSAR (2025) - https://www.ramsar.org/sites/default/files/documents/library/key_ris_e.pdf (accessed on 01 February 2025).
RHS, 2025- https://www.rhs.org.uk/plants/ 256070/ tulipa-hungarica/details (accessed 20.01.2025).
Stevanović, V. (2011). *Tulipa hungarica* (errata version published in 2021). *The IUCN Red List of Threatened Species* 2011: e.T162072A196620267. https://dx.doi.org/10.2305/IUCN.UK.2011-1.RLTS.T162072A196620267.en (accessed on 01 February 2025).
USDA - *Tulipa hungarica*, United States Department of Agriculture. Agricultural Research Service, https://npgsweb.ars-grin.gov/gringlobal/taxon/taxonomydetail?id=40738 (accessed 25 February 2025)
WFO - https://www.worldfloraonline.org/taxon/wfo-0000770355 (accessed 20.01.2025).
Wilson, B. (2021). *Welcome to the Balkans! IUCN SSC Wild Tulip Specialist Group*, https://tulipconservation.com/2021/04/02/welcome-to-the-balkans/ (accessed on 05.03.2025).
Flora, 2020, https://npdjerdap.rs/en/flora/ (accessed in 25 January 2025).

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

INFLUENCE OF SOME FERTILIZERS ON THE GROWTH AND FLOWERING OF *CROSSANDRA INFUNDIBULIFORMIS* (L.) NEES PLANTS

Carmen NICU, Manuela MANDA

University of Craiova, Faculty of Horticulture,
13 Al. I. Cuza Street, Craiova, Romania

Corresponding author email: manda_manu@yahoo.com

Abstract

Crossandra infundibuliformis (L.) Nees is a highly valued ornamental perennial species, with attractive orange flowers and dark green leaves, cultivated in temperate regions as an indoor plant. The objective of this study was to evaluate the influence of three fertilizers, in concentration of 0.2% (Bionat Plus, Green Feed and Cropmax), on vegetative growth and flowering of plants. The results indicated that the foliar application of these fertilizers caused a significant increase in most of the studied morphological parameters, compared to the control plants. The highest values of plant height (40.35 cm), number of shoots per plant (7.81), leaf length (8.78 cm), leaf width (4.63 cm), number of flowers per spike (18.93), and spike length (8.62 cm) were recorded in plants sprayed with Bionat Plus. Plants fertilization with Cropmax increased the number of spikes per plant, and also promoted the earlier flowering, recording the lowest number of days from planting to flowering (81.33 days).

Key words*: Crossandra, foliar fertilization, growth, flowering.*

INTRODUCTION

Crossandra infundibuliformis (L.) Nees is a beautiful and popular perennial species in the Acanthaceae family. It is also known as the firecracker plant, which grows as a subshrub up to 1 m tall. The leaves are simple, ovate to lanceolate, opposite, glossy, 3-12 cm long and deep-green in colour, with wavy edges. The attractive, delicate fan-shaped flowers, with five asymmetrical petals, in various shades of orange, yellow, red or pink, depending on the cultivar, are arranged in dense terminal spike inflorescences, consisting of prominent, hairy, overlapping, ovate-oblong, green bracts. The flowers are unscented and begin to open gradually from the base of the spike. The plants typically bloom from spring to autumn, but in warm, tropical climates, the flowering occurs abundantly throughout the year.

Crossandra prefers light, fertile soils, rich in organic matter, moist, well-drained, with a pH ranging from 5.8 to 6.5, in full sun to partial shade.

It requires optimal light and temperatures conditions, such as 4-6 hours of direct sunlight daily and between 20-30°C, for vigorous vegetative growth and blooming. The plants are sensitive to low temperature and frost, and do not tolerate lack of humidity.

This species is propagated through seeds in spring, and vegetatively by stem or shoot tip cuttings of 8-10 cm length, during early spring or late summer. For successful rooting, it is recommended to treat the cuttings with some rooting hormones. Rehana et al. (2020; 2023) obtained the best results, in terms of rooting percentage, root and shoot formation, and subsequently for various growth and flowering parameters, in Crossandra var. 'Arka Shravya', using terminal cuttings treated with 3000 ppm IBA, in quick dip method.

Crossandra can also be multiplied through micropropagation, from axillary and apical buds (Girija et al., 1999), callus and shoot tips cultures (Hewawasam et al., 2001; 2004), nodal segments (Kalaivani et al., 2019). The performance of different genotypes cultivated in various regions exhibits variability depending on the climatic conditions, growth habitat and genetic potential (Gowsika et al., 2019). Therefore, it is important to evaluate and estimate the magnitude of variation among the different genotypes concerning the morphological and yield traits, to develop new cultivars of Crossandra (Bhosale et al., 2018;

Dhivya & Balakirshnan, 2019; Vinodh & Kannan, 2020; Hosagoudar et al., 2022; Srinivasulu et al., 2023).

Adequate fertilisation is essential for promoting optimal growth and flowering of the plants, and as well as for achieving maximum yield and quality. Therefore, to maintain vigorous plants with healthy foliage and to stimulate blooming, it is necessary to identify the nutrient deficiencies, which can cause various problems affecting the plant growth and development. Nitrogen, phosphorus and potassium are the main macronutrients that plants require throughout their growth cycle. Micronutrients are also essential for plant growth and flowering, and for various physiological processes, but in smaller quantities. To choosing the right fertilizers with micronutrients will take into account the plants requirement, soil conditions and specific nutrient deficiencies identified. The foliar application of macronutrients and different micronutrient combinations has shown positive effect on plant growth, yield and quality of flowers in gladiolus (Fahad et al., 2014), dahlia (Kashif et al., 2014), tuberose (Patel et al., 2017), and rose (Hafiz et al., 2020).

Integrated nutrient management plays an important role in maximizing the biological potential for improving floricultural crop productivity, by using adequate amounts of fertilizers in balanced proportions (Kumar & Chaudhary, 2018). The optimum quantity and nutrient application method should be determined based on soil fertility status and local conditions (Niu et al., 2021).

Numerous researches have been conducted on the nutrition of crossandra plants with the aim to investigate the effects of different levels of organic and chemical fertilizers (Aruna et al., 2007; Gowthami et al., 2018; Priyanka et al., 2018; Gowtham & Karuppaiah, 2024), as well as biofertilizers (Vaingankar & Rodrigues, 2015; Vithu et al., 2018; Bhavanisanker & Vanangamudi, 1999) on growth, flowering, yield, and quality attributes. The results showed that various types of fertilizers, applied both as foliar sprays and on soil significantly improved all these studied parameters.

Crossandra infundibuliformis is a popular ornamental plant appreciated for its bright orange flowers with an interesting shape, as well as for the glossy dark green foliage. This plant is grown outdoors in the tropical climates, in parks and gardens, and it is used in flowerbeds, borders, mass plantings, and in containers on patios. Additionally, it can be cultivated in pots as an indoor plant. The appearance and architecture of the plant have an important role at the plants cultivated in pots. Crossandra has an irregular growth habit, therefore to produce dwarf and marketable potted plants, various cultural techniques are required for a compact growth such as pinching (Bharathi et al., 2018) and/or application of plant growth regulators (Sundar et al., 2008; Beese & Sarvanan, 2020; Sreekala et al., 2000; Meghana et al., 2022, 2023). In India, the loose flowers of crossandra, either alone or in combination with jasmine, marigold and scented rose flowers are commonly used in garlands, and as hair adornments (Naveena & Thamaraiselvi, 2020; Remya et al., 2022).

Many ornamental species including crossandra have medicinal properties and can be used in complementary and alternative medicine (Mangroliya et al., 2022). The plant contains various bioactive compounds such as alkaloids, flavonoids, phenolic acids, glycosides, tannins, saponins, carbohydrates, terpenoids, minerals and other nutrients with therapeutic benefits (Sumalatha, 2012; Sangekar & Devarkar, 2017; Selvakumar, 2015; Selvakumar et al., 2016; Vasantha et al., 2022; Krupa & Hegde, 2024; Sajeena et al., 2024). It has been used in traditional medicine in India and many other countries around the world for the treatment of several diseases (Hari Vishva et al., 2022; Chavhan et al., 2023). Numerous studies have shown that the ethanolic and methanolic extracts of different parts of the plant (leaves, flowers and roots) presented significant pharmacological activities, such as antioxidant (Sharmila & Gomathi, 2011; Patil et al., 2014), antibacterial and antifungal (Elamathi et al., 2011; Madhumitha & Saral, 2011; Pulipati et al., 2013; Kumar et al., 2018), anti-inflammatory (Dhamija et. al, 2015), anti-arthritic (Sanakattula et al., 2019), antidiabetic, anthelmintic and anticancer (Vadivel & Panwal, 2016; Vasantha et al., 2023; Remya et al., 2023) activity.

Silver nanoparticles biosynthesized using aqueous flower extracts have demonstrated

anticancer properties (Manivasagan et al., 2019). The objective of this study was to investigate the influence of three fertilizers foliar application, on the growth and flowering of *Crossandra infundibuliformis*, to produce potted plants with high aesthetic value.

MATERIALS AND METHODS

The research on the influence of foliar application of fertilizers on vegetative growth and flowering of crossandra potted plants was carried out in the Floriculture Research Area, Faculty of Horticulture, University of Craiova, under greenhouse conditions. The biological material used consisted of rooted shoot tip cuttings, that were transplanted into 14 cm diameter plastic pots (1 plant/pot), filled with a mixture of peat and perlite (3:1), in May 2023. The cuttings were pinched after planting in pots to stimulate branching and obtain more compact plants. Three fertilizers were applied foliarly, bimonthly, until the flowering stage. The experimental variants were: T_1 (untreated control plants), T_2 (Green Feed 0.2%), T_3 (Bionat Plus 0.2%) and T_4 (Cropmax 0.2%). The foliar applications were carried out early in the morning, for maximum absorption of nutrients by the plants. The experiment was laid out in a randomized complete block design with three replications and four treatments.

The observations and measurements on the growth and flowering characteristics of the plants, such as plant height (cm), number of branches per plant, length and width of the leaves (cm), spike length (cm), number of spikes per plant, number of flowers per spike, and the number of days from planting to flowering were recorded. The obtained data were statistically analysed using one-way analysis of variance, and differences between treatments applied were compared by Duncan's multiple range test at the 5% probability level.

RESULTS AND DISCUSSIONS

Vegetative growth parameters

Foliar fertilizers have an important role in improving the quality, yield and metabolism of plants. The efficiency of foliar application of nutrients is influenced by many factors such as physico-chemical properties of spray solutions, environmental conditions (temperature, light, humidity, soil nutrition), the physiological status and the characteristics of the plants (Fernández et al., 2013). Foliar fertilization has the advantage as nutrients can be applied in controlled amounts and at specific growth stages, being absorbed through the leaves. It serves as a complementary fertilization strategy to enhance nutrient efficiency, minimize negative soil impacts, and correct nutritional deficiencies (Bindraban et al., 2015; Niu et al., 2021).

The results presented in Table 1 revealed that treatments with foliar fertilizers increased all vegetative growth parameters of crossandra plants. Statistical analysis of the data indicated a significant effect of fertilizers applied on plant height. The highest value was recorded in plants treated with Bionat Plus (40.82 cm), followed by Cropmax (38.53 cm), compared to the control variant, which had the lowest plant height value (29.73 cm). There were no significant differences between the treatments, for the other vegetative parameters analyzed. The maximum values for the number of branches per plant (7.81), leaf length (8.78 cm) and leaf width (4.63 cm) were recorded in plants sprayed with Bionat Plus 0.2%, while the minimum values of these growth features were observed in untreated plants.

Karuppaiah (2019) revealed that foliar spray of ferrous sulfate 0.5%, individually or in combination with bioregulators, significantly influenced the growth attributes in *Crossandra infundibuliformis* plants. Biju et al. (2020) reported that all vegetative and yield characteristics of crossandra plants recorded the highest values, compared to the control, by foliar application of $MnSO_4$ (0.5%) and $FeSO_4$ (0.5%) micronutrient mixture. Similarly, Lenin et al. (2021) studied the effect of micronutrients on the growth of crossandra cv. 'Delhi' and obtained the best results in plants sprayed with $ZnSO_4$ (0.5%) + $FeSO_4$ (0.5%).

Foliar fertilization is used to improve the visual quality and productivity of many ornamental plants. El-Nashar & El-Tony (2020) reported a significant increase in various vegetative growth characteristics, such as plant height, number of branches and leaves per plant, and stem diameter, as well as in flowering parameters in carnation plants grown in pots

and sprayed with chemical fertilizer solutions at the level of 4 $g \cdot L^{-1}$. Sharaf & El-Naggar (2003) evaluated the response of carnation plants to phosphorus and boron foliar fertilization under greenhouse conditions and concluded that the best growth and flowering results were achieved through foliar application of 200 mg/L^{-1} P_2O_5 in combination with 50 mg/L^{-1} B. The foliar application of the inorganic fertilizer Growmore on Syngonium plants at the concentration of 2.0 ml/L, significantly increased all growth parameters, pigment content, and nitrogen, phosphorus, and potassium ions content in the leaves (El-Quesni et al., 2010). Artur et al. (2022) showed that nitrogen foliar fertilization at a concentration of 20 $g \cdot L^{-1}$ applied twice a month, increased the height and diameter of *Anthurium maricense* plants grown in pots.

Table 1. The effect of foliar application of fertilizers on the growth of *Crossandra infundibuliformis* plants

Treatment	Plant height (cm)	Number of branches/ plant	Leaf length (cm)	Leaf width (cm)
T_1	29.73 c	5.66 a	6.93 a	3.85 a
T_2	32.61 bc	6.24 a	7.56 a	4.12 a
T_3	40.35 a	7.81 a	8.78 a	4.63 a
T_4	37.86 ab	6.13 a	8.61 a	4.41 a

Means with same letter(s) in a column are statistically non-significant at the 5% significance level according to Duncan's multiple range test.

Flowering parameters

The influence of foliar fertilizers on the number of spikes per plant, spike length, number of flowers per spike and the number of days to flowering is presented in Table 2. Foliar fertilization had a positive effect on the quality indicators of crossandra plants. The results indicated significant differences among the treatments, regarding the number of spikes per plant. The maximum value (8.27) was observed in T_4 (Cropmax 0.2%), while the minimum value (4.13) was recorded in the control plants. Application of foliar nutrients significantly increased the spike length for T_3 (8.62 cm), followed by T_2 (7.83 cm). The lowest average value of this parameter (6.47 cm) was observed in T_1 (untreated plants). The number of flowers per spike was also increased as a result of the foliar nutrition and ranged from 13.66 in control plants to 18.93 in plants that were treated with Bionat Plus 0.2%.

The plants sprayed with foliar fertilizers had an earlier flowering compared to the control plants. The analysis of the obtained data showed significant differences for the number of days from the planting in pots to flowering, in the treatment with Cropmax 0.2%, where the minimum value (81.33 days) was recorded.

Table 2. The effect of foliar application of fertilizers on flowering of *Crossandra infundibuliformis* plants

Treatment	Number of days to flowering	Number of spikes per plant	Spike length (cm)	Number of flowers per spike
T_1	92.16 b	4.13 c	6.47 b	13.66 b
T_2	89.65 b	6.25 b	7.83 a	17.51 a
T_3	87.42 ab	6.31 b	8.62 a	18.93 a
T_4	81.33 a	8.27 a	7.56 ab	14.25 b

Means with same letter(s) in a column are statistically non-significant at the 5% significance level according to Duncan's multiple range test.

CONCLUSIONS

The results indicated that the used foliar fertilizers improved various growth and flowering parameters of crossandra potted plants. Foliar application of Bionat Plus 0.2%, significantly increased the plant height, spike length and the number of flowers per spike, compared to the control variant. The plants sprayed with Cropmax 0.2% had the highest number of spikes per plant and bloomed earlier.

REFERENCES

Artur, A.G., Sousa Teixeira, D.B., Silva Martins, T., Taniguchi, C.A.K., Ribeiro Castro, A.C. (2022). Fertilization for potted foliage anthurium. *Journal of Plant Nutrition*, 45(9), 1370-1377.

Aruna, P., Rajangam, J., Geetha Rani, P., Manivannan, M.I. (2007). Nutrient studies in crossandra (*Crossandra infundibuliformis* L. Nees). *The Asian Journal of Horticulture*, 2(2), 169-177.

Beese, S., Sarvanan, S.S. (2020). Studies on effect of plant growth regulators on plant growth and flower yield of crossandra (*Crossandra infundibuliformis* L.) var. Arka Ambara. *International Journal of Chemical Studies*, 8(6), 1560-1567.

Bharathi, T.U., Manjunatha Rao T., Aswath, C. (2018). Assessing the suitability of crossandra (*Crossandra infundibuliformis* (L.) Nees) as potted plant.

International Journal of Current Microbiology and Applied Sciences, 7(12), 1028-1035.

Bhavanisanker, K., Vanangamudi, K. (1999). Integrated nutrient management in crossandra (*Crossandra infundibuliformis* L.). *South Indian Horticulture*, 47(1/6), 125-129.

Bhosale, P.B., Kadam, M.B., Katwate, S.M., Pawar, B.G. (2018). Evaluation of different genotypes of crossandra. *International Journal of Applied Research,* 4(3), 204-206.

Bindraban, P.S., Dimkpa, C., Nagarajan, L., Roy, A., Rabbinge, R (2015). Revisiting fertilisers and fertilisation strategies for improved nutrient uptake by plants. *Biology and Fertility of Soils*, 51(8), 897-911.

Biju, A., Bargavi, G., Bhavani, K., Chinjumol, K., Divyadharshini, V., Indu Rani, C., Jeeva, S., Masilamani, P. (2020). Effect of micronutrients on growth and flower yield in Delhi crossandra (*Crossandra infundibuliformis* (L.) Nees). *Multilogic in Science*, X (XXXIV), 812-814.

Chavhan, P., Shahare, P.D., Sheikh, M.T., Kalambe, P.S., Nakhate, Y.D. (2023). *Crossandra infundibuliformis*: A review study on ethnobotany, phytochemical investigation and pharmacology. *International Journal of Creative Research Thoughts*, 11(5), 564-574.

Dhamija, I., Suhag, A., Kumar, S., Chaudhary, H. (2015). Evaluation of anti-inflammatory activity of herbal methanolic floral extract of *Crossandra infundibuliformis* (Linn.) Nees. *International Journal of Biology, Pharmacy and Allied Sciences*, 4(4), 2058-2068.

Dhivya, M., Balakirshnan, S. (2019). Genetic variability and heritability studies in *Crossandra infundibuliformis* (L.) Nees. *Plant Cell Biotechnology and Molecular Biology*, 20(23-24), 1434-1438.

Elamathi, R., Deepa, T., Kavitha, R., Kamalakannan, P., Sridhar, S., Suresh Kumar, J. (2011). Phytochemical screening and antimicrobial activity of leaf extracts of *Crossandra infundibuliformis* (L.) Nees on common bacterial and fungal pathogens. *International Journal of Current Science*, 1(3), 72-77.

El-Nashar, Y.I., El-Tony, F.E.H. (2020). Response of carnation (*Dianthus chinensis* x *barbatus*) to different levels of foliar spraying of amino acid and chemical fertilizers. *Acta Scientific Agriculture*, 4(7), 102-113.

El-Quesni, F.E.M., Mahgoub, M. H., Kandil, M.M. (2010). Impact of foliar spray of inorganic fertilizer and bioregulator on vegetative growth and chemical composition of *Syngonium podophyllum* L. plant at Nubaria. *Journal of American Science*, 6(8), 288-294.

Fahad, S., Ahmad, M.Kh., Anjum, M.A., Hussain S. (2014). The effect of micronutrients (B, Zn and Fe) foliar application on the growth, flowering and corm production of gladiolus (*Gladiolus grandiflorus* L.) in calcareous soils. *Journal of Agricultural Science and Technology*, 16(7), 1671-1682.

Fernández, V., Sotiropoulos, T., Brown, P.H. (2013). Foliar Fertilisation: Principles and Practices. Paris: International Fertilizer Industry Association (IFA).

Girija, S., Ganapathi, A., Vengadesan, G. (1999). Micropropagation of *Crossandra infundibuliformis* (L.) Nees. *Scientia Horticulturae,* 82(3-4), 331-337.

Gowsika, S., Kavitha, M., Sankari, A., Vetrivelkalai, P. (2019). Evaluation of Crossandra (*Crossandra infundibuliformis* (L.) Nees.) genotypes for morphological and yield traits. *International Journal of Chemical Studies*, 7(3), 3394-3396.

Gowtham, S., Karuppaiah, P. (2024). Effect of different levels of nitrogen in combination with nano urea on growth and yield of crossandra (*Crossandra infundibuliformis* L.) cv. Lakshmi. *Annals of Plant and Soil Research,* 26(2), 250-255.

Gowthami, L., Nageswararao, M.B., Umajyothi, K., Umakrishna, K. (2018). Influence of nitrogen and potassium on spike characters of crossandra (*Crossandra infundibuliformis* L.). *International Journal of Chemical Studies*, 6(5), 3223-3224.

Hafiz, M.B., Rohoma, T., Muhammad, A., Syed, M.A., Haseeb, I., Muhammad, S.U., Farman, A.M., Rai, I.A., Muhammad, I. (2020). Does foliar application of macro and micronutrients have any impact on roses production? *Annals of Reviews and Research*, 6(1), 1-5.

Hari Vishva, P., Abdul Azim, A., Vanitha, M., Ganesan, S., Gokulnath, K. (2022). Pharmacognostical studies and pharmacological activities of *Crossandra infundibuliformis*. *International Journal of Research and Development in Pharmacy & Life Sciences*, 8(5), 140.

Hewawasam, W.D.C.J., Bandara, D.C., Abeyarathne, W.M. (2001). In-vitro propagation of *Crossandra infundibuliformis* var. Danica through shoot tip and callus culture. *Tropical Agricultural Research*, 13, 328-340.

Hewawasam, W.D.C.J., Bandara, D.C., Abeyarathne, W.M. (2004). New phenotypes of *Crossandra infundibuliformis* var. Danica through *in vitro* culture and induced mutations. *Tropical Agriculture Research*, 16, 253-270.

Hosagoudar, A.S., Champa, B.V., Naveen, P.M. (2022). Evaluation of crossandra genotypes for vegetative, floral and yield parameters for commercial production. *The Pharma Innovation Journal*, 11(6), 2554-2557.

Kalaivani, K., Rajadurai, K.R., Hemaprabha, K., Kannan, M. (2019). *In vitro* propagation of crossandra (*Crossandra infundibuliformis* (L.) Nees.) var. Arka Shravya and Pondicherry local through direct organogenesis. *International Journal of Chemical Studies*, 7(2), 1606-1609.

Karuppaiah, P. (2019). Effect of bioregulators and FeSO4 on growth, yield and quality of crossandra (*Crossandra infundibuliformis* L.). *Horticulture International Journal*, 3(1), 1-5.

Kashif, M., Rizwan, K., Khan, M.A., Younis, A. (2014). Efficacy of macro and micro-nutrients as foliar application on growth and yield of *Dahlia hybrida* L. (Fresco). *International Journal of Chemical and Biochemical Sciences*, 5, 6-10.

Krupa, N., Hegde, K. (2024). Integrating pharmacological insights, pharmacognostical applications, and phytochemical analysis of

Crossandra infundibuliformis: A comprehensive review. *Journal of Emerging Technologies and Innovative Research*, 11(8), 638-643.

Kumar, M., Chaudhary, V. (2018). Effect of integrated sources of nutrients on growth, flowering, yield and soil quality of floricultural crops: A review. *International Journal of Current Microbiology and Applied Sciences,* 7(3), 2373-2404.

Kumar, G.S., Reddy, G., Mujahid, S.K, Ashwini, T., Chary, V.M. (2018). Extraction, phytochemical studies and *in vitro* screening of the leaves and flowers of *Crossandra infundibuliformis* against Mycobacterium tuberculosis. *Asian Journal of Research in Pharmaceutical Sciences*, 8(4), 247-252.

Lenin, A.R., Rizwana Begum, Z., Kalaiselvan, S., Kannan, R. (2021). Effect of micronutrients on growth of Crossandra (*Crossandra infundibuliformis* L.) cv. Delhi. *International Journal of Botany Studies*, 6(6), 68-71.

Madhumitha, G., Saral, A.M. (2011). Preliminary phytochemical analysis, antibacterial, antifungal and anticandidal activities of successive extracts of *Crossandra infundibuliformis*. *Asian Pacific Journal of Tropical Medicine*, 4(3), 192-195.

Mangroliya, R.M., Patel, J.J., Patel, N.A. (2022). Medicinal value of ornamental plants. *International Journal of Agriculture Sciences*, 14(8), 11600-11603.

Manivasagan, V., Bhavani, K., Swathy, R., Ramesh Babu, N.G. (2019). Biosynthesis of silver nanoparticles from *Crossandra infundibuliformis* and its anticancer activity. *International Journal for Research Trends and Innovation*, 4(2), 81-85.

Meghana, K., Raju, D.V.S., Dorajee Rao, A.V.D., Ravindra Babu, M., Subbramamma, P. (2022). Influence of growth retardants on growth and flowering of crossandra (*Crossandra infundibuliformis*) under potted conditions. *The Pharma Innovation Journal*, 11(8), 1302-1304.

Meghana, K., Raju, D.V.S., Dorajee Rao, A.V.D., Ravindra Babu, M., Subbramamma, P. (2023). The effect of growth inhibitors on the growth, roots, and flowering attributes of crossandra (*Crossandra infundibuliformis*) in pot. *Biological Forum - An International Journal*, 15(11), 36-38.

Naveena, N., Thamaraiselvi, S.P. (2020). Value addition in fresh flowers - An overview. *Biotica Research Today*, 2(8), 844-846.

Niu J., Liu C., Huang M., Liu K., Yan D. (2021). Effects of foliar fertilization: A review of current status and future perspectives. *Journal of Soil Science and Plant Nutrition*, 21, 104-118.

Patel, T.D., Viradia, R.R., Tejashwini, C.R., Patel, H.V., Patel, U.R. (2017). Studies on effect of foliar application of micronutrient (Fe & Zn) on growth, flowering quality and yield of tuberose (*Polianthes tuberosa* L.) CV. Prajwal. *International Journal of Chemical Studies*, 5(6), 93-97.

Patil, K.G., Jaishree, V., Tejaswi, H. (2014). Evaluation of phenolic content and antioxidant property of *Crossandra infundibuliformis* leaves extracts. *American Journal of Plant Sciences*, 5(9), 1133-1138.

Pulipati, S., Ghanta, V., Sai Prudhvi, D., Srinivasa Babu, P. (2013). Evaluation of phytochemical and antimicrobial properties of *Crossandra infundibuliformis* (L) Nees flower extract. *International Journal of Biological and Pharmaceutical Research*, 4(8), 588-591.

Priyanka, T.K.., Kamble, B.S., Subiya, R.K., Anuradha, R.W., Gasti, V.D. (2018). Interactive study of spacing and different levels of nutrients on flowering and yield attributes of crossandra (*Crossandra undulaefolia* Salisb.). *Journal of Pharmacognosy and Phytochemistry*, 7(1), 487-490.

Rehana, S.K., Madhavi, M., Dorajee Rao, A.V.D., Subbaramamma, P. (2020). Effect of cutting types and IBA treatments on success of vegetative propagation in crossandra (*Crossandra infundibuliformis* L.) var. Arka Shravya. *Journal of Pharmacognosy and Phytochemistry*, 9(2), 1469-1475.

Rehana, S., Madhavi, M., Dorajee Rao, A.V.D., Subbaramamma, P. (2023). Efficacy of IBA treatments on field performance of terminal and semi hardwood cuttings of crossandra (*Crossandra infundibuliformis*) var. Arka Shravya. *Journal of Eco-Friendly Agriculture*, 18(1), 93-101.

Remya, S.B., Rakesh Kumar Jat, Subash Chandran, M.P., Aparna, P. (2022). A review on cultivation, propagation and harvesting of *Crossandra infundibuliformis*. *International Journal of Research Publication and Reviews*, 3(4), 971-978.

Remya, S.B., Rakeshkumar, J., Anoopa, J.L. (2023). Exploring the phytochemical properties and anticancer activity ethanolic extract of *C. infundibuliformis*. *World Journal of Pharmaceutical and Medical Research*, 9(12), 72-81.

Sajeena, Ch., Mohammed Dilshad Kalliyath, Mohammed Nihad P., Mohammed Shabeeb, Mufeedha, K.P., Muhammed Salih, K.T., Celestin Baboo, R.V., Sirajudheen, M.K. (2024). A review on *Crossandra infundibuliformis*. *World Journal of Pharmaceutical Research*, 13(12), 547-559.

Sanakattula, S., Pavani Reddy, P., Rajitha Reddy, C., Sowjanya, A., Vinod Kumar, G. (2019). In vitro anti-arthritic activity of *Crossandra infundibuliformis* leaf extract. *World Journal of Pharmaceutical Research*, 8(6), 553-558.

Sangekar, S.N., Devarkar, V.D. (2017). Pharmacognostical studies in *Crossandra infundibuliformis* (L.) Nees. *Bioscience Discovery*, 8(3), 346-351.

Sharaf, A.I., El-Naggar, A.H. (2003). Response of carnation plant to phosphorus and boron foliar fertilization under greenhouse conditions. *Alexandria Journal of Agricultural Research*, 48(1), 147-158.

Selvakumar, S. (2015). Preliminary phytochemical analysis of aerial parts of *Crossandra infundibuliformis*. *Journal of Chemical and Pharmaceutical Research,* 7(12), 784-787.

Selvakumar, S., Gangatharan, S., Rao, M.R.K. (2016). Preliminary phytochemical screening of root extracts of *Crossandra infundibuliformis*. *Research Journal of Pharmacy and Technology*, 9(2), 131-134.

Sharmila, N., Gomathi, N. (2011). Antibacterial, antioxidant activity and phytochemical studies of *Crossandra infundibuliformis* leaf extracts. *International Journal of Phytomedicine*, 3(2), 151-156.

Sreekala, C., Mathew, K.L., George, T.S., Rajeevan, P.K. (2000). Growth and flowering of crossandra as influenced by paclobutrazol and cycocel (CCC). *Horticultural Journal,* 13(2), 65-70.

Srinivasulu, G.B., Mukund Shiragur, Shirol, A.M., Basavaraja, N., Gangadharappa, P.M., Pavankumar (2023). Genetic variability studies in crossandra (*Crossandra infundibuliformis*). *International Journal of Current Microbiology and Applied Sciences*, 12(09), 318-325.

Sumalatha, K. (2012). Pharmacognostical and phytochemical studies of *Crossandra infundibuliformis* L. (Acanthaceae) leaf. *International Journal of Phytopharmacy Research*, 3(1), 35-47.

Sundar, S.T.B., Sadasakthi, A., Kumar, G.A., Priya, M.V. (2008). Effect of growth regulators on growth and flowering in triploid crossandra (*Crossandra infundibuliformis*) cv. Delhi. *Research on Crops*, 9(2), 335-337.

Vadivel, E., Panwal. S.V. (2016). *In vitro* anticancer and insecticidal activity of *Crossandra infundibuliformis*. *Journal of Chemical and Pharmaceutical Research*, 8(5), 260-264.

Vadivel, E., Panwal, S.V. (2016). Antidiabetic and anthelmintic activity of *Crossandra infundibuliformis. International Journal of Pharmacy and Technology*, 8(3), 16508-16514.

Vaingankar, J.D., Rodrigues, B.F. (2015). Effect of arbuscular mycorrhizal (AM) inoculation on growth and flowering in *Crossandra infundibuliformis* (L.) Nees. *Journal of Plant Nutrition*, 38(10), 1478-1488.

Vasantha, G., Bhargavi, D., Yashodha, A., Sharmila Roy, N., Shaik, S.A. (2022). A plant review on *Crossandra infundibuliformis*. *ANVESAK*, 52(1), 375-383.

Vasantha, G., Sharmila Roy, N., Yashodha, A., Bhargavi, D., Shaik, S.A. (2023). Qualitative tests for preliminary phytochemical screening and antihelmintic activity of root extract of *Crossandra infundibuliformis*. *Journal of Advanced Zoology*, 44(3), 717-724.

Vinodh, S., Kannan, M. (2020). Variability studies in Crossandra (*Crossandra infundibuliformis*). *Journal of Pharmacognosy and Phytochemistry*, 9(1), 312-314.

Vithu, G.N., Varu, D.K., Patel, D., Aghera Sameer, R. (2018). Efficacy of biofertilizers to improve flower production: A review. *Bulletin of Environment, Pharmacology and Life Sciences,* 8(1), 9-17.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

THE INFLUENCE OF SUBSTRATE TYPE ON THE MORPHOLOGICAL AND ORNAMENTAL CHARACTERS OF SOME VARIETIES OF *IPOMOEA BATATAS* GROWN IN CONTAINERS

Alina-Ștefana OZARCHEVICI[1], Irina CARA[1], Gerard JITĂREANU[1], Bogdan-Ionel CIOROIU[2], Maria APOSTOL[1], Lucia DRAGHIA[1]

[1]"Ion Ionescu de la Brad" Iași University of Life Sciences,
3 Mihail Sadoveanu Alley, 700490, Iași, Romania
[2]Romanian Academy, Iași Branch, Research Centre for Oenology,
9H Mihail Sadoveanu Alley, Iași, Romania

Corresponding author email: lucia.draghia@iuls.ro

Abstract

In this study, three ornamental varieties of Ipomoea batatas (`Heart Bronze`, `Black`, `Heart Lime`) were studied to evaluate the influence of substrate type on some morpho-decorative characters. The plants were grown in 70 L containers in which four types of substrate were used: garden soil + peat, garden soil + peat + hydrogel, garden soil + peat + coconut fiber, garden soil + peat + coconut fiber + hydrogel. `Heart Bronze` was characterized by a richer vegetative mass (2-6 times more leaves than the other two varietys) and `Heart Lime` by longer branching. In all varieties, the substrate consisting of garden soil + peat favored stem branching, and in the varieties 'Heart Lime' and 'Heart Bronze' it favored the stem length growth. To increase the number of leaves/plant, the substrate of garden soil + peat + hydrogel can be recommended for all varieties, although good results were also obtained with the substrate containing coconut fiber. The results show that the presence of hydrogel, which helps in solubilization of nutrients and their uptake, ensured the formation of more leaves.

Key words*: sweet potato, ornamental varieties, morphological characters.*

INTRODUCTION

The *Ipomoea* L. genus, family *Convolvulaceae*, comprises numerous species, including sweetpotato (*Ipomoea batatas* (L.) Lam), a plant cultivated mainly in tropical regions of Latin America, Africa and Asia as a food crop (Zhang et al., 2009; Nimmakayala et al., 2011; Roullier et al., 2013; He and Qin, 2020; Wood et al., 2020; Jiang et al., 2022; Xiong and Kaluwasha, 2022). Central America is considered to be the main center of sweetpotato origin, without excluding the existence of secondary centers in China, Southeast Asia and East Africa (Aguoru et al., 2015; Loebenstein, 2009).

Among the 14 species placed in the Batatas section of the *Ipomoea* genus, sweetpotato is the only one used in food crops for its tuberous roots and edible leaves (Wood et al., 2020; Jiang et al., 2022; Xiong and Kaluwasha, 2022).

Recent studies indicate the worldwide presence of more than 35000 taxa of *I. batatas* in *ex situ* collections (Roca et al., 2007; Kim et al., 2018, Jackson et al., 2020). An impressive collection is held by the International Potato Center in Peru (Nimmakayala et al., 2011; Xiong and Kaluwasha, 2022).

I. batatas is a perennial plant, but usually cultivated as an annual in about 117 countries (Gruneberg et al., 2015; Widaryanto and Saitama, 2017; Behera et al., 2022; Nimmakayala et al., 2011). Due to the rich content of starch, protein, fiber, sugars, β-carotene, vitamins, fatty acids, essential minerals, etc. in the tuberous roots, young shoots, leaves and petioles, it is a global staple food with high nutritional value (Bovel-Benjamin, 2007; Nimmakayala et al., 2011; He and Qin, 2020; Wood et al., 2020; Jiang et al., 2022). It also has numerous medicinal or industrial uses (stationery, textiles, etc.), depending on the needs of the production area (Gruneberg et al., 2015).

It has tuberous roots and stems up to 7 m long, green or purple, with high branching power. Leaves, alternate, long petiolate, have large, variously shaped blades (Huaman, 1991; Behera et al., 2022; Jiang et al., 2022). The color palette

of leaves, stems, and tuberous roots includes shades of white, yellow, orange or brown, pink, reddish-purple, or deep purple (Jiang et al., 2022). *I. batatas* is usually propagated vegetatively, by stem cuttings or tuberous roots, but recent studies have also admitted shoots by *in vitro* propagation (Cioloca et al., 2020; Jiang et al., 2022; Behera et al., 2022; Tadda et al., 2022). It prefers sunny sites, well-drained soils with a pH of 5.5-6.5 and temperatures of about 21-26°C. It is highly adaptable to different soil and climatic conditions (Behera et al., 2022; Nimmakayala et al., 2011). Studies on the influence of the type of substrate (perlite, peat, coconut fiber, etc.), including hydroponic culture, both on vegetative mass or tuberous root production and for rooting cuttings, have shown that the use of a light substrate that allows good aeration and retains soil moisture, such as coconut fiber or perlite, leads to better results (Cruz de Souza et al, 2022; Stoian et al., 2022; Mohd et al., 2022; Perez - Pazos et al., 2023). In terms of nutrients, potassium, has been shown to have a beneficial effect on the taste, shape, size, color or texture of sweet potatoes (Darko et al., 2020).

In recent years, sweetpotato has become a popular crop in the ornamental sector due to the variety of foliage colors and shapes, stem length and habit diversity in the approximately 6000 identified varietys, mostly in the USA and South Korea (Lee et al., 2021; Gruneberg et al., 2015). The plant offers various potential uses in hanging pots, pots, containers and window boxes, ground coverings, color patches and wall cladding, pergolas, gazebos, green wall landscaping, etc. (Lee et al., 2021; Cruz de Souza et al., 2022; Ozarchevici et al., 2024).

The majority of studies in *I. batatas* focus predominantly on factors influencing nutrient storage and accumulation in the tuberous roots, but there is a considerable lack of knowledge on above-ground morphology (Somda and Kays, 1990).

The aim of this work was to evaluate the influence of different substrate types on the morpho-decorative characters of some ornamental varieties of *I. batatas* grown in containers.

MATERIALS AND METHODS

The study was carried out in the period 2023-2024, under the experimental field conditions of the Floriculture Discipline, Iasi University of Life Sciences (IULS), Romania. Three ornamental varietys of *I. batatas* (first factor) and four types of container substrate (second factor) were used, with the experiments being organized in randomized blocks with three replications. The plant containers were kept in unprotected conditions, the experimental crops were annuals with a similar growing season as field plants (May-October).

Plant material

The sweetpotato varietys used as experimental material were 'Heart Lime' (HL), 'Heart Bronze' (HB) and 'Black' (B), used in the text with the abbreviated names in brackets.

Variety characterization was adapted from Huaman's (1991) method, based on the use of plant descriptors of *I. batatas*. The method has been adapted and used by other authors in studies on the morphology of sweet potato varieties (Cruz de Souza et al., 2022; Jackson et al., 2020). From the total descriptors, only those that helped in the general description of the studied varieties with reference to stem and leaf characters were selected. The parameters used for stem description were: plant twining capacity, plant growing habit (spreading - >75 cm; semi-compact – 50-75 cm; compact - <50 cm), plant covering capacity (high - 75-90%; medium - 50-70%), vine predominant color.

Table 1. Characterization of the sweet potato varietys vines

Cv.	Descriptors			
	PTC	PGH	PCC	VPC
HL	Non-twining	Spreading	High	Green
HB	Slightly Twinning	Semi-compact	High	Mostly purple
B	Non-twining	Compact	Medium	Dark purple

PTC – plant twining capacity, PGH – plant grow habit, PCC – plant covering capacity, VPC – vine predominant colour

The characters that most differentiated the varieties from each other were those related to growth habit (PGH) and the predominant color of the stems and their branches (VPC). Also, the plant covering capacity ranged from high (HB and HL) to medium in the case of variety B (Table 1).

Characterization of leaves was done according to the following criteria: leaf shape, leaf lobes type, leaf lobes number, mature leaf color. Leaf shape ranged from triangular to almost divided, number of lobes from 1 to 5 and the color from green to dark purple (Table 2, Figure 1).

Table 2. Characterization of the sweet potato varietys leaves

Cv.	Descriptors			
	LS	LLT	LLN	MLC
HL	Triangular	Very slight	1-3	Green
HB	Hastate	Slight	3	Mostly purple
B	Almost divided	Deep	5	Full purple

LS – leaf shape, LLT – leaf lobes type, LLN – leaf lobes number, MLC – mature leaf colour



'Heart Lime' 'Heart Bronze' 'Black'

Figure 1. Leaf appearance of *I. batatas* varieties

Growing substrates

The substrates were composed of three basic components (garden soil, peat, coconut fiber) and hydrogel. As in the case of the sweet potato varietys, abbreviated names have been used, in brackets. The garden soil (GS) was a cambic chernozem with a sand-loamy texture and a weakly alkaline pH (7.8). The peat (P) was SuliFlor (SF2) with pH = 5.5-6.5 and medium structure (0-20 mm), improved with complex fertilizers (1.5 kg/m^3 NPK 14-16-18) and additives based on limestone and dolomite powder. Dehydrated coconut fiber (C) had a pH = 5.5-6.5 and a water absorbtion capacity of 650-850%. Granular hydrogel (H), an environmentally friendly potassium-based hydroabsorbate, had a neutral pH and a filtration surface density of 30-60 mesh. In the execution of the substrate variants, the mixtures consisted of equal volumetric parts of the presented components, except for the hydrogel, added 2 g/L. The following substrate variants were obtained, for which the abbreviations corresponding to the components were used: garden soil + peat (GSP), garden soil + peat + hydrogel (GSPH), garden soil + peat + coconut fiber (GSPC), garden soil + peat + coconut fiber + hydrogel (GSPCH). The determination of the main physico-chemical indices of the substrates (Table 3 and Table 4) was carried out at the Research Institute for Agriculture and Environment (ICAM) of the Iasi University of Life Sciences.

The substrate chemical composition were analyzed following the standard provided by the National Institute for Research and Development for Pedology, Agrochemistry and Environmental Protection (ICPA, Bucharest). Soil pH was measured in 1:2.5 soil to water suspension using a pH meter (Mettler-Toledo, Greifensee, Switzerland). Available phosphorus (P) and potassium (P) were extracted in aqueous solution using the colorimetric molybdenum blue method and measured spectrophotometrically with a Specord Plus UV–Vis device (Analytikjena, Jena Germany). The potassium and microelements (Zn, Cu, Fe, Mn) quantification was performed using Atomic Absorption Spectrometry (AAS ContrAA 700, Analytikjena, Jena, Germany).

Table 3 presents the granulometric composition of substrates where we noted that there is a redistribution of fractions within the added substrate which facilitate the plant growth. The incorporation of peat, coconut fiber and hydrogel improved the water retention capacity of sand-loamy soils texture and increase their nutrient holding ability.

Table 3. Granulometric composition of substrates

Substr.	CS 2.0-0.2%	FS 0.2-0.002%	D 0.02-0.002%	CC <0.002%	*PC <0.01%*
GSP	-	34.8	45.8	19.4	*54.2*
GSPH	-	35.3	38.5	26.2	*52.3*
GSPC	-	37.0	35.8	27.2	*53.7*
GSPCH	-	39.4	28.7	31.9	*46.2*

CS – Coarse sand, FS – Fine sand, D – Dust, CC – Colloidal clay, PC – Physical clay.

The influence of substrate granulometric composition was also identified by Stoian M. (2022), who obtained different results in terms of yield and vegetative mass using peat and 2 type of perlite and recorded the best results in the mixture with better porosity wich allowed a proper oxygenation.

Table 4. Chemical characteristics of substrates

Substr.	pH	P ppm	K ppm	Zn ppm	Cu ppm	Mn ppm	Fe ppm
GSP	6.04	231	262	5.20	13.20	42.26	72.79
GSPH	5.84	190	401	5.21	13.28	45.10	81.91
GSPC	5.97	190	255	6.51	11.53	45.96	57.86
GSPCH	6.09	212	469	6.08	13.07	48.99	66.47

Table 4 presents the substrate chemical composition and reflect the differences between the components added, highlighting the changes observed in terms of fertility management and maintenance of physico-chemical balance. The GSP, GSPH, GSPC and GSPCH substrate

maintained stable soil pH showing slight acidification. A medium content in phosphorus and potassium was displayed demonstrated the mobilization and element availability mediated by different substrate compositions.
The experimental crops were established annually by planting cuttings in 70 L containers (3 plants/container). The determinations and observations were carried out during the vegetative period of the plants and aimed to monitor some biometric indices that define the morpho-decorative particularities of the plants (number of branches and leaves per plant, stem length).
Interpretation of the results was carried out using statistical analysis in order to obtain a series of indices on the direction, strength and relationship between the analyzed morphological characters. The Kolmogorov-Smirnov test was used to check the normality condition and the Mann-Whitney test was chosen to compare the means of two selections from independent populations, since the data were found to be non-normalized. The scatter plot and mathematical modeling by linear regression were also used. Statistical testing was performed at 0.05 level of significance using MS EXCEL professional application from MS OFFICE 2020 package.

RESULTS AND DISCUSSIONS

The observations and determinations carried out aimed both to evaluate the decorative effect of the three sweet potato varieties grown in containers and the influence of substrate type on some morphological characters of the plants.

Substrate influence on plant morphology

The *I. batatas* varieties presented above were analyzed in the context of identifying the influence of substrates with different compositions on some morpho-decorative characters: stem length, number of branches, number of leaves/plant. The results of the biometric determinations were analyzed in correlation with the four types of culture substrate: garden soil + peat (GSP), garden soil + peat + hydrogel (GSPH), garden soil + peat + coconut fiber (GSPC), garden soil + peat + coconut fiber + hydrogel (GSPCH).

Interpretation of the results was based on statistical analysis on the variables. It was established both the dependence of plant characters on the growing substrate and the significance of the differences generated by the type of substrate.
The use of graphical representation of the calculated values (mean values) for a small number of plants in the experiment performed, may lead to a slightly distorted conclusion. Therefore, the recommended non-parametric statistical test was the Mann-Whitney test which will indicate in detail the similarities between the compared substrate pairs (medians compared). The dependence between plant character and substrate type was determined, based on the *p-value* resulting from the pairwise comparison of substrates. Values of *p-value* < 0.05 (marked with *) indicate significant differences in relation to the analyzed substrates pair, which means that the respective substrates influence the character differently. Values of *p-value* > 0.05 highlight similarities in the effect of the substrates, in which case it is possible to choose one or the other of them.
The variation trend as well as the minimum and maximum values of the characters according to the type of substrate were analyzed by means with the "stock" type graphs for each variety.

Substrate influence on stem length

Statistical analysis (Table 5) showed both similarities of results (*p-value* > 0.05) and significant differences (*p-value* < 0.05), depending on the variety.

Table 5. Dependence between the length of stem and the type of substrate

Cv.	Substr.	GSPH	GSPC	GSPCH
HL	GSP	0.03074*	0.04363*	0.0268*
	GSPH		0.04746*	0.26435
	GSPC			0.05705
HB	GSP	0.41294	0.09342	0.49202
	GSPH		0.04846*	0.30854
	GSPC			0.03673*
B	GSP	0.025*	0.03074*	0.04746*
	GSPH		0.02872*	0.04846*
	GSPC			0.0548

*Level of significance=0.05

In HL, significant differences were found between all substrate types, except GSPCH–GSPH (*p-value* = 0.26435) and GSPCH–GSPC (*p-value* = 0.05705) between which similarities were found and where substrate choice options may be suggested. These data are partially confirmed also by the mean, maximum and minimum values of the biometric determinations plotted in Figure 2. Very similar values of the stem lengths of the GSPH and GSPCH substrates are observed, suggesting the possibility of choosing the substrate with fewer components (GSPH). Although on the graph the GSPC substrate does not suggest similarity with GSPCH, statistical calculation (Table 5) does not exclude the alternative hypothesis. From the data presented for this variety, the GSP substrate is recommended to obtain plants with longer stems, while the other variants are not excluded.

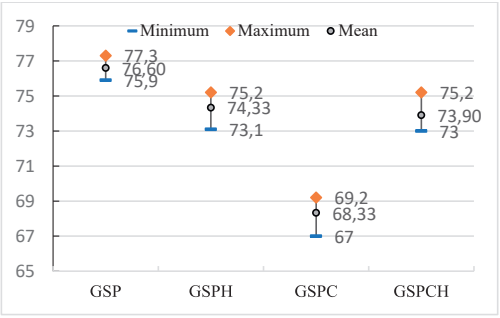


Figure 2. Minimum and maximum values for the length of stem according to the type of substrate (HL)

The HB variety was characterized by a very low dependence of the stem length in the GSP substrate compared to GSPH and GSPCH substrates, the differences recorded being nonsignificant. The highest similarities, with *p-value* = 0.41294–0.49202, were between GSP and GSPH, respectively GSPCH, justifying the choice of the simpler substrate (GSP) (Table 5). Figure 3 also shows similar stem length values in GSP, GSPH and GSPCH substrates, with an average of ca. 69 cm, suggesting that the use of a simple mixture of only peat and garden soil is equally effective. A high level of similarity was also recorded between GSPH and GSPCH (*p-value* = 0.30854), with the recommendation to choose the substrate with fewer components (GSPH). In this variety, for the variants in which the GSPC substrate was compared with the GSPH and GSPCH substrates, the differences were significant (Table 5), also highlighted in Figure 3, where it can be seen that only the GSPH and GSPCH substrates favored stem length growth. The data presented in Figure 3 also suggest the tendency of stem length towards maximum values, regardless of substrate type, as well as the higher variability of this trait in plants grown on GSP.

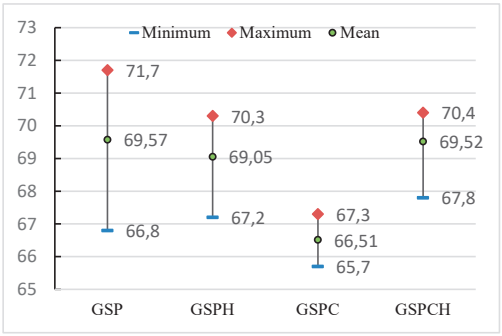


Figure 3. Minimum and maximum values for the length of stem according to the type of substrate (HB)

The influence of substrate on stem length at B variety was relatively different from the others. The only statistically recorded similarity from the comparison of substrate pairs was in the case of GSPC–GSPCH, but the very low *p-value* (0.0548), close to the threshold of significant differences, is less recommending the substitution of one substrate for the other (Table 5).

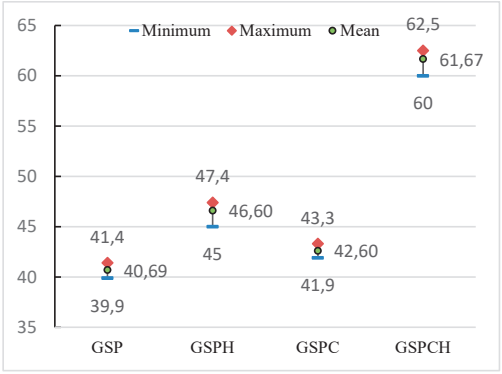


Figure 4. Minimum and maximum values for the length of stem according to the type of substrate (B)

Although the graphical representation (Figure 4) suggests distorted conclusions compared to the statistical calculation (explained by the lower number of plants), the conclusion suggested by the statistical calculation can be maintained. Another

peculiarity of the stem length of B variety was the small range of variation between minimum and maximum (1.4-2.5 cm), indicating a greater uniformity of this character within each variant. In all substrate types, the variation trend was towards the maximum values.

Substrate influence on the degree of stem branching

The branching capacity of plants used as ground cover or creeping plants, such as *I. batatas*, is an essential feature of their decorative value. In the present study, the analysis of this character was done similar to stem length in order to determine the degree of influence and the level of similarity of the effect of culture substrates. Table 6 summarizes the results on the dependence of branching degree on the substrate type.

Table 6. Dependence between the number of branches and the type of substrate

Cv.	Substr.	GSPH	GSPC	GSPCH
HL	GSP	0.00001*	0.00001*	0.01831*
	GSPH		0.49202	0.00149*
	GSPC			0.00042*
HB	GSP	0,00326*	0,49202	0,00001*
	GSPH		0,00326*	0,00023*
	GSPC			0,00001*
B	GSP	0,00082*	0,49202	0,11123
	GSPH		0,00082*	0,00027*
	GSPC			0,11123

*Level of significance=0.05

For HL, the statistical analysis showed significant differences in the number of branches/plant between most substrate pairs, especially in the case of GSP compared to GSPH and GSPC, where a very low *p-value* (0.00001) was recorded.

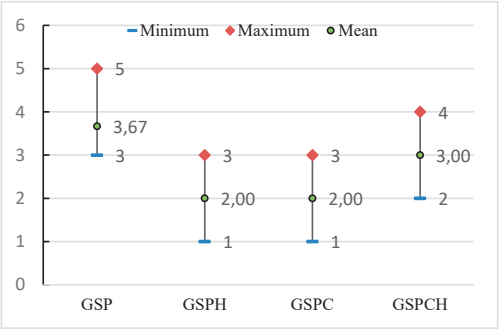


Figure 5. Minimum and maximum values for the number of branches/pl. according to the type of substrate (HL)

Nonsignificant differences (*p-value*=0.49202) of the results obtained for this variety, i.e. similarity of the effect of substrates, were only between GSPH and GSPC. The way in which the effect of each substrate on the number of branches was manifested is also observed in Figure 5. In accordance with the data from Table 6, the median indicates the highest degree of favorability of the substrate GSP, with maximum of 5 branches/plant, the recorded results not being similar to other substrates.

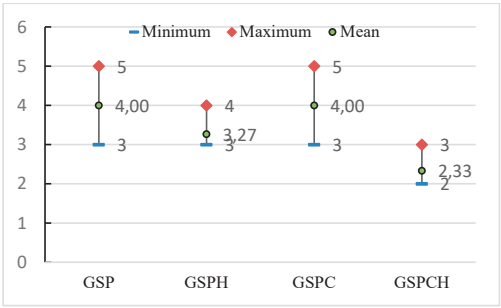


Figure 6. Minimum and maximum values for the number of branches/pl. according to the type of substrate (HB)

Also in HB, the differences resulting from the comparison of substrate pairs were significant, with the exception of GSP and GSPC substrates. The level of significance also suggests the substrates that determine the largest differences in the results, as is the case between GSP and GSPCH, or GSPC and GSPCH, with *p-value* = 0.00001 (Table 6). The data in Figure 6 confirm these aspects, in that the GSP and GSPC substrates gave the best results and similar effect, suggesting the possibility of their mutual substitution. It is also obvious that substrates containing hydrogel (GSPH and GSPCH) are not recommended for increasing the number of branching in HB (Figure 6).

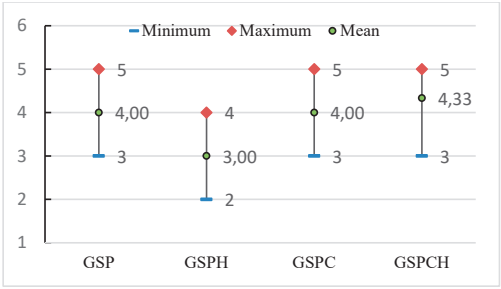


Figure 7. Minimum and maximum values for number of branches/pl. according to the type of substrate (B)

In B variety, several substrate pairs with similar effects on branching number (*p-value* > 0.05) were recorded, such as GSP with GSPC and GSPCH, or GSPC with GSPCH. In all the comparisons involving the GSPH substrate, the differences in the results were significant, indicating the different action of this substrate compared to the others (Table 6).

The Mann-Whitney test data are also supported by the graphical representations (Figure 7). The maximum and minimum values of the number of branches were similar in the GSP, GSPC and GSPCH variants (ranging from 3 to 5), with very small differences of the mean (4-4.33). The fewest branching were formed at the plants grown in the GSPH substrate. The results obtained at this variety indicate that for better branching of the plants it is recommended to choose one of the substrates GSP, GSPC or GSPCH, but from the economic point of view the simpler substrate (GSP) is indicated.

It can be observed that the addition of coconut fiber in the mixture improved the nutrient source and the physico-chemical characters of the substrates which led to an increase in biomass, which was also found in the research done by Perez-Pazos (2023), who also used coconut fiber in the rooting mixture. It is thus understood that each type of mixture influences differently the characters in each variety. A similar situation can be observed to that found by Mohd (2022), who used five types of sweetpotato mixtures including coconut fiber. The best results in terms of vegetative growth was in the substrate where coconut fiber was used in higher proportions to retain moisture.

Substrate influence on number of leaves/plant

Rich leaf mass and high stem branching are two characters that complement each other in obtaining *I. batatas* plants with high decorative value. Therefore, when considering the number of leaves/plant, the choice of culture substrates should be made according to the maximum favorability they can have on this character. In addition, in order to give cultivators the possibility to choose between substrates with different compositions but similar effect on the plants, the significance of the differences between the compared substrate pairs was also calculated for this character (between GSP and GSPCH) and HB (between GSPH and GSPC).

In contrast to the other characters analyzed, the number of leaves/plant showed significant differences between most of the substrate pairs compared for all varieties (Table 7). From the statistical calculation, similarities appeared only for HL. According to the level of significance of the differences (Table 7) and the values indicating the mean, minimum and maximum range of leaf number (Figure 8), it can be seen that the influence of substrates was quite different in HL. The greatest differences (*p-value* = 0.00001) were recorded by comparing the results from the substrate GSPH with those from the substrates GSPC and GSPCH (Table 7), the graph (Figure 8) indicating the direction of influence: GSPH determined the formation of the highest number of leaves (mean 143.6 leaves/plant), while GSPC and GSPCH were less favorable, with mean values of 96.2-106.67 leaves/plant.

Table 7. Dependence between the number of leaves and the type of substrate

Cv.	Substr.	GSPH	GSPC	GSPCH
HL	GSP	0,00008*	0,00453*	0,12302
	GSPH		0,00001*	0,00001*
	GSPC			0,02938*
HB	GSP	0,00001*	0,00001*	0,00001*
	GSPH		0,49202	0,01463*
	GSPC			0,00001*
B	GSP	0,00001*	0,00001*	0,00017*
	GSPH		0,00001*	0,01191*
	GSPC			0,00001*

*Level of significance=0.05

In this variety, the results recommend the use of GSPH substrate. The similarity between GSPC and GSPCH excludes the possibility of choosing between them, both having poorer results.

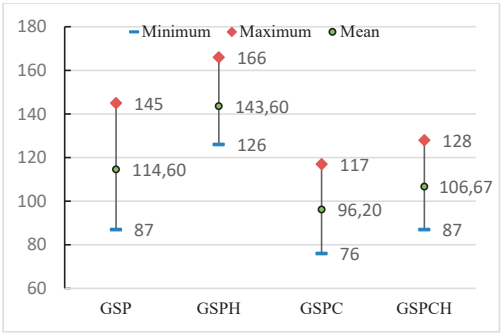


Figure 8. Minimum and maximum values for the no. of leaves/pl. according to the type of substrate (HL)

For HB variety, plants grown in GSP substrate showed the worst results in terms of leaf number, although there was some uniformity of plants, with a relatively narrow range between maximum and minimum values (698–738) compared to other substrates (Figure 9). As a result, the differences obtained from comparing GSPH with all other substrates were significant, within *p-value* = 0.00001 (Table 7). The only similarity was between GSPH and GSPC (Table 7), but of no practical importance in terms of choice (in both cases, the results were less good).

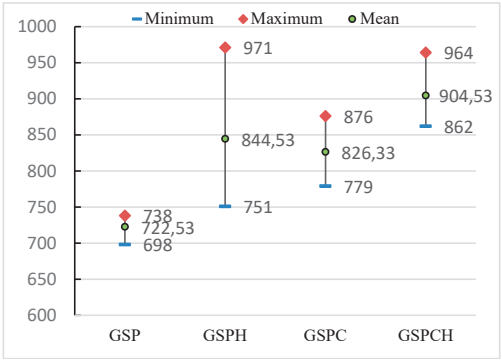


Figure 9. Minimum and maximum values for the no. of leaves/pl. according to the type of substrate (HB)

The data in Figure 9 provide further information on the influence of each substrate on leaf number in HB: higher plant uniformity in GSPC and GSPCH substrates, but with the highest mean values in GSPCH (904.5 leaves/plant); large amplitude between maxima and minima in GSPH, with maxima up to 971 leaves/plant, but with means below GSPCH and trending towards minima (Figure 9).

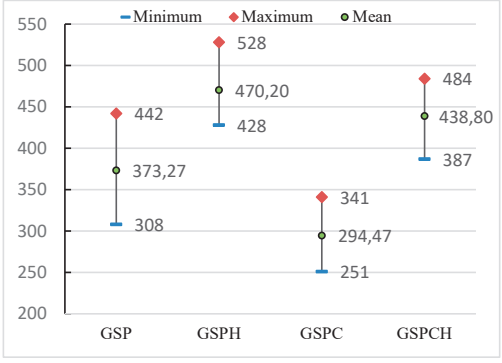


Figure 10. Minimum and maximum values for the no. of leaves/pl. according to the type of substrate (B)

The influence of substrate on the number of leaves/plant in B variety was characterized by significant differences in all combinations of substrate pairs analyzed, so that no similarities were recorded (Table 7). character, GSPH substrate is recommended for B variety. The results presented in Figure 10 indicate a ranking of substrate favorability in terms of number of leaves/plant in B variety, i.e. the highest mean values (470.2) in the GSPH substrate, ranging from a maximum of 528 to a minimum of 428 leaves/plant, with a trend towards minimum values. Relatively good results for this character were also obtained in GSPCH substrate, with an average of 438.8 leaves/plant. Less favorable was the GSPC substrate, with an average of only 294.5 leaves/plant. Due to the maximum results for this character, the substrate GSPH is recommended for B variety.

Analysis of correlations between morphological characters of I. batatas varieties

Detailed assessment of the relationship between the analyzed characters (as variables) was performed by calculating the coefficient of determination (R^2), i.e. the proportion of the total variation of the dependent variable that can be explained by the independent variable. The coefficient of determination was determined in the following combinations: number of branches (BN) and stem length (SL), number of branches (BN) and number of leaves/plant (LN), stem length (SL) and number of leaves/plant (LN).

For each combination, the corresponding linear regressions were constructed, illustrated by graphs only for the cases that recorded the maximum values of the coefficient of determination (R^2). Pearson correlation coefficients (r) for pairs of characters indicate predominantly nonsignificant indirect relationships (Table 8).

Table 8. Pearson correlation coefficient results

Variety/ Character	HL		HB		B	
	SL	LN	BL	LN	SL	LN
BN	0.53*	-0.10	-0.19	-0.61*	0.20	-0.21
SL		0.40*		-0.073		0.49*

SL - Stem length, BN - Branches no., LN - Leaves no.

In HL there were positive correlations of medium intensity between BN/SL (r=0.53; R^2=0.2838) and SL/LN (r=0.40; R^2=0.1565). Between BN and LN the correlation was negative nonsignificant (r=0.10; R^2=0.0096).

The graphical representation was given only for the BN/SL pair of characters with the highest R^2 value (Figure 11).

Correlations between characters at HB were negative, but significant only in the BN/LN pair (r=-0.61), indicating that the number of leaves decreased in proportion to the increase in the number of branches and vice versa (Figure 12).

In B variety, BN/SL (r=0.20) and SL/LN (r=0.49) were positively correlated, while BN/LN were negatively correlated (r=0.21). The only correlation with statistically assured results and graphically represented was between SL/LN (Figure 13), with 24% dependence (R^2=0.2366).

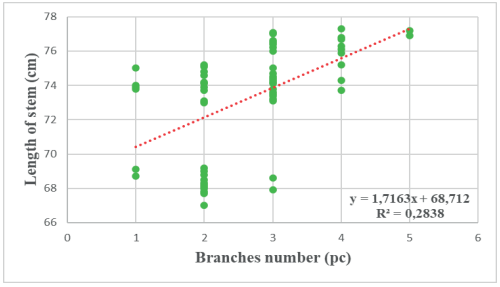


Figure 11. Correlation between number of branches/pl and stem length (HL)

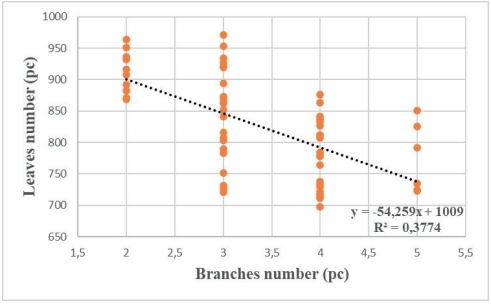


Figure 12. Correlation between number of branches/pl and number of leaves/pl (HB)

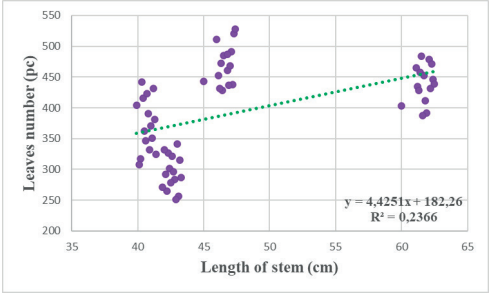


Figure 13. Correlation between number of leaves/pl. and length of stem (B)

CONCLUSIONS

The study carried out on the three varieties of *I. batatas* grown in containers showed that the type of culture substrate used influenced certain morphological characters of the plants, i.e. their decorative appearance.

The reaction of each variety to the composition of the substrates used allowed the formulation of recommendations indicating the variants by which the results for the characters analyzed (stem length and branching degree, number of leaves/plant) could be maximized, in correlation with the way the plants were used. The statistical analysis provided the possibility of establishing the similarity of the effect of certain substrates and choosing those that provide efficiency by a reduced number of components.

In terms of stem branching, the substrate composed of garden soil + peat (GSP) was effective for all varieties, and it also favoured stem length growth in 'Heart Lime' (HL) and 'Heart Bronze' (HB), a characteristic that is well exploited when plants are used for vertical decoration.

To increase the number of leaves/plant, a very important characteristic for cover plants, the substrate of garden soil + peat + hydrogel (GSPH) can be recommended for all varieties, although good results were also obtained with the substrate containing coconut fiber (GSPCH), but considered less efficient due to its complexity and additional costs. The results show that the presence of hydrogel, which helps in solubilization of nutrients and their uptake, ensured the formation of more leaves.

In the analyzed variants the favorable effect of peat (P) was noted, which played a role in increasing the degree of aeration of substrates. In most cases, the efficiency of coconut fiber (C) was ensured in combination with hydrogel (H).

ACKNOWLEDGEMENTS

This research work was carried out with the support of "Ion Ionescu de la Brad" Iasi University of Life Sciences (IULS), Romania, and Research Institute for Agriculture and Environment (ICAM) of IULS.

REFERENCES

Aguoru, C.U., Uhia, P., & Olasan J.O. (2015). Varietal Characterisation and Taxonomic Evaluation of Sweet Potato (*Ipomoea batatas*) Using Macro- and Micromorphological Evidence. *Open Access Library Journal*, 2, https://www.researchgate.net/publication/282484054.

Behera, S., Chauhan, V.B.S., Pati, K., Bansode, V., Nedunchezhiyan, M., Verma, A.K., Monalisa, K., Naik, P.K., & Naik, S.K. (2022). Biology and biotechnological aspect of sweet potato (*Ipomoea batatas* L.): a commercially important tuber crop. *Planta*, 256(40), https://link.springer.com/article/10.1007/s00425-022-03938-8.

Bovel-Benjamin, A.C. (2007). Sweet potato: a review of its past, present, and future role in human nutrition. *Advances in Food and Nutrition Research* 52, 1–59, DOI: 10.1016/S1043-4526(06)52001-7.

Cioloca, M., Tican, A., Popa, M., & Bărăscu, N. (2020). Optimization of sweet potato (*Ipomoea batatas* l.) *In vitro* cultivation by using the contaminated cultures for obtaining new shoots in greenhouse conditions, Iași, RO: *Lucrări științifice, seria Agronomie*, *USV, 63(2), 125 – 130.*

Cruz de Souza, M., Carlos de Oliveira Silva, J., Alves da Silva, E., Ferreira de Oliveira, M.E., Carvalho de Andrade Junior, V., Valquiria dos Reis, M. (2022). Characterization of sweet potato genotypes with landscaping potential. *Euphytica* 218(156), https://doi.org/10.1007/s10681-022-03107-8.

Darko, C., Yeboah, S., Amoah, A., Opoku, A., Berchie, J.N. (2020). Productivity of sweet potato (*Ipomoea batatas* (L) Lam) as influenced by fertilizer application in different agro-ecologies in Ghana, *Scientific African* 10(e00560), https://doi.org/10.1016/j.sciaf.2020.e00560.

Gruneberg, W.J., Ma, D.,Mwanga, R.O.M., Carey, E.E., Huamani, K., Diaz, F., Eyzaguirre, R., Guaf, E., Jusuf, M., Karuniawan, A., Tjintokohadi, K., Song, Y.S., Anil,S.R., Hossain, M., Rahaman, E., Attaluri, S.I., Some, K., Afuape, S.O., Adofo, K., Lukonge, E., Karanja, L., Ndirigwe, J., Ssemakula, G., Agili, S., Randrianaivoarivony, J.M., Chiona, M., Chipungu, F., Laurie, S.M., Ricardo, J., Andrade, M., Rausch Fernandes, F., Mello, A.S., Khan, M.A., Labonte D.R., Yencho, G.C. (2015). Advances in sweetpotato breeding from 1992 to 2012 (Chapter 1, 1-67) in *Potato and Sweetpotato in Africa: Transforming the Value Chains for Food and Nutrition Security*, *CAB International*.

He, J., & Qin, L. (2020). Growth and photosynthetic characteristics of sweet potato (*Ipomoea batatas*) leaves grown under natural sunlight with supplemental LED lighting in a tropical greenhouse. *Journal of Plant Physiology* 252(153239), https://doi.org/10.1016/j.jplph.2020.153239.

Huaman, Z. (1991). *Descriptors for sweet potato*. Rome, I: International Board for Plant Genetic Resources.

Jackson, D.M., Harrison, H.F., Jarret, R.L., Wadl, P.A. (2020). Phenotypic variation in leaf morphology of the USDA, ARS sweetpotato (*Ipomoea batatas*) germplasm collection. *HORTSCIENCE* 55(4), 465–475, https://doi.org/10.21273/HORTSCI14703-19.

Jiang, T., Ye, S., Liao, W., Wu, M., He, J., Mateus, N., Oliveira, H. (2022). The botanical profile, phytochemistry, biological activities and protected-delivery sybranches for purple sweet potato (*Ipomoea batatas* (L.) Lam.): An up-to-date review. *Food Research International,* 161(111811), https://www.sciencedirect.com/science/article/pii/S0963996922008699.

Kim, J.H., Kim, J.H., Jo, W.S., Ham, J.G., Chung, I.K., Kim, K.M. (2016). Characterization and development of EST-SSR markers in sweet potato [*Ipomoea batatas* (L.) Lam]. *3 Biotech,* 6(243). <https://doi.org/10.1007/s13205-016-0565-9>.

Lee, J.K., Kim, D.Y., Park, S.H., Woo, S.Y., Nie, H., Kim, S.H. (2021). Particulate Matter (PM) Adsorption and Leaf Characteristics of Ornamental Sweet Potato (*Ipomoea batatas* L.) Varietys and Two Common Indoor Plants (*Hedera helix* L. and *Epipremnum aureum* Lindl. & Andre). *Horticulturae*, 8(26). https://doi.org/10.3390/horticulturae8010026.

Loebenstein, G. (2009). *Origin, Distribution and Economic Importance. The Sweetpotato (Chapter 2)*, 9-12, Springer, https://link.springer.com/chapter/10.1007/978-1-4020-9475-0_2.

Mohd, Y.S., Raship, I., Nordin, N.I., Mohamed Nor, M.E., Zainal Abidin, S.N., Abdul Latiff, Z.A., Towhid, M.F., Samsudin, N.S. (2022). Effects of substrates on growth, yield and tuber quality of sweet potato cultivated using soilless culture system, *AGROFOR International Journal*, 7(3), 10.7251/AGRENG2203133M.

Nimmakayala, P., Vajja, G., Reddy, U.R. (2011). Ipomoea (Chapter 7, 123-132), in *Wild Crop-Relatives: Genomic and Breeding Resources. Industrial Crops*, https://link.springer.com/chapter/10.1007/978-3-642-21102-7_7.

Ozarchevici, A.S., Apostol, M., Chiruță, C., Bernardis, R.R., Draghia, L. (2024). The behavior of some decorative varieties of *Ipomoea batatas* in different culture systems and types of substrate. București, RO: *Scientific Papers*. Series B, Horticulture, Vol. LXVIII (1), 666- 675.

Perez-Pazos, J, Rosero, a., Vergara, E., Gamez, R. (2023). Response of Sweet Potato to Substrates and Acclimatization Conditions in the Greenhouse to Produce High-quality Planting Material. *Horticulture Journal,* 9(4), 451-463, doi: 10.2503/hortj.QH-017.

Roca, W.M., Montesino, G.R., Campilan, D., Laliberte, B., Renoso, D., Rao, R., de Chavez, H. (2007). Global strategy for ex-situ conservation of sweet potato genetic resources. *Sweetpotato Genetic Resources Conservation – CIP Genebank. CIP*, Lima, Peru, <https://www.croptrust.org/wpcontent/uploads/2015/05/SweetPotato-Strategy-FINAL-12Dec07.pdf>.

Roullier, C., Duputie, A., Wennekes, P., Benoit, L., Bringas, V.M.F., Rossel, G., Tay, D., McKey, D., Lebot, V. (2013). Disentangling the Origins of Cultivated Sweet Potato (*Ipomoea batatas* (L.) Lam.). *PLoS ONE* 8(5), e62707. doi:10.1371/journal.pone.0062707.

Somda, Z.C., Kays, S.J. (1990). Sweet Potato Canopy Architecture: Branching Pattern, *J. AMER. Soc. HORT. SCI*. 115(1), 33-38.

Stoian, M., Doltu, M., Drăghici, E.M. (2022). The influence of the type of substrate on the production of sweet potatoes. Timișoara *Journal of Horticulture, Forestry and Biotechnology*, 26(1), 46-50.

Tadda, S.A., Kui, X., Yang, H., Li, M., Huang, Z., Chen, X., Qiu, D. (2022). The Response of Vegetable Sweet Potato (*Ipomoea batatas* Lam) Nodes to Different Concentrations of Encapsulation Agent and MS Salts. *Agronomy*, 12(19). https:// doi.org/10.3390/ agronomy12010019.

Widaryanto, E., & Saitama, A. (2017). Analysis of plant growth of ten varieties of sweet potato (*Ipomoea batatas* L.) cultivated in rainy season, *Asian J. Plant Sci*., 16, 193-199, DOI: 10.3923/ajps.2017.193.199.

Wood, J.R.I., Munoz-Rodriguez, P., Williams, B.R.M., Scotland, R.W. (2020). A foundation monograph of Ipomoea (Convolvulaceae) in the New World, *PhytoKeys* 143, 1–823, doi: 10.3897/phytokeys.143.32821.

Xiong, X., & Kaluwasha, W. (2022). Sweet Potato (*Ipomoea batatas*) Biology and Importance in U.S., *Agriculture,* 26(5), https://juniperpublishers.com/artoaj/ ARTOAJ.MS.ID.556346.php.

Zhang, I., Wang, Q., Liu, Q., Wang, Q. (2009). Sweetpotato in China (Chapter 15, 325-326), In: *Loebenstein, G., Thottappilly, G. (eds) The Sweetpotato.* Springer, Dordrecht. https://doi.org/ 10.1007/978-1-4020-9475-0_15.

ICPA București, Evaluarea conţinutului de nutrienţi din sol (cap. 6.1). https://icpa.ro/documente/coduri/ Evaluarea_continutului_de_nutrienti_din_sol.pdf.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

SPREAD AND STABILITY OF ORNAMENTAL ALIEN PLANT SPECIES ON URBAN AND PERI-URBAN RIVERBANKS OF BUCHAREST, ROMANIA

Maria RAICU[1, 2], Mihaela URZICEANU[3, 4], Petronela CAMEN-COMĂNESCU[1], Florin TOMA[2]

[1]Botanic Garden "D. Brândză", University of Bucharest, Romania
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, Romania
[3]Faculty of Biology, University of Bucharest, Romania
[4]Reasearch Institute of the University of Bucharest (ICUB), Bucharest, Romania

Correspondence author email: mariana-mihaela.urziceanu@bio.unibuc.ro

Abstract

Ornamental horticulture is one of the most significant pathways for the introduction of alien plant species globally. Many ornamental species, initially cultivated in gardens, have demonstrated invasive potential and begin their spread in riparian habitats. While a majority of these introduced plants have not yet become naturalized in local ecosystems, some species exhibit significant invasive potential under favorable environmental conditions or climate change.
This study examines the occurrence and stability of alien ornamental plant populations along the riverbank zones of urban and peri-urban waterways in Bucharest, focusing on the Argeș River, Dâmbovița River, and Colentina lake chain. The results highlight the presence of species with varying degrees of invasiveness in Romania, including, Ailanthus altissima, Alcea rosea, Hemerocallis fulva, Humulus scandens, Ipomoea purpurea, Reynoutria japonica and Solidago canadensis. These species have established stable populations influencing local biodiversity. The analysis brings into focus key aspects such as introduction pathways, invasion status, spread mechanisms, distribution across the three rivers, and relative abundance, offering valuable insights into the dynamics of alien plant invasions within urban and peri-urban riverbanks.

Key words*: alien, horticulture, biodiversity, river, urban.*

INTRODUCTION

Urban and peri-urban riverbanks represent highly dynamic ecosystems that function as transitional zones between aquatic and terrestrial habitats (Richardson et al., 2007). These ecosystems are considered "critical transition zones" due to their susceptibility to both natural and anthropogenic influences, including the introduction of alien plant species (Ewel et al., 2001; Richardson et al., 2007). Their hydrological dynamism, frequent disturbances, and high connectivity facilitate the dispersal of seeds and propagules, creating opportunities to establish and spread alien flora (Priede, 2008; Rusnák & Kidová, 2022). Horticulture plays a significant role in shaping urban biodiversity, acting as a primary pathway for introducing and spreading alien plant species (Van Kleunen et al., 2018; Hulme et al., 2018). Many ornamental species, initially cultivated for their aesthetic and functional value in gardens and green spaces, have demonstrated the capacity to escape from cultivation and establish populations in riparian habitats (Raicu et al., 2024). Depending on their ecological adaptability, these species may either integrate into existing plant communities with minimal impact or exhibit invasive behavior, leading to significant ecological disruptions (Richardson et al., 2007).

The expansion of ornamental plant species along urban riverbanks is influenced by multiple interacting factors, including modified hydrological regimes, soil disturbances, and the presence of diverse seed dispersal mechanisms such as hydrochory, anemochory, and human-mediated transport (Säumel & Kowarik, 2010). While some species establish stable populations without significantly altering ecosystem functions, others can outcompete native vegetation, disrupt trophic interactions, and modify riparian habitat structure (Strayer, 2010). The ecological consequences of such invasions underscore the need for a comprehensive understanding of the mechanisms driving the establishment and persistence of alien ornamental species in riparian landscapes. This study assesses alien

ornamental plant species' distribution, spread, and stability along urban and peri-urban riverbanks in Bucharest and its surrounding areas. By mapping the occurrence of alien ornamental plant species along the Argeș, Dâmbovița, and Colentina lake chain and analyzing their reproductive and dispersal strategies, we seek to evaluate their long-term establishment potential and ecological impact. The findings of this research can contribute to a deeper understanding of plant community dynamics in urban riparian ecosystems and provide valuable insights for biodiversity conservation and invasive species management in anthropogenic landscapes.

MATERIALS AND METHODS

Study area

The study focuses on the lower sections of the Argeș, Dâmbovița, and Colentina Rivers, which are hydrologically interconnected in the Bucharest region (Figure 1). The Argeș River (350 km) originates in the Făgăraș Mountains and flows southeast, collecting the Dâmbovița River (286 km), which also originates in the Făgăraș Mountains and passes through Bucharest before merging with the Argeș at Budești locality. The Colentina Rivers, a tributary of the Dâmbovița, forms a chain of artificial lakes before joining it (Radu & Stoiculescu, 2010).

Survey

Observation points were established at 2 km intervals along the lower courses of the Argeș and Dâmbovița Rivers. For the Colentina River, one observation point was selected for each of its ten major lakes (Cernica, Pantelimon, Fundeni, Plumbuita, Tei, Floreasca, Herăstrău, Băneasa, Grivița and Străulești), given the difficult access due to the private properties surrounding them. In each site, vegetation was surveyed using 100 m² plots. Species abundance was initially estimated using the Braun-Blanquet scale, which was subsequently converted into percentage values representing mean abundance. The study was conducted in August and September 2023.

Data analysis

Species frequency was calculated as the proportion of plots where the species occurred, relative to the total number of plots per river, and was categorized as: very rare (<10%), rare (10-25%), moderate (25-50%), frequent (50-75%), and very frequent (>75%). General frequency was determined across all rivers. Mean abundance was calculated as the average percentage cover in the plots where the species was present. Abundance categories were defined as follows: very low (<1%), low (1-10%), moderate (10-30%), high (30-50%), and very high (>50%). Additionally, species traits were recorded for each taxon, including invasiveness status in Romania, commercial use in Romania, life form, biogeographical origin, seed dispersal mode, and reproductive strategy (Raicu et al, 2024). These traits were used to assess ecological behavior and establishment potential across river banks.

In addition to trait-based and distributional analyses, a visual map was created to illustrate the spatial occurrence of ten selected ornamental alien species along the studied riverbanks. The selection was not based on frequency or abundance but rather on aesthetic and cultural relevance, as these species are commonly cultivated in green spaces and ornamental gardens due to their showy flowers (Figures 1, 2). To ensure clarity and avoid visual overcrowding, taxa with extensive distributions were excluded from the map.

RESULTS AND DISCUSSIONS

Species Inventory and Functional Traits

A total of 26 alien ornamental plant species were recorded along the banks of the Argeș, Dâmbovița, and Colentina rivers, each characterized by a set of functional traits (Table 1). Of the 26 taxa studied, 24 are marketed in Romania, especially for landscaping and decorative purposes in public and private green spaces. The species *Phytolacca americana, Reynoutria japonica,* are not for sale in specialty stores in Romania, perhaps because they have a high degree of invasiveness and are known from this point of view (Raicu et al., 2024).

More than half of the species (15 species) are classified as invasive in Romania (*Acer negundo*, *Ailanthus altissima*, *Amorpha fruticosa*, *Elaeagnus angustifolia*, *Fraxinus pennsylvanica*, *Helianthus tuberosus*, *Humulus scandens*, *Lycium barbarum*, *Morus alba*, *Oenothera biennis*, *Parthenocissus inserta*, *Phytolacca americana*, *Reynoutria japonica*,

Robinia pseudoacacia, *Solidago canadensis*). Others are casual (8 taxa) or naturalized (3 taxa) without being formally listed as invasive (eg, *Aesculus hippocastanum*, *Campsis radicans*, *Commelina communis*, *Hemerocallis fulva*, *Ipomoea purpurea*, *Salix babylonica*, *Symphoricarpus albus*) (Table 1).

The surveyed alien ornamental plant species exhibit diverse functional traits that reflect their potential ecological impact and adaptability to urban and peri-urban riparian environments (Table 1). Phanerophytes – including trees, shrubs, and lianas – represent the majority, with 16 species identified (e.g., *Acer negundo*, *Ailanthus altissima*, *Amorpha fruticosa*, *Fraxinus pennsylvanica*, *Lycium barbarum*, *Parthenocissus inserta*, *Robinia pseudoacacia*, *Salix babylonica* etc.). These woody plants generally exhibit high adaptability to disturbed environments and possess mechanisms for long-distance dispersal, often facilitated by human activity (Rundel et al., 2014; Pyšek et al., 2020). Hemicryptophytes are also well represented, with 5 taxa, such as *Alcea rosea*, *Helianthus tuberosus*, *Hemerocallis fulva*, *Phytolacca americana*, and *Solidago canadensis*, frequently characterized by rapid growth, high reproductive rates, and effective dispersal strategies (Popay, 2022). Additionally, 3 taxa were identified as therophytes or geophyte-hemicryptophytes (*Oenothera biennis*, *Reynoutria japonica, Commelina communis*), and 2 taxa as climbing annual (*Humulus scandens*, *Ipomoea purpurea*). The species *Humulus scandens* and *Ipomoea purpurea* exhibit vigorous growth and effective seed dispersal via water, wind, animals, and humans, enhancing their establishment potential along watercourses (Dumitrașcu et al., 2024; Astegiano et al., 2010). Biogeographically, most of the species originated primarily from North America (13 species) and Asia (11 species), regions recognized as major sources of ornamental plants in Europe (Van Kleunen et al., 2018). North American species, including *Acer negundo*, *Amorpha fruticosa*, and *Solidago canadensis*, have shown considerable invasive potential in European riparian systems due to their broad ecological tolerances and effective dispersal abilities (Richardson et al., 2007). Similarly, East Asian taxa such as *Ailanthus altissima*, *Reynoutria japonica*, and *Morus alba* demonstrate aggressive colonization abilities, significantly altering native community structures and ecological processes (Landenberger et al., 2007; Miroshnyk et al., 2025).

Seed dispersal mechanisms of the surveyed alien ornamental plant species include anemochory (wind dispersal), hydrochory (water dispersal), zoochory (animal-mediated dispersal), and anthropochory (human-mediated dispersal) (Table 1). Species such as *Fraxinus pennsylvanica* and *Ailanthus altissima* utilize primarily wind and water for long-distance dispersal (Säumel and Kowarik, 2013). In contrast, taxa such as *Elaeagnus angustifolia*, *Morus alba*, and *Symphoricarpos albus* exhibit zoochorous, often facilitated by birds, or anthropochore dispersal strategies, which enhance their potential for establishment in new areas (Richardson et al., 2007).

Most of the species exhibit both vegetative and generative reproduction, significantly increasing their potential for persistence and spread in dynamic riparian habitats. Species with efficient vegetative propagation via rhizomes or stolons - such as *Reynoutria japonica*, *Helianthus tuberosus*, and *Hemerocallis fulva* - are capable of forming dense monospecific stands, facilitating rapid clonal expansion even in the absence of viable seed production (Mandák et al., 2004; Barney et al., 2006). For instance, *Reynoutria japonica* spreads primarily through rhizome fragments and, despite limited sexual reproduction in many introduced populations, often reaches high dominance through vegetative means (Bailey et al., 2007). Similarly, *Helianthus tuberosus* propagates extensively through underground tubers, enabling rapid colonization of riverbanks and anthropogenic habitats (Abdalla et al., 2014). Field observations confirmed the presence of these two species at a single site along Lake Griviţa (Colentina chain), where they form compact, expanding patches (Figure 2D). In the same location, *Helianthus tuberosus* was found growing in association with *Reynoutria japonica*, contributing to the formation of dense and persistent plant communities. Similarly, *Hemerocallis fulva* was recorded as an isolated population along the Argeș River, yet composed of numerous individuals (Figure 2H). It was located near semi-natural riparian vegetation dominated by

Rubus caesius, *Populus alba*, *Populus nigra*, *Salix* spp., *Cornus sanguinea*, and *Rosa canina*, suggesting a higher ecological compatibility with habitat type NATURA 2000 92A0 – *Salix alba* and *Populus alba* galleries.

Some taxa, such as *Campsis radicans* (Figure 2E), *Parthenocissus inserta*, and *Commelina communis* (Figure 2F), combine vegetative propagation (via layering or stem fragmentation) with seed reproduction, offering a dual strategy that enhances resilience to environmental stress and mechanical disturbance. *Campsis radicans* was observed expanding along the shores of Lake Plumbuita (Colentina chain), where it colonizes disturbed riverbank slopes. *Parthenocissus inserta* is relatively abundant on the banks of the Dâmbovița River, particularly in urban sections, and also occurs frequently along the Colentina riverbanks, often climbing over concrete banks. *Commelina communis* was recorded outside the urban core, along the Dâmbovița River near Chitila locality, where it grows within vegetation assignable to the *Rubi-Salicetum cinereae* association, indicating its capacity to integrate into semi-natural habitats.

In contrast, species such as *Ipomoea purpurea* (Figure 2G) and *Alcea rosea* (Figure 2C) rely predominantly on generative reproduction. Their seeds exhibit long-term viability, allowing them to persist in the soil and germinate when conditions become favorable, even in the absence of established adult individuals (Sohrabi et al., 2025). This reproductive strategy is well-suited to disturbed environments, where periodic substrate exposure facilitates seedling establishment. *Ipomoea purpurea* was observed with moderate abundance but low frequency along the Argeș River, growing both in human-disturbed zones and within patches of spontaneous vegetation, reflecting its ability to colonize a range of habitats through seed dispersal. Similarly, *Alcea rosea* was recorded on the banks of the Dâmbovița River, particularly in peripheral areas of Bucharest, where it was found growing in cracks between concrete plates along flood protection dikes. Its presence in these highly modified, ruderal environments highlights the role of seed persistence and germination in enabling colonization of artificial substrates and periodically disturbed sites.

Overall, the predominance of multiple dispersal vectors and mixed reproductive strategies among alien ornamental plants observed along Bucharest's urban and peri-urban rivers underscores their high ecological plasticity and potential for long-term establishment. These functional traits collectively illustrate why ornamental alien species have successfully established populations along the riparian habitats studied. Understanding these traits provides crucial insights into their potential ecological impacts and aids in the development of effective management and conservation strategies in urban riverine ecosystems (Pyšek et Prach, 1993).

Distribution Patterns and Abundance

Alien species distribution varies substantially among rivers, influenced by urbanization intensity, habitat connectivity, and riparian management (Guida-Johnson et al., 2017). Colentina showed the highest species richness (17 taxa), followed by Argeș (15 taxa) and Dâmbovița (13 taxa) (Table 1)

Table 1 also presents the frequency and mean abundance of each species along the three rivers. Several species demonstrated strong patterns of establishment:

- *Humulus scandens* was found in 16 plots on Dâmbovița (40%) with a very high mean abundance (55%), but was much less common on Argeș (10 plots; 20%) and rare on Colentina (10%).
- *Ailanthus altissima* occurred widely, with 14 plots on Argeș (28%), 10 on Dâmbovița (25%), and 5 on Colentina (50%), suggesting high ecological plasticity.
- *Robinia pseudoacacia* formed dense stands along the Argeș River, particularly in peri-urban areas subject to human disturbance, where it reached abundance values exceeding 60% in several plots. In some cases, these occurrences were associated with former or existing *Robinia* plantations.
- Conversely, *Alcea rosea* and *Campsis radicans* were observed in only a few locations (5 and 1 plots, respectively), with low abundance (<5%).

The Colentina River, characterized by its artificial lakes and landscaped banks, hosted the most prominent ornamental species, like *Campsis radicans*, *Reynoutria japonica*, *Solidago canadensis*.

A better visualization of the spatial occurrence of the 10 most visually prominent ornamental alien species present on the riverbanks can be seen in the distribution map (Figure 1). The selected species refers to *Phytolacca americana* (Figure 2A), *Solidago canadensis* (Figure 2B), *Alcea rosea* (Figure 2C), *Helianthus tuberosus, Reynoutria japonica* (Figure 2D), *Campsis radicans* (Figure 2E), *Commelina communis* (Figure 2F), *Ipomoea purpurea* (Figure 2G), *Hemerocallis fulva* (Figure 2H), and *Oenothera biennis*. Their mapping highlights isolated but often strategically placed occurrences, typically near human settlements, gardens, or urban parks that facilitate accidental introduction and establishment (Barudanović et al., 2021). While these species currently show low frequency and localized abundance (Table 1), their spatial presence in accessible and dynamic urban-riparian mosaics may facilitate further spread under favorable ecological or anthropogenic conditions. Thus, Figure 1 serves not only as a visual tool but also as an early warning signal for species potentially transitioning from casual occurrence to broader naturalization.

The mapping results of the ten ornamental species highlight the proximity of several species identified along the studied rivers to protected natural areas. Specifically, *Alcea rosea* was found along the Colentina River near the protected area ROSCI0308 Lake and Forest Cernica, while *Hemerocallis fulva*, *Ipomoea purpurea*, and *Oenothera biennis* occur on the banks of the Argeș River close to Comana Natural Park (Figure 1). This spatial proximity may enhance propagule pressure, increasing the likelihood of invasive colonization of adjacent natural habitats (Fensham et al., 2013). Species such as *Oenothera biennis* and *Hemerocallis fulva* are already recognized internationally for their invasive potential in riparian ecosystems, capable of rapidly outcompeting native vegetation and altering community structure (Mihulka et al., 2006). Consequently, the presence of ornamental alien species near protected areas emphasizes the urgent need for continuous monitoring, preventive actions, and targeted management interventions to preserve biodiversity and ecosystem integrity in these vulnerable urban and peri-urban riparian zones.

The presence of ornamental alien species along the urban and peri-urban riverbanks of Bucharest highlights the dual ecological role of riparian zones: as both conduits for dispersal and hotspots of invasion. The patterns observed in this study confirm that horticulture remains a key pathway for the introduction of alien flora in European cities (van Kleunen et al., 2018). Species cultivated in gardens, parks, and roadside plantations can readily escape cultivation and establish in nearby natural or semi-natural areas, especially where disturbance, fragmentation, and hydrological connectivity create favorable conditions (Säumel & Kowarik, 2010; Castro-Díez et al., 2014).

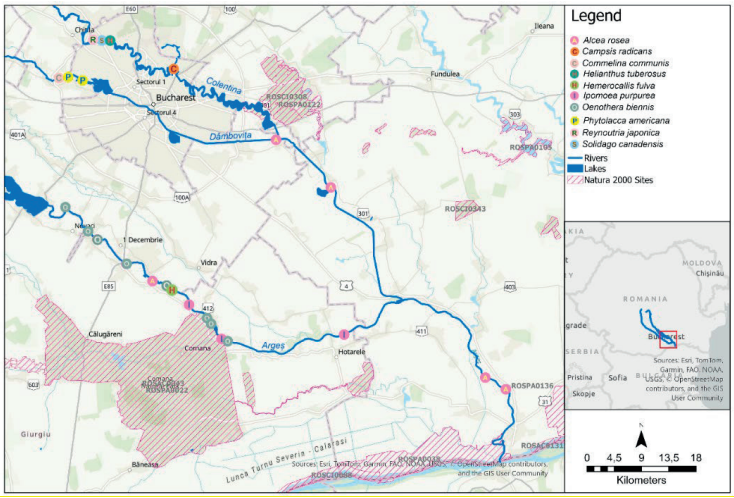


Figure 1. Distribution of selected ornamental alien plant species along urban and peri-urban riverbanks of Bucharest, Romania

Table 1. Functional traits, frequency and abundance of ornamental alien plant species recorded along riverbanks/

No.	Taxon	Inv. status	Life Form	Origin	Seed dispersal	Repr.	Frequency	Abundance
1	*Acer negundo* L	i	Ph	N Am.	An, Ap, Hy	V, G	AG: Rare (16.0%), DB: Rare (22.5%), CL: Moderate (40.0%), General: Rare (21.0%)	AG: Low (2.5%), DB: Low (1.5%), CL: Low (2.5%), General: Low (2.1%)
2	*Aesculus hippocastanum* L.	c	Ph	Balk	Ap, Z	V, G	AG: Very rare (0.0%), DB: Very rare (0.0%), CL: Very rare (10.0%), General: Very rare (2.0%)	AG: Very low (0.0%), DB: Very low (0.0%), CL: Low (5.0%), General: Low (5.0%)
3	*Ailanthus altissima* (Mill.) Swingle	i	Ph	As	An, Ap, Hy	V, G	AG: Moderate (28.0%), DB: Moderate (22.5%), CL:Rare (50.0%), General: Moderate (24.0%)	AG: High (16.88%), DB: High (15.56%), CL: Low (1.9%), General: High (11.98%)
4	*Alcea rosea* L.	c	H	As	Ap, Z	G	AG: Very rare (6.0%), DB: Very rare (5.0%), CL: Very rare (0.0%), General: Very rare (4.0%)	AG: Very low (0.5%), DB: Very low (0.5%), CL: Very low (0.0%), General: Very low (0.5%)
5	*Amorpha fruticosa* L.	i	Ph	N Am	An, Hy, Z	V, G	AG: Moderate (28.0%), DB: Moderate (12.5%), CL: Rare (30.0%), General: Moderate (20.0%)	AG: High (16.88%), DB: High (24.5%), CL: Low (2.0%), General: High (16.19%)
6	*Campsis radicans* (L.) Bureau	c	Ph	N Am	An, Ap, Hy	V, G	AG: Very rare (0.0%), DB: Very rare (0.0%), CL: Very rare (10.0%), General: Very rare (2.0%)	AG: Very low (0.0%), DB: Very low (0.0%), CL: Low (5.0%), General: Low (5.0%)
7	*Commelina communis* L.	n	T	Temp As	Ap, Hy, Z	V, G	AG: Very rare (0.0%), DB: Very rare (2.5%), CL: Very rare (0.0%), General: Very rare (0.5%)	AG: Very low (0.0%), DB: Very low (0.5%), CL: Very low (0.0%), General: Very low (0.5%)
8	*Elaeagnus angustifolia* L.	i	Ph	As	An, Ap, B, Ez, Hy	V, G	AG: Very rare (4.0%), DB: Very rare (0.0%), CL: Very rare (0.0%), General: Very rare (2.0%)	AG: Very low (0.5%), DB: Very low (0.0%), CL: Very low (0.0%), General: Very low (0.5%)
9	*Fraxinus pennsylvanica* Marshall	i	Ph	N Am	An, Hy	V, G	AG: Rare (12.0%), DB: Moderate (42.5%), CL: Very rare (10.0%), General: Rare (24.0%)	AG: Very low (0.5%), DB: Low (0.74%), CL: Very low (0.5%), General: Low (0.71%)
10	*Gleditsia triacanthos* L.	i	Ph	N Am	An, Ap	V, G	AG: Rare (12.0%), DB: Rare (17.5%), CL: Very rare (10.0%), General: Rare (14.0%)	AG: High (6.67%), DB: Moderate (3.64%), CL: Very low (0.5%), General: Low (4.02%)
11	*Helianthus tuberosus* L.	i	H	N Am	An, Hy, Z	V, G	AG: Very rare (0.0%), DB: Very rare (0.0%), CL: Very rare (10.0%), General: Very rare (2.0%)	AG: Very low (0.0%), DB: Very low (0.0%), CL: Very low (0.5%), General: Very low (0.5%)
12	*Hemerocallis fulva* (L.) L.	c	H	E As	Ap, Hy, Z	V, G	AG: Very rare (2.0%), DB: Very rare (0.0%), CL: Very rare (0.0%), General: Very rare (0.5%)	AG: Low (5.0%), DB: Very low (0.0%), CL: Very low (0.0%), General: Low (5.0%)
13	*Humulus scandens* (Lour.) Merr.	i	T	E As	Hy	G	AG: Moderate (20.0%), DB: Frequent (40.0%), CL: Rare (10.0%), General: Moderate (26.0%)	AG: Moderate (17.9%), DB: Very high (55.0%), CL: Very high (87.5%), General: High (41.1%)
14	*Ipomoea purpurea* (L.) Roth	c	T	Trop-Am	An, Ap, Hy, Z	G	AG: Rare (10.0%), DB: Very rare (0.0%), CL: Very rare (0.0%), General: Very rare (4.0%)	AG: Moderate (8.3%), DB: Very low (0.0%), CL: Very low (0.0%), General: Low (8.3%)
15	*Lycium barbarum* L.	i	Ph	E As	An, Ap, Hy, Z	V, G	AG: Very rare (0.0%), DB: Very rare (0.0%), CL: Very rare (10.0%), General: Very rare (2.0%)	AG: Very low (0.0%), DB: Very low (0.0%), CL: Moderate (17.5%), General: Moderate (17.5%)
16	*Morus alba* L.	i	Ph	E As	Ap, Hy, Z	V, G	AG: Moderate (30.0%), DB: Rare (15.0%), CL: Very rare (20.0%), General: Moderate (22.0%)	AG: Low (4.88%), DB: Very low (0.5%), CL: Very low (0.5%), General: Low (2.85%)
17	*Morus nigra* L.	n	Ph	SW As	Ap, Hy, Z	V, G	AG: Rare (14.0%), DB: Very rare (7.5%), CL: Very rare (0.0%), General: Rare (10.0%)	AG: Low (2.92%), DB: Very low (0.5%), CL: Very low (0.0%), General: Low (2.0%)
18	*Oenothera biennis* L	i	Th	N Am	Ap, Hy, Z	V, G	AG: Rare (16.0%), DB: Very rare (0.0%), CL: Very rare (0.0%), General: Rare (6.0%)	AG: Low (1.94%), DB: Very low (0.0%), CL: Very low (0.0%), General: Low (1.94%)

No.	Taxon	Inv. status	Life Form	Origin	Seed dispersal	Repr.	Frequency	Abundance
19	*Parthenocissus inserta* (A. Kerner) Fritsch	i	Ph	N Am	Ap, Hy, Z	V, G	AG: Very rare (0.0%), DB: Rare (17.5%), CL: Moderate (70.0%), General: Moderate (28.0%)	AG: Very low (0.0%), DB: Moderate (8.79%), CL: Moderate (7.14%), General: Moderate (8.0%)
20	*Parthenocissus quinquefolia* (L.) Planch.	c	Ph	N Am	Ap, Hy, Z	V, G	AG: Very rare (2.0%), DB: Very rare (0.0%), CL: Very rare (0.0%), General: Very rare (0.5%)	AG: Low (5.0%), DB: Very low (0.0%), CL: Very low (0.0%), General: Low (5.0%)
21	*Phytolacca americana* L.	i	H	N Am	Ap, Ez, Hy,	V, G	AG: Very rare (0.0%), DB: Very rare (5.0%), CL: Very rare (0.0%), General: Very rare (2.0%)	AG: Very low (0.0%), DB: Very low (0.5%), CL: Very low (0.0%), General: Very low (0.5%)
22	*Reynoutria japonica* Houtt.	i	GH	E As	An, Ap, Hy	V (main), G	AG: Very rare (0.0%), DB: Very rare (0.0%), CL: Very rare (10.0%), General: Very rare (2.0%)	AG: Very low (0.0%), DB: Very low (0.0%), CL: Low (5.0%), General: Low (5.0%)
23	*Robinia pseudoacacia* L.	i	Ph	E As	An, Ap, B, Hy, Z	V, G	AG: Moderate (28.0%), DB: Very rare (2.5%), CL: Very rare (10.0%), General: Moderate (18.0%)	AG: Very high (13.0%), DB: Very low (0.5%), CL: Very low (0.5%), General: Low (10.3%)
24	*Salix babylonica* L.	c	Ph	E As	Ap	V, G	AG: Rare (12.0%), DB: Rare (7.5%), CL: Rare (30.0%), General: Rare (12.0%)	AG: Low (3.58%), DB: Moderate (14.3%), CL: Low (2.0%), General: Low (6.0%)
25	*Solidago canadensis* L.	i	H	N Am	An, Ap	V, G	AG: Very rare (0.0%), DB: Very rare (0.0%), CL: Very rare (10.0%), General: Very rare (2.0%)	AG: Very low (0.0%), DB: Very low (0.0%), CL: Very low (0.5%), General: Very low (0.5%)
26	*Symphoricarpos albus* (L.) S.F.Blake	c	Ph	N Am	Ap, Z	V, G (less)	AG: Very rare (0.0%), DB: Very rare (0.0%), CL: Very rare (20.0%), General: Very rare (4.0%)	AG: Very low (0.0%), DB: Very low (0.0%), CL: Very low (0.5%), General: Very low (0.5%)

Abbreviations: Invasive status (Inv. status) (in Romania): c- casual, n- naturalized, i – invasive. Life form: G – Geophyte, H - Hemicryptophyte, Ph – Phanerophyte, T – Therophyte, TH – Hemitherophytes. Origin: Am – America, As – Asia, Balk – Balkan Peninsula, Trop – Tropical, N – North, E – East, S – South, W – West. Seed dispersal: An = Anemochore, Hy = Hydrochore, Ap = Anthropochore, Z = Zoochore, Ez = Endozoochore, B = Barochore, Reproduction (Repr.): G = Generative, V = Vegetative, V (main) = Vegetative (mainly), G (less) = Generative (less...)



Figure 2. Representative alien ornamental plant species with high decorative value observed along urban and peri-urban riverbanks in Bucharest: (A) *Phytolacca americana*, (B) *Solidago canadensis*,(C) *Alcea rosea*, (D) *Reynoutria japonica* and *Helianthus tuberosus*, (E) *Campsis radicans*, (F) *Commelina communis*, (G) *Ipomoea purpurea*, (H) *Hemerocallis fulva*

The high occurrence of species such as *Ailanthus altissima*, *Amorpha fruticosa*, and *Humulus scandens* - all capable of vegetative spread - demonstrates the relevance of reproductive strategies in determining invasion success. Their frequent association with highly disturbed areas supports earlier findings that propagule pressure and human activity are major drivers of ornamental species escape (Essl et al., 2019; Pyšek et al., 2020). Interestingly, several species with strong horticultural value (*Campsis radicans*, *Alcea rosea*, *Hemerocallis fulva*) were recorded with low frequency, yet their distribution near human-modified landscapes may signal early stages of naturalization. This underlines the importance of early detection and monitoring efforts, particularly for taxa that are not yet widespread but possess traits associated with invasiveness. The distinct distribution patterns between rivers also reflect differences in land use and hydrological structure. The Colentina, with its string of urban lakes and recreational spaces, hosted a larger number of decorative species with low abundance but high aesthetic value, likely due to landscape plantings. In contrast, the more naturalized

sections of the Argeș and Dâmbovița featured higher cover values of aggressive invaders such as *Amorpha fruticosa*, *Robinia pseudoacacia*, *Humulus scandens*, *Parthenocissus inserta*.

CONCLUSIONS

This study highlights the presence and ecological behavior of 26 ornamental alien plant species established along the riparian corridors of Bucharest. Our results show that both urban design and ecological traits shape the distribution and persistence of these species. Frequent and abundant taxa such as *Ailanthus altissima*, *Amorpha fruticosa*, and *Humulus scandens* demonstrate high adaptability and suggest a strong potential for long-term establishment and expansion. Conversely, species with restricted presence but high aesthetic value (*Alcea rosea*, *Campsis radicans, Hemerocallis fulva, Solidago canadensis* etc.) exemplify the risk of unnoticed transitions from cultivation to naturalization. The urban and peri-urban riverbanks of the Argeș, Dâmbovița, and Colentina rivers act as both entry points and dispersal corridors for alien ornamental plant species. While certain taxa remain limited in distribution, others exhibit traits associated with invasiveness, especially in disturbed or unmanaged riparian stretches. Tailored management interventions, based on site-specific data and continuous botanical monitoring, are needed to mitigate the risks posed by these species and to preserve the ecological integrity of riparian habitats in Bucharest.

REFERENCES

Abdalla, N., Domokos-Szabolcsy, É., El-Ramady, H., Hodossi, S., Fári, M., Ragab, M., & Taha, H. (2014). Jerusalem artichoke (*Helianthus tuberosus* L.): A review of *in vivo* and *in vitro* propagation. *International Journal of Horticultural Science*, 20(3-4), 131-136.https://doi.org/10.31421/IJHS/20/3-4/1148

Astegiano, J., Funes, G., & Galetto, L. (2010). Comparative studies on plant range size: linking reproductive and regenerative traits in two *Ipomoea* species. *Acta Oecologica*, *36*(5), 454-462.

Bailey, J.P., Bímová, K. & Mandák, B. (2007) The potential role of polyploidy and hybridisation in the further evolution of the highly invasive *Fallopia* taxa in Europe. *Ecol Res* **22**, 920–928 (2007). https://doi.org/10.1007/s11284-007-0419-3

Barney, J. N., Tharayil, N., DiTommaso, A., & Bhowmik, P. C. (2006). The biology of invasive alien plants in Canada. 5. *Polygonum cuspidatum* Sieb. & Zucc. [= *Fallopia japonica* (Houtt.) Ronse Decr.]. *Canadian Journal of Plant Science*, 86(3), 887–905. https://doi.org/ 10.4141/P05170

Barudanović, S., Zečić, E., Macanović, A., Duraković, B., & Mašić, E. (2021). Invasive alien plant species in global perspectives with special references to Bosnia and Herzegovina. Invasive Alien Species: Observations and Issues from Around the World, 3, 215-252.

Castro-Díez, P., Godoy, O., Saldaña, A., & Richardson, D.M. (2014). Predicting invasiveness of Australian acacias on the basis of their native climatic affinities, life history traits and human use. *Diversity and Distributions*, 20(7), 798-808. https://doi.org/ 10.1111/j.1472-4642.2011.00778.x

Dumitrașcu, M., Cîșlariu, A. G., Mihai, D. C., & Sârbu, A. (2024). Anatomical insights into the adaptability and invasiveness of *Humulus scandens* in Romanian flora. *Notulae Scientia Biologicae*, *16*(4), 12157-12157. https://doi.org/10.55779/nsb16412157

Essl, F., Bacher, S., Blackburn, T. M., et al. (2019). Drivers of future alien species impacts: An expert-based assessment. *Global Change Biology*, 26(2), 488–502. https://doi.org/10.1111/gcb.15199

Ewel, K.C., Cressa, C., Kneib, R.T., Lakes, P.S., Levin, L.A.,Palmer, M.A., Snelgrove, P. & Wall, D.H. (2001). Managing critical transition zones. *Ecosystems,* 4, 452-460.

Fensham, R. J., Donald, S., & Dwyer, J. M. (2013). Propagule pressure, not fire or cattle grazing, promotes invasion of buffel grass *Cenchrus ciliaris*. *Journal of Applied Ecology*, *50*(1), 138-146. https://doi.org/ 10.1111/1365-2664.12009

Guida-Johnson, B., Faggi, A. M., & Zuleta, G. A. (2017). Effects of urban sprawl on riparian vegetation: is compact or dispersed urbanization better for biodiversity? *River Research and Applications*, *33*(6), 959-969. https://doi.org/10.1002/rra.3138

Hulme, P.E., Brundu, G., Carboni, M., Dehnen-Schmutz, K., Dullinger, S., Early, R., … Verbrugge, L.N. H. (2018). Integrating invasive spe cies policies across ornamental horticulture supply chains to prevent plant invasions. *Journal of Applied Ecology,* 55, 92–98. https://doi Org/10.1111/1365-2664.12953

Landenberger, R. E., Kota, N. L., & McGraw, J. B. (2007). Seed dispersal of the non-native invasive tree *Ailanthus altissima* into contrasting environments. *Plant Ecology*, 192, 55-70. http://dx.doi.org/ 10.1007/s11258-006-9226-0

Mandák et al., 2004: Mandák, B., Pyšek, P., & Bímová, K. (2004). History of the invasion and distribution of Reynoutria taxa in the Czech Republic: A hybrid spreading faster than its parents. *Preslia*, 76(1), 15–64.

Mihulka, S., Pyšek, P., Martínková, J., & Jarošík, V., 2006. Invasiveness of Oenothera congeners alien to Europe: Jack of all trades, master of invasion? *Perspectives in Plant Ecology, Evolution and Systematics*, 8(2), 83-96. https://doi.org/10.1016/ j.ppees.2006.08.00 3

Miroshnyk, N., Grabovska, T., & Roubík, H. (2025). The spread of the invasive species *Reynoutria japonica* Houtt. will both expand and contract with climate change: results of climate modelling for 14 European countries. *Pest Management Science*. https://doi.org/10.1002/ps.8732

Popay, I. (2022). *Helianthus tuberosus* (Jerusalem artichoke). CABI Compendium, CABI Compendium. https://doi.org/10.1079/cabicompendium.26716

Priede, A. (2008). Distribution of some invasive alien plant species in riparian habitats in Latvia. *Botanica Lithuanica* (1392-1665), *14*(3).

Pyšek, P., & Prach, K. (1993). Plant invasions and the role of riparian habitats: a comparison of four species alien to Central Europe. *Journal of biogeography*, 413-420.

Pyšek, P., Hulme, P. E., et al. (2020). Scientists' warning on invasive alien species. *Biological Reviews*, 95(6), 1511–1534. doi: 10.1111/brv.12627

Radu, M., & Stoiculescu, R. C. (2010). Landscape changes in Colentina river basin (between Buftea and the confluence with Dambovita) as reflected in cartographic documents (1791-2000). *Present Environment & Sustainable Development*, *4*(2).

Raicu, M., Camen-Comănescu, P., Urziceanu, M., Anastasiu, P., & Toma, F. (2024). The Role of Ornamental Horticulture in Plant Invasion: A Case Study in Romania. *Scientific Papers. Series B. Horticulture*, *68*(2).

Richardson, D. M., Holmes, P.M., Esler, K.J., Galatowitsch, S.M., Stromberg, J.C., Kirkman, S.P.,....& Hobbs, R.J. (2007). Riparian vegetation: degradation, alien plant invasions, and restoration prospects. *Diversity and distributions*, *13*(1), 126-139. https://doi.org/10.1111/j.1366-9516.2006.00314.x

Rundel, P. W., Dickie, I. A., & Richardson, D. M. (2014). Tree invasions into treeless areas: mechanisms and ecosystem processes. *Biological invasions*, 16, 663-675. https://doi.org/10.1007/s10530-013-0614-9

Rusnák, M., Goga, T., Michaleje, L., Šulc Michalková, M., Máčka, Z., Bertalan, L., & Kidová, A. (2022). Remote sensing of riparian ecosystems. *Remote Sensing*, *14*(11), 2645.

Säumel, I., & Kowarik, I. (2010). Urban rivers as dispersal corridors for primarily wind-dispersed invasive tree species. *Landscape and Urban Planning*, *94*(3-4), 244-249.

Säumel, I., & Kowarik, I. (2013). Propagule morphology and river characteristics shape secondary water dispersal in tree species. *Plant Ecology*, *214*, 1257-1272. https://doi.org/10.1007/s11258-013-0249-z

Sohrabi, S., Yazlık, A., Bazos, I., Gherekhloo, J., Kati, V., Kitiş, Y. E., Arianoutsou, M., Kortz, A., & Pyšek, P. (2025). Alien species of Ipomoea in Greece, Türkiye and Iran: Distribution, impacts and management. *NeoBiot*a, 97, 135–160. https://doi.org/10.3897/neobiota.97.131827

Strayer, D. L. (2010). Alien species in fresh waters: ecological effects, interactions with other stressors, and prospects for the future. *Freshwater biology, 55, 152-174.* https://doi.org/10.1111/j.1365-2427.2009.02380.x

Van Kleunen, M., Essl, F., et al. (2018). The changing role of ornamental horticulture in alien plant invasions. *Biological Reviews*, 93(3), 1421–1437. https://doi.org/10.1111/brv.12402

.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

THE MORPHOLOGICAL STRUCTURE OF THE RENEWAL BUD IN *KNIPHOFIA NELSONII* MAST.

Irina SFECLĂ

"Alexandru Ciubotaru" National Botanical Garden (Institute) of the State University of Moldova, 18 Pădurii Str., Chişinău, Republic of Moldova

Corresponding author email: irina.sfecla@gb.usm.md

Abstract

The study of the ornamental plants morphogenesis is of great importance because it allows the control of growth and development processes, and correction, adjustment and application of cultivation technology. The architecture of the bioform is determined by the vegetative organs. The vital form of the plant is an indicator of vital processes in the organism (Cernei 1994). During most of the year, physiological processes are concentrated in underground organs. Due to this aspect, research was carried out on the rhizome of Kniphofia nelsonii Mast. (red hot poker), in particular, of the buds of renewal. During this study were examined: the morphological aspects of the root; the morphological aspect of the rhizome; the type of renewal buds; its development method during the plant`s vegetative and dormant periods (August, November and February); their shape and disposition; presence or absence of cataphylls; their number, shape, size and their protective role; the type of proliferation and foliation.

Key words*: Kniphofia Mast., morphology, renewal bud.*

INTRODUCTION

Currently, the sustainable use, conservation and the enrichment of the diversity of ornamental plant species cultivated *ex situ* is of vital importance. Through ornamental plants, humans ensure a pleasant life, decorating his place of residence, work, cities, rural localities, etc. The study of the behavior of plants in *ex situ* conditions, mobilized from various floristic regions of the globe in areas with pedoclimatic conditions different from those of origin, is always relevant both theoretically and practically. The above-mentioned outlines the need to study the biological potential of plants in the pedoclimatic conditions of the Republic of Moldova. The introduction of plants, appreciated as a vast ecological and phytogeographic experimentation, provides a multitude of data for the researcher, and the analysis of the data allows drawing conclusions on the adaptive potency and ecological amplitude of organisms (Cernei, 1994a).

Research on the introduction of representatives of the genus *Kniphofia* Moench in the Republic of Moldova was initiated in 1979, by acclimatizing the species *Kniphofia uvaria* (L.) Oken within the ornamental plant collection of the Al. Ciubotaru National Botanical Garden (Institute) (NBGI), obtained through the international seed exchange (*Index Seminum*) (Chernej, 1987; Sfeclă, 2016, 2021). Later, through the *Index Seminum* and purchases, other representatives of the genus *Kniphofia* also ennobled the NBGI exhibitions, such as: *K. ensifolia* Baker; *K. tuckii* Baker; *K. nelsonii* Mast.; *K. citrina* Baker; *K. sarmentosa* Baker; *K. galpinii* Baker; *K. hirsuta* Codd; *K. thodei* Baker; *K. ´*Royal Standart*´* (Syrbu and Sfekla, 2008; Syrbu et al., 2009; Sfeclă, 2016; 2017; Sfeclă and Sîrbu, 2018). As a result of the experimentation of induced mutagenesis in *K. nelsonii*, a form was selected and the variety *K. nelsonii* `Micul Prinț` was patented (Sfeclă and Sîrbu, 2019; Sfeclă et al., 2019; Sfeclă et al., 2022, Sîrbu et al., 2023). Currently, the collection of the genus *Kniphofia* in NBGI lists 11 taxa.

In the process of introducing plants, some of the main indicators are their phenological phases, growth dynamics, ontogenesis and morphogenesis. These indicate the level of adaptability of the species to local conditions.

The study of the morphogenesis of ornamental plants is paramount, as it allows the control of both growth and development processes, including the adjustment and correct application of cultivation technology. The

architectonics of the biomorph is determined by the vegetative organs. The vital form of the plant is an indicator of the vital processes in the organism (Cernei, 1994b). For most of the year, physiological processes are concentrated in the underground organs. In this regard, research has been carried out on the rhizome, in particular, on the renewal buds.

The renewal bud represents the initial phase of the monocarpic shoot – the main element that attributes the kniphofias to the group of hemicryptophyte herbaceous plants. According to the definition proposed by O. Schuepp (1938) (cited by Shilova, 1988), the bud represents the proportions unit of growth, including the development of the growth cone. According to the data presented by T. Serebrjakova (1971), in the buds, the development of leaf primordia occurs, as well as the first stages of growth of leaf blades, internodes, etc. Primary morphogenesis also takes place here.

The species of the genus *Kniphofia* Moench do not differ in ecological and geographical characteristics and present a similar ontogenetic program. The differences are only in quantitative characteristics such as the size of the plant in certain ontogenetic stages, the number of leaves, the thickness in the area of the crown, etc. In this regard, research was carried out on the rhizome and especially on the renewal bud of *Kniphofia nelsonii*. During this study, the following were examined: morphological aspects of the root; morphological appearance of the rhizome; type of renewal buds; mode of its development during the vegetative and dormant periods of the plant (August, November and February); their shape and arrangement; presence or absence of cataphylls; number, shape, size and their protective role; type of proliferation and foliation.

***Kniphofia nelsonii* Mast.** 1892, Gardeners Chronical, 1: 554; Baker, 1896, Flora Capensis, 6: 280; Berger, 1908, Pflanzenreich, 4: 53 (Baker 1896; Berger 1908; Codd 1968, 2005) – *Kniphofia triangularis* subsp. *triangularis* (Germishuizen and Meyer, 2003) (Figure 1).

Herbaceous, acaulescent, rosulate plants. Orthotropic, thick, abbreviated rhizomes, from which the tuberized roots start laterally. Leaves in rosettes of 12-16, arranged distich-alternate, glabrous, canaliculate-linear, rigid, erect or geniculate, green-glauce, 40-50 cm long, 2 cm wide and ca. 2 mm thick, with three prominent veins (one central more furrowed and two lateral), V-shaped in cross section, the margin and the keel serrated. Cylindrical, simple, accreting floral peduncles, several flowerless bracts are present below the inflorescence. Inflorescences – terminal spiciform racemes, cylindrical, oblong, dense, narrowed towards the apex, acropetal flowering. Membranous, oblong-lanceolate, three-veined bracts. Floral buds first erect, then horizontal, dense, red. Actinomorphic, bisexual, pendulous, deflected flowers, looser than floral buds, yellow-green, with a green vein on each lobe of the perigone, odorless. Pedicels 2-3 mm, patent, curved, accreting in the fruiting phase. Perigone tubular, narrowed above the ovary, the 6 perigonial lacinia flat, ovate, slightly recurved. Stamens – 6, bifid, all fertile, inserted at the base of the ovary, included, exserted in anthesis. Styles exserted. Stigmata trilobate. Ovary trilocular, glabrous, ovoid, truncated at the base, narrowed at the tip. Fruit – dehiscent, subglobose capsule (Sfeclă, 2017).

Bioecology: perennial, V-VI, hemicryptophyte, xeromesophyte (Pînzaru and Sîrbu, 2016). n = 6, 12, 24 (Nayak, 1988).

Phytogeographic element: South Africa

General distribution: Free State, Kwa Zulu-Natal, Lesotho, Eastern Cape provinces. It is found at altitudes of 910 - 2895m. (Germishuizen and Meyer, 2003).

A characteristic of kniphofias, originating from South Africa, is the decorativeness of the flowers and leaves, which allows their use in landscape architecture and bouquet art, through the production of flowers. Within green spaces they can be framed in various elements, such as: solitary specimens, small groups around water bodies, borders and mixed borders (Sfeclă, 2015). *Kniphofia* is one of the cut flower crops that has grown in popularity in recent years, both in the floriculture industry and among consumers (Hettiarachchi and Balas, 2005). This popularity is felt globally, not locally. As a result of research into the specific ornamental assortment existing on the floricultural market in the city of Chisinau, knifofia was not highlighted (Dica et al., 2018).

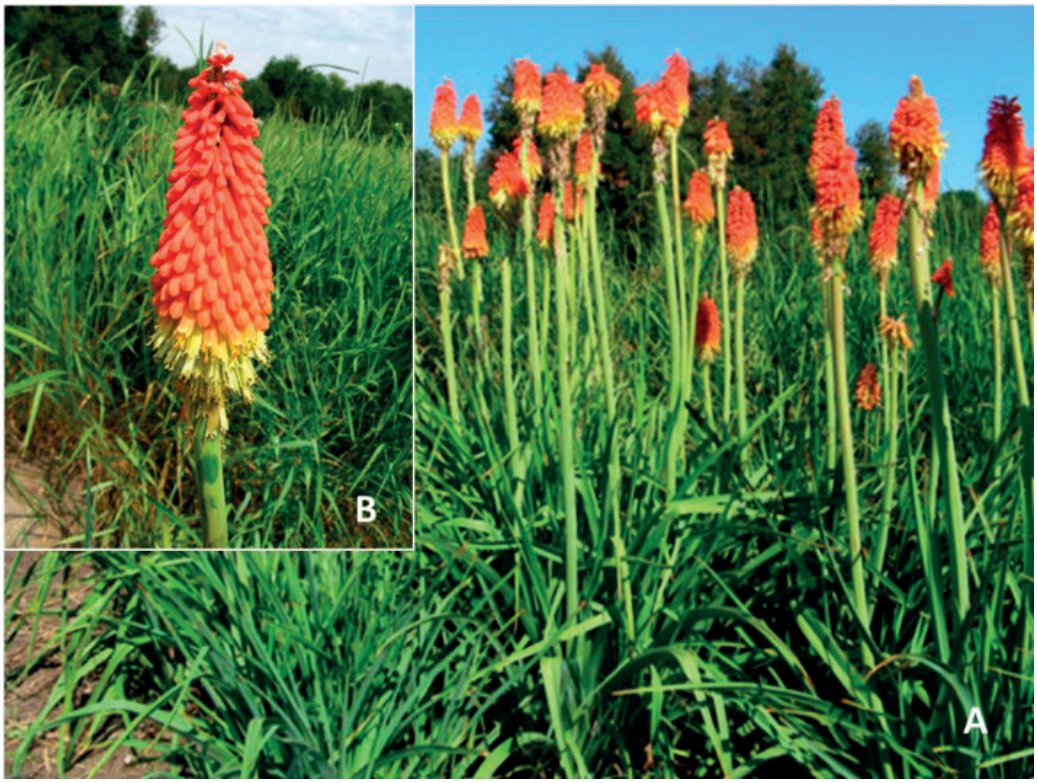


Figure 1. *Kniphofia nelsonii* Mast. in the collections of the Ornamental Plants Laboratory of the NBGI
A – group of plants; B – the inflorescence in the blooming phase

MATERIALS AND METHODS

The research was carried out within the Ornamental Plants Laboratory of the NBGI.

The morphological description was carried out with the help of the works: "*Atlas po opisatel'noj morfologii vysshih rastenij*" (Fjodorov and Artjushenko, 1962); "*Lexicon Botanicum Polyglottum*" (Váczy, 1980) and "*The Kew Plant Glossary: An Illustrated Dictionary of Plant Terms*" (Beentje, 2016).

The scientific name of the species is cited according to the publication "*Authors of plant names*" by R. Brummitt and C. Powell (1992) and global data networks, such as: *International Plant Names Index* (IPNI) (2020) and *Global Biodiversity Information Facility* (GBIF) (2020). The morphology of the renewal bud was analyzed in accordance with the principles of examining morphological processes proposed by T. Serebrjakova (1971). The morphological survey was carried out according to the following model: extraction of specimens from the soil (February, August and November) and preparation of buds in laboratory conditions.

Their thorough research included morphometry, photofixation and description of the structural features of the composing elements. During the work process a manual magnifying glass, an optical microscope Biolam-D-12 and a digital microscope VWR VisiScope-BL254T1 were used.

The sections through the growth cone were manually made with a blade. The study was carried out on the basis of fresh material (Figure 2).

For a more extensive confirmation, the results of the research were consolidated with photographic fixation and analytical drawings.



Figure 2. The process of preparing renewal buds

RESULTS AND DISCUSSIONS

The rhizome is a metamorphosed, underground stem (Fjodorov and Artjushenko, 1962; Morariu, 1973). The rhizome of *Kniphofia*'s constitutes the multiannual basis of these plants. It is the organ of renewal and vegetative propagation as well as a place where the plant deposits reserve substances (Serebrjakov, 1952, 1962).

As a result of the morphological study of the rhizome, we found that it is simple, orthotropic, abbreviated, 4-6 cm thick and 3-5 cm long, reddish-brown in color, from which 12-25 tuberous roots with a diameter of 0.5-0.7 cm radially start.

This aspect suggests that the rhizome represents an effective mechanism of survival and vegetative renewal, which allowed kniphofias to adapt to the specific environmental conditions of the Republic of Moldova. The dimensions and characteristics of the rhizome, including the thickness and number of tuberous roots, indicate a resource conservation strategy, essential in variable pedoclimatic conditions.

Morphological survey of renewal buds in *Kniphofia nelsonii* was carried out in February, August and November, according to the following model: extraction of specimens from the soil, preparation of buds in laboratory conditions. Depending on the position of the buds, in kniphofia we distinguish: apical buds, axillary buds and adventive buds (Figure 3).

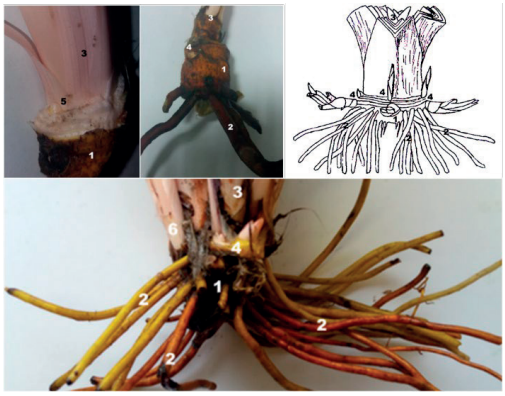


Figure 3. General appearance of the underground part of *Kniphofia nelsonii* Mast.:
1 – rhizome; 2 – tuberous roots; 3 – apical bud covered by persistent leaves; 4 – dormant buds; 5 – axillary bud; 6 – shoot developed from axillary bud

The apical bud (growth cone) (Figure 3) is mixed, solitary, open, with 2-5 leaves in the vegetative phase. It is protected by the base of the persistent leaves of the previous monocarpic shoot. In August, the apical bud has a conical shape and yellowish color. If the specimen has not flowered this year, the bud is placed on the rhizome in the center, otherwise at the base of the floral axis. At this time, leaf primordia and the apical meristem are present (Figure 4 A).

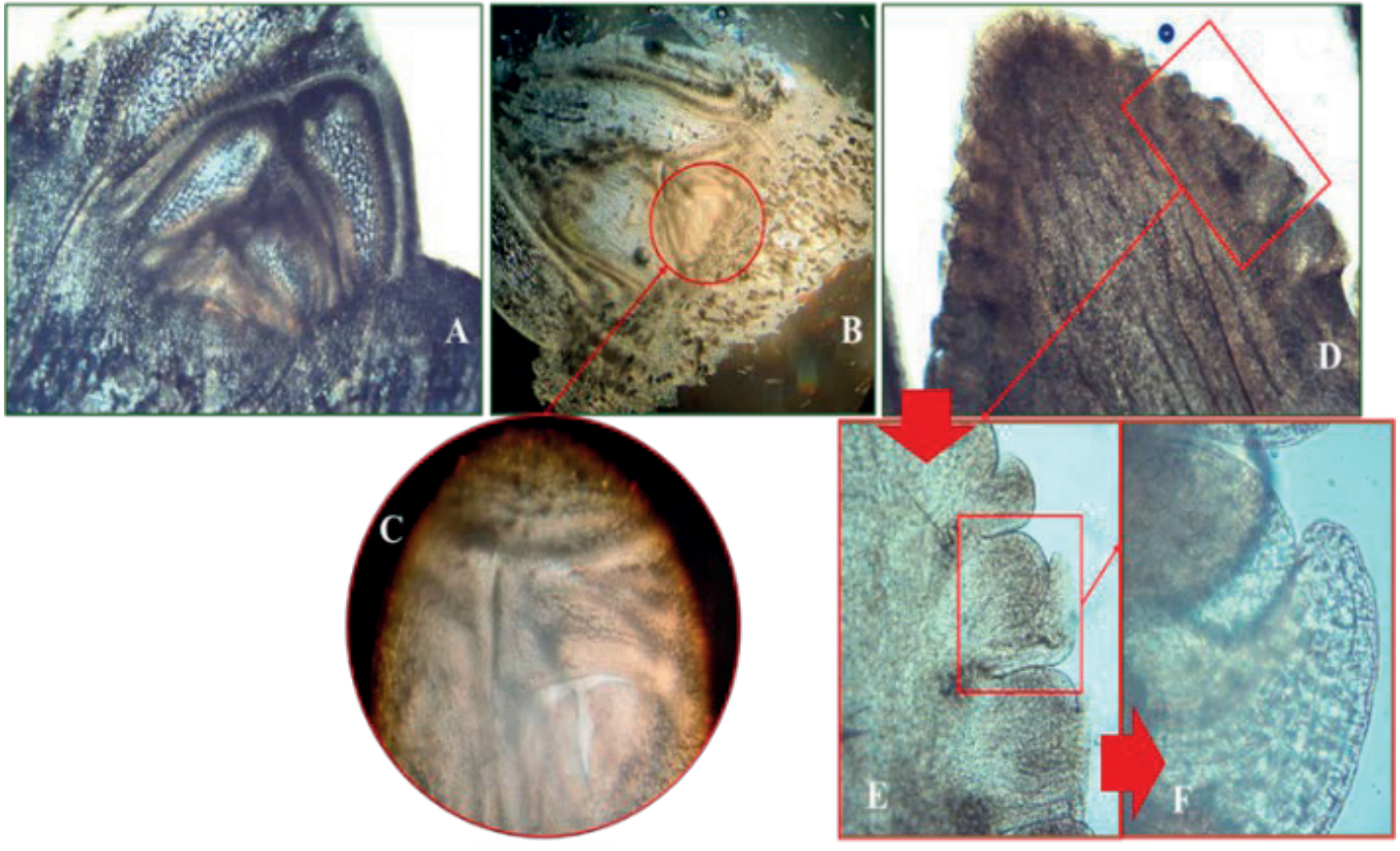


Figure 4. Anatomical structure of an apical bud:

A – apical bud examined in August (40x); B – apical bud examined in November (40x); C – differentiation of the inflorescence in the apical part (60x); D – apical bud examined in February (40x); E – inflorescence differentiated into apical bud examined in February (40x); E – differentiation of the floral primordium (60x); F – floral primordium and bract differentiated (90x)

The buds that were analyzed in November indicate that some essential changes have occurred, such as the presence of 4-6 yellow-green embryonic leaves. The differentiation of the inflorescence begins in the apical part (Figure 4 B-C).

For the apical renewal bud studied in February, it was found that the floral axis is differentiated. The inflorescence presents floral buds, as well as numerous elongated and thickened bracts (Figure 4 C-F).

The morphological changes observed in the apical bud, including the differentiation of inflorescences in the winter months, highlight the dynamic aspects of plant development. This information is viable for understanding the appropriate frequency in the reproductive cycle, which may allow for the optimization of cultivation technology and more efficient planning of the flowering period.

Axillary buds (Figure 3) which develop in the leaf axil, are solitary, and closed. Of all these type of buds developed by the plant in a vegetative season, only a few will develop seedlings in the following year. The others turn into dormant buds (Figures 3, 4, 5 A-B) and remain in the latent phase indefinitely. As a result of the morpho-anatomical analysis of the axillary buds, it was found that they are vegetative (Figure 5 E-F) and present 5-7 protective membranes (Figure 5 C). The distichous arrangement of the leaves is evident when examining the cross-section of the apical and axillary buds (Figure 5 E).

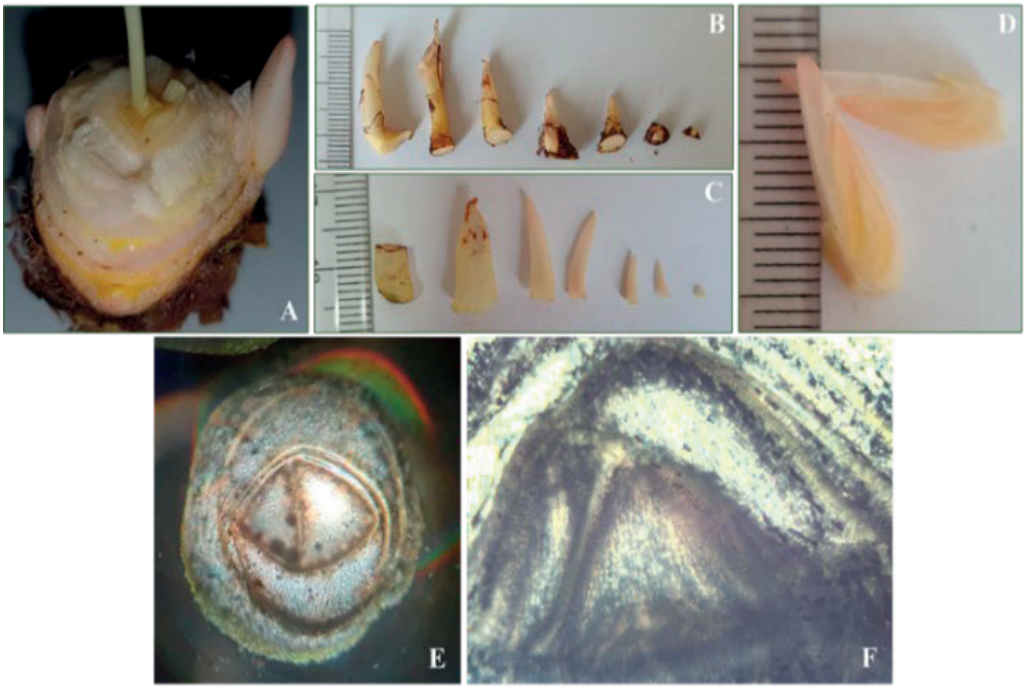


Figure 5. Axillary bud:

A – general appearance of the rhizome with apical bud and axillary buds; B – axillary buds at different stages of development; C – protective membranes of axillary buds; D – axillary bud in longitudinal section; E – axillary bud in transverse section (20x); F – anatomical structure of an axillary bud (40x)

Observations on the morphogenesis of renewal buds suggest that *Kniphofia* species, such as *K. nelsonii*, exhibit increased adaptability to environmental conditions. The identification of apical and axillary buds at different stages of development demonstrates the ability of plants to cyclically adapt to seasonal variability. The presence of dormant and active buds suggests an efficient strategy for managing reserve

substances, which could influence the success of these plants under environmental stress.

CONCLUSIONS

In the pedoclimatic conditions of the Republic of Moldova, *Kniphofia nelsonii* Mast. retains its biomorph (perennial, hemicryptophyte), which is an important indicator of the species' adaptation. This adaptability suggests an increased ecological efficiency, relevant for the use of this ornamental species in landscaping green spaces.

Observations on renewal buds in different periods of the year (February, August and November) revealed processes of inflorescence differentiation, confirming that *Kniphofia nelsonii* presents a clear seasonal cyclicity in morphological development. These data substantiate the technology of cultivation of this species in *ex situ* conditions.

ACKNOWLEDGEMENTS

This research work was carried out with the support of "Al. Ciubotaru" National Botanical Garden (Institute) of the State University of Moldova and also was financed from Project 010101 "Research and conservation *ex situ* and *in situ* of plant diversity in the Republic of Moldova".

REFERENCES

Baker, J. (1896). Flora Capensis. In: Sistematic discription of the plants of the Cape colony, Caffraria, and Port natal [online]. Kent, pp. 275-285, from https://doi.org/10.5962/bhl.title.821

Berger, A. (1908). Liliaceae-Asphodeloideae-Aloineae. In: Pflanzenreich. Regni vegetalis conspectus [online]. Leipzig. pp. 2-71, from https://bibdigital.rjb.csic.es/viewer/11043/?offset=#page=72&viewer=picture&o=bookmark&n=0&q=

Beentje H. (2016). The Kew Plant Glossary: An Illustrated Dictionary of Plant Terms. UK: Royal Botanic Gardens Kew. 192 p. ISBN 978-1-84246-605-6.

Brummitt, R., Powell, C. (1992). *Authors of plant names. Kew: Royal Botanic Gardens*. 731 p. ISBN 1 842460 85 4.

Cernei, E. (1994a). Cu privire la caracteristica fitogeografică a erbaceelor perene ornamentale din Grădina Botanică, Ch. In: *Conferința științifică a botaniștilor. Ch.: Știința*, p. 104.

Cernei, E. (1994b). Contribuții la studiul morfologic al stânjeneilor în perioada autumnală. In: *Congresul I al botaniștilor din Moldova. Chișinău: Știința*, pp. 82-83. ISBN 5-376-01849-0.

Chernej, E. (1987). Kniphofija jagodnaja (*Kniphofia uvaria* (L) – perspektivnoe dekorativnoe rastenie v Maldavii. V: Nauchnye osnovy ozelenenija gorodov i sel Moldavii. Kishinjov, ss. 156-157.

Codd, L. (1968). The South African Species of Kniphofia (Liliaceae). In: *Bothalia*, vol. 9 [online], pp. 363-519. Disponibil: doi.org/10.4102/abc.v9i3/4.1790

Codd, L. (2005). Asphodelaceae: *Kniphofia*. In: Flora of Southern Africa: The Republic of South Africa, Basutoland, Swaziland and South West Africa, Vol. V, Part 1 [online]. 94 p. ISBN 1-919976-03-5, from https://doi.org/10.5962/bhl.title.119879

Dica, A., Sfeclă, I., Șabarov, D., Slivca, V. (2018). The analysis of flower markets in Chișinău. In: *Journal of botany*, Nr. 2 (17). Chișinău, pp. 90-94. ISSN 1857-095X, from https://ibn.idsi.md/sites/default/files/imag_file/90-94_9.pdf

Fjodorov, A, Artjushenko, Z. (1962). Atlas po opisatel'noj morfologii vysshih rastenij: Stebel' i koren'. Moskva – Leningrad: AN SSSR, 350 s.

GBIF [online]. GBIF Home Page, (2017-2020), from https://www.gbif.org

Germishuizen, G., Meyer N. (2003). *Plants of southern Africa: an annotated checklist*. Strelitzia 14. Pretoria: National Botanical Institute, pp. 1008-1010. ISBN 1-919795-99-5.

Hettiarachchi, M., Balas, J. (2005). Postharvest handling of cut kniphofia (Kniphofia uvaria Oken 'Flamenco') flowers. In: *ISHS Acta Horticulturae*, 669: VIII International Symposium on Postharvest Physiology of Ornamental Plants [online], pp. 359-366, from https://www.actahort.org/books/669/669_47.htm

IPNI (2013-2020) [online]. International Plant Names Index, from http://www.ipni.org.

Morariu, I. (1973). *Botanica generală și sistematică*. București: Ceres, 568 p.

Nayak, S. (1988). Response to in vitro methodology and chromosome study of some liliaceous taxa: tz. doct. of philosophy science. Calcutta, 200 p.

Pînzaru, P., Sîrbu, T. (2016). *Flora vasculară din Republica Moldova (lista speciilor și ecologia)*. Chișinău, 261 p. ISBN 978-9975-76-185-7.

Sfeclă, I. (2015). *Kniphofia* Moench species of perspective for arrangement of green areas of Republic of Moldova. In: *Lucrări stiințifice seria Horticultura*, Iași, Romania, NR. 1, Vol. 58, pp. 141-145. ISSN–L=1454-7376, from https://ibn.idsi.md/sites/default/files/imag_file/Vol%2058_1_2015%2822%29.pdf

Sfeclă, I. (2016). Genul *Kniphofia* Moench – istorie și actualitate. In: Simpozionul Științific, Conservarea diversității plantelor *in situ* și *ex situ*. Iași, pp. 48-49.

Sfeclă, I. (2017). Genul *Kniphofia* Moench în colecția de plante netradiționale a Grădinii Botanice (Institut) a A.Ș.M. In: *Știința agricolă*, Nr. 2, Chișinău: UASM, pp. 50-56. ISSN 1857-0003, from https://sa.uasm.md/index.php?journal=sa&page=article&op=view&path[]=559

Sfeclă, I., Sîrbu, T. (2018). *Kniphofia galpinii* Baker – o specie decorativă nouă în Grădina Botanică Națională

(Institut) „Alexandru Ciubotaru”. In: *Lucrări științifice a UASM*: Materialele simpozionului științific internațional „Horticultura modernă – realizări și perspective”, 4-6 octombrie 2018. Chișinău: UASM, pp. 492-497. ISBN 978-9975-64-296-5, from https://ibn.idsi.md/sites/default/files/imag_file/492-497_0.pdf

Sfeclă, I., Sîrbu, T. (2019). Inducerea mutațiilor la *Kniphofia triangularis* Kunth. In: *Abstract book International Scientific Sympozion (Vth Edidion)* “Advanced biotechnologies – achievements and prospects”, October 21-22. Chișinău: Print-Caro, p. 59. ISBN 978-9975-56-695-7, from https://ibn.idsi.md/sites/default/files/imag_file/59-59_13.pdf

Sfeclă, I., Sîrbu, T., Sfeclă, V. (2019). Plant variety patent application. Nr. 516, 02.12.2019.

Sfecla, I. (2021) International seed exchange (*Index Seminum*) in the National Botanical Garden (Institute) “Al. Ciubotaru”. In: *Yesterday’s heritage – implications for the development of tomorrow’s sustainable society. Ed. 3*, Chișinău, pp. 195-197. ISSN: 2558 – 894X, from https://ibn.idsi.md/sites/default/files/imag_file/p-98-99.pdf

Sfeclă, I., Sîrbu, T. Sfeclă, V. Plant variety patent *Kniphofia nelsonii* Mast. ‘Micul Prinț’, nr. 384, 31.03.2022, from https://db.agepi.md/soideplante/Details.aspx?id=v%202019%200014

Sîrbu, T. Sfecla, Irina, Manole, S., Șabarov, D., Lupan, A. (2023). Soiuri noi de plante ornamentale. In: *Intellectus*, nr. 1, pp. 161-170. ISSN 1810-7079, from https://doi.org/10.56329/1810-7087.23.1.16

Serebrjakov, I. (1952). Morfologija vegetativnyh organov vysshih rastenij. Moskva: Sov. nauka, 391 s.

Serebrjakov, I. (1962). Jekologicheskaja morfologija rastenij. Zhiznennye formy pokrytosemennyh i hvojnyh. M.: Vysshaja shkola, 378 s.

Serebrjakova, T. (1971). Tipy bol'shogo zhiznennogo cikla i struktura nadzemnyh pobegov u cvetkovyh rastenij. V: Bjull. MOIP, otd. biol., T.76., ss. 105-116.

Shilova, N. (1988). Ritmy rosta i puti strukturnoj adaptacii tundrovyh rastenij. Leningrad: Nauka, 212 s.

Syrbu, T., Sfekla, I. (2008). Perspektivnyj mnogoletnik dlja zeljonogo stroitel'stva Moldovy. V: Materialy HV Mezhdunarodnogo simpoziuma „Netradicionnoe rastenievodstvo. Selekcija. Ohrana prirody. Jekologija i zdorov'e”. Simferopol': OAO „Simferopol'skaja gorodskaja tipografija”, ss. 249-252.

Syrbu, T., Sfekla, I., Kuchurka, T. (2009). Hovye vidy travjanistyh rastenij dlja zeljonogo stroitel'stva Moldovy V: Materiali Drugoï Mizhnarodnoï naukovoï konferenciï. Tom 2. Doneck, ss. 299-301. UDK 581.522.4:632:727.6:634.0.27.

Váczy, C. (1980). *Lexicon Botanicum Polyglottum*. București: Editura Științifică și Enciclopedică, 1017 p.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

RESEARCH ON THE IMPACT OF THE GROWING MEDIUM ON PRODUCTION AND QUALITY OF *GERBERA HYBRIDA* FLOWERS IN PROTECTED CULTIVATION

Ioana Andra VLAD[1], Laviniu Ioan-Nuțu BURESCU[1], Bartha SZILÁRD[1], Călin Gheorghe PĂȘCUȚ[1], Goji GYÖZÖ[2], Florin-Alexandru REBREAN[3]

[1]University of Oradea, 26 General Magheru, Oradea, Romania
[2]Technological High School "Ștefan Manciulea", 3 Piața 1848, Blaj, Romania
[3]University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, 3-5 Calea Mănăștur, Cluj-Napoca, Romania

Corresponding author emails: laviniuburescu@gmail.com, ggyozo2000@yahoo.com

Abstract

Their high productive potential, tenderness and long lasting of flowers in water place the gerbera among the highly valued species for cut flowers grown in protected cultivation regime. Several surveys carried out among European flower growers and buyers rank gerbera 5th after carnations, roses, tulips and chrysanthemums (Frandin P. et all, 1990). Since in Romania, gerbera flowers have beenmuch appreciated and sought after by buyers at greenhouses in Sântandrei, Bihor, in the period 2020-2022 we conducted a research whose goal was to increase the productivity of gerbera plants and flower quality. To achieve the proposed goal, we used the Romeo hybrid variety cultivated on different substrates in six experimental working variants, each variant comprising four repetitions. The statistical analysis of the obtained results shows that the protocols related to variants 5 and 4 provide us with major concrete data regarding the increase of productivity and flower quality. We draw six conclusions which synthesize our research results.

Key words*: Gerbera hybrida, growing medium, peat, leaf soil, sand, fir bark, flower quality.*

INTRODUCTION

The genus *Gerbera* comprises several perennial herbaceous species that grow spontaneously in South Africa, Asia and South America. The species *Gerbera jamesonii* and *Gerbera viridifolia* laid the basis for obtaining numerous hybrids known as *Gerbera hybrida*. The plants appear in the form of rosettes formed by numerous deeply notched (penatifid) leaves, hairy petioles emerging from short rhizomes with easily trailing growth. Numerous roots penetrate the soil to a depth of 50-70 cm. Flower stalks are 20-90 cm long. Inflorescences are flower-heads of 5-18 cm in diameter with ligulate marginal flowers arranged radially and having tubular flowers in the middle. The fruit is a compressed achene (Garibaldi, 1993).

In recent years, through hybridization and selection, valuable hybrids with large flowers and long stems which are also resistant to preservation in water have been obtained (Șelaru, 1996).

Large flowers are today more of a requirement in Eastern Europe, while in the Western Europe people prefer the uniformity of the flower size even if average.

The flower colour and shape (involute, semi-involute, simple) are elements dictated more by trends and observed by breeders.

The plant presents particularities related to propagation and culture, the knowledge and mastery of which are required for the management of a correct technology and obtaining high and quality productions (Vlad, 2011).

The flowers are used for interior decoration, being kept in vases for 8-14 days. Gerbera can also be planted in green areas where it acts as an annual flowering plant (Viard, 2015).

MATERIALS AND METHODS

The working hypothesis we use is that by using a valuable, hybrid, resistant to both diseases and nematodes, by ensuring an appropriate mineral nutrition and by ensuring sustained

cultural hygiene, top production performance and economic efficiency can be achieved.

The main objectives through which we reached the proposed goal were the following ones:

- Observing a rigorous control of plant nutrition, comparing the culture on the organic growing medium with the classic plant growing in soil;
- Ensuring culture hygiene during the plant growing, especially by combating the common red spider mite, thrips, wilting and powdery mildew.

From the analysis of the state of research on gerbera culture in protected cultivation, the necessity and opportunity of the experiences undertaken in this study emerges based on the fact that domestic research works has not approached so far the issue in a complex manner by correlating the main factors that impact, to the greatest extent, the success of the culture: the hybrid used, the shading, and the culture system that controls the hydro-mineral regime.

The factors surveyed concerned the classic system of plant growing in soil.

This study is part of the current context with regard to the concerns of specialists to increase the production and quality of gerbera flowers.

We worked with the ROMEO red-coral hybrid, with the involute inflorescence and the diameter of 12-15 cm, obtained from the company Rosmaring, Obuda, Budapest.

The working experience comprised six variants, with 4 repetitions each, namely:

V1 - Soil culture system using classical technology;

V2 - Culture in substrate consisting of 40% peat, 20% beech leaves, 20% old manure, and 20% fir bark;

V3 - Culture in substrate consisting of 50% peat, 15% beech leaves, and 15% old manure, 20% fir bark;

V4 - Culture in substrate consisting of 60% peat, 10% beech leaves, 10% old manure, and 20% fir bark;

V5 - Culture in substrate consisting of 70% peat, 5% beech leaves, 5% old manure, and 20% fir bark;

V6 - Culture in substrate consisting of 70% peat, and 30% fir bark.

For the culture in the soil of the greenhouse, the following preparation works were done:

- Soil scarification at a depth of 50-60 cm;
- Basic fertilization following lab soil analysis with 15 kg/m^2 of peat and 15 kg/m^2 of well-decomposed cattle manure, the manure of other animals being not recommended, and with 20 g/m^2 of ammonium nitrate, 40 g/m^2 of superphosphate, 30 g/m^2 of potassium sulphate and 10 g/m^2 of magnesium sulphate;
- Plowing with a Wikon rotary tiller at a depth of 30-35 cm;
- Soil milling to a depth of 15-17 cm;
- Soil modelling in ridges with a width at the base of 60 cm and a height of 40 cm (Ţepardei, 2002).

The culture substrate from variants 2-6 was placed in 40 cm high ridges on polyethylene foil. Each variant was allocated an area of 120 m^2 for culture and four repetitions (Figure 1). The fir bark was chopped and the components of the substrates were mixed evenly. The pH was corrected to 6.5 for each variant. In variants 5 and 6, where the largest amount of peat was used for pH correction, an amount of 5 kg of calcium carbonate per m^3 was required (Leonardi, 1997).

PROTECTION AREA	4	3	2	1	PROTECTION AREA	ALLEY	PROTECTION AREA	4	3	2	1	PROTECTION AREA
	2	1	4	3				6	5	6	5	
	6	5	6	5				2	1	4	3	

Figure 1. Experiment placement draw

For the fertilization of the substrates, we made use of the following doses calculated per m^3:

- Superphosphate 2 kg; ammonium nitrate 0.5 kg, magnesium sulphate 1.5 kg, potassium sulphate 1.5 kg, borax 20 g, copper sulphate 25 g, iron sulphate 50 g;
- The basic fertilizers were mixed in powder form with the substrate, and those fertilizers containing microelements were dissolved in water and administered uniformly (Budoi, 2001; Davidescu & Davidescu, 1999).
- Two rows of plants were planted on a ridge, providing a density of 8 stems/m^2. The planting was done at the beginning of October, and maintenance works consisted of the following:
- Loosening the soil and substrate once a month without disturbing the plants;

- Drip irrigation of soil, and sprinkling over the period May-September;
- Fertilization (preceded by labanalyses on a monthly basis), with free-chlorine complex fertilizers that were dissolved and introduced into the drip irrigation system;
Throughout the growing period (i.e. three years) the mineral elements in the soil and culture substrate were maintained within the limits shown in Table 1 below:

Table 1. Content in mineral elements

Content in aqueous extract (1:5) in mg/100 g substrate						pH
N	P_2O_5	K_2O	CaO	MgO	Mineral residue %	
15-25	10-14	30-40	35-45	10-12	0.25-0.50	6.0-6.5

- Removal of aged leaves - was carried out on a timely basis by pulling them individually. In the summer this maintenance work was carried out twice a once, and a once month for the rest of the year. Flower production and flower quality decrease if senescent leaves are not removed and an optimal ratio between the leaves count and the flower count on a plant is not maintained (Zaharia, 1995).
- The activity of the gerbera root system was optimal at a temperature of 20°C. The higher temperature forced flowering but reduced flower quality (small flower heads, thin stems with low shelf life). Although it presents high requirements for light, during the summer we had to provide a light shading. Gerbera grows and blooms normally at a humidity of 60% in the substrate and 70% in the air (Burzo et al., 2005). To combat the "vascular disease" withering caused by three pathogens (i.e. *Phytophtora cryptogea*, *Verticillium* sp. and *Fusarium oxysporum*) monthly treatments were made with Previcur 0.15%, Fundazol 0.05%, Topsin 0.1%, and Ronilan 0.1% (Gheorgieș et al., 2003).
Powdery mildew (triggered by *Oidium erysiphoides*) occurred in the months and was combated with Rubigan 0.1%, and Afugan 0.05%.
With regard to the pests, the red spider mite (*Tetranychus urticae*) was the most common, and fought with Omite 0.08%, Torque 0.08% and Mitox 0.1%. Thrips (*Franklinella occidentalis*) also created problems for the elimination of which treatments were made with Methomex 0.06%, Sherpa 0.03%, Phosdrin 0.1%, and Thiodan 0.1%.

The flowers were harvested when they were fully opened and the neck of the flower stems was rigid. Harvesting was done by pressing the flower stem (left to right) twist and pull. No watering was performed in the harvest day (Vidalie, 2020).
In order to differentiate the variants of the Romeo hybrid culture, observations and determinations were made with regard to the total production, the flower quality and the economic efficiency.

RESULTS AND DISCUSSIONS

As one may notice from Table 2, the production of flowers harvested and sold ranged between 155 stems/m^2 for Variant 1 - Soil culture system using classical technology and 211 stems/m^2 for Variant 5 - Culture in substrate consisting of 70% peat, 5% beech leaves, 5% old manure, and 20% fir bark.

Table 2. Total production of Gerbera flowers obtained in the first year after planting under the influence of the growing medium

Variants	Flower production		±D	Significance of difference
	Absolute no of stems/m^2	Relative %		
V1 - Soil culture system using classical technology (control variant)	155	100	-	-
V2 - Culture in substrate consisting of 40% peat, 20% beech leaves, 20% old manure, and 20% fir bark	177	114	22	x
V3 - Culture in substrate consisting of 50% peat, 15% beech leaves, 15% old manure, and 20% fir bark	192	123	37	xx
V4 - Culture in substrate consisting of 60% peat, 10% beech leaves, 10% old manure, and 20% fir bark	205	132	50	xxx
V5 - Culture in substrate consisting of 70% peat, 5% beech leaves, 5% old manure, and 20% fir bark	211	136	56	xxx
V6 - Culture in substrate consisting of 70% peat, and 30% fir bark.	201	129	46	xx

DL 5% - 21.92; DL 1% - 32.26; DL 0.1% - 48.6

There is a 36% production increase in Variant 5, compared to the control variant (i.e. Variant 1), and between 14% in Variant 2 and 32% in the other variants.

The statistical analysis indicates a very significant difference between variants 5 (culture in substrate consisting of 70% peat, 5% beech leaves, 5% old manure, and 20% fir bark), 4 (Culture in substrate consisting of 60% peat, 10% beech leaves, 10% old manure, and 20% fir bark), and the control Variant 1 (Soil culture system using classical technology).
Between variants 6, 3 and the control variant the difference is clearly significant, while between Variant 2 and the control variant the difference is significant.
The yield of gerbera flowers is favourably and qualitatively impacted by the culture substrate (see Table 3 below).

Table 3. Quality of Gerbera flowers obtained in the first year after planting under the impact of the growing medium

Variants	Flower production		
	Total (stems/m^2)	of which of top quality	
		Absolute (stems/m^2)	Relative %
V1 - Soil culture system using classical technology (control variant)	155	116	75
V2 - Culture in substrate consisting of 40% peat, 20% beech leaves, 20% old manure, and 20% fir bark	177	138	78
V3 - Culture in substrate consisting of 50% peat, 15% beech leaves, and 15% old manure, and 20% fir bark	192	152	81
V4 - Culture in substrate consisting of 60% peat, 10% beech leaves, 10% old manure, and 20% fir bark	205	178	87
V5 - Culture in substrate consisting of 70% peat, 5% beech leaves, 5% old manure, and 20% fir bark	211	192	91
V6 - Culture in substrate consisting of 70% peat, and 30% fir bark.	201	170	85

In the case of Variant 1, cultivated in the soil according to the classical technology, the percentage of top quality flowers with the length of the floral stems over 50 cm and the diameter of the flowers over 13 cm, was only 75% of the total plants harvested, while in Variant 5 (culture in substrate consisting of 70% peat, 5% beech leaves, 5% old manure, and 20% fir bark), out of the total flowers harvested, 91% were categorised as top quality. In the other variants, the percentage of top quality flowers ranged between 87% and 78%, higher than in the case of control variant.
From the data presented in Table 4, it results that the highest economic efficiency in terms of profit was obtained in the cause of Variant 5 (789 lei/m^2), closely followed by Variant 4 (684 lei/m^2). In the other variants the profit obtained is lower than in the optimal variants.

Table 4. Economic efficiency of Gerbera cultivation under the influence of the growing medium

Variants	Expenses incurred lei/m^2	Flower production stems/m^2	Production value lei/m^2	Profit lei/m^2
V1 - Soil culture system using classical technology (control variant)	1562	155	2170	608
V2 - Culture in substrate consisting of 40% peat, 20% beech leaves, 20% old manure, and 20% fir bark	1873	177	2531	658
V3 - Culture in substrate consisting of 50% peat, 15% beech leaves, and 15% old manure, and 20% fir bark	2130	192	2803	673
V4 - Culture in substrate consisting of 60% peat, 10% beech leaves, 10% old manure, and 20% fir bark	2452	205	3136	684
V5 - Culture in substrate consisting of 70% peat, 5% beech leaves, 5% old manure, and 20% fir bark	2481	211	3270	789
V6 - Culture in substrate consisting of 70% peat, and 30% fir bark.	2341	201	3015	674

The selling price was variable during the year, ranging between 5 and 17 lei/plant. The expenses analysis shows very high levels in terms of the cost of heating with methane gas and other expenses that include indirect expenses and interest related to contracted loans.

From the economic efficiency perspective, although the production expenses were higher in the case of Variant 5 due to the increase in production and quality, the profit was higher than in the control variant i.e. Variant 1 by 181 lei/m^2, i.e. 1800000 lei/ha.

CONCLUSIONS

- Cultivation of gerbera in protected regime is a profitable activity for flower businesses but resulting in differentiated outcomes depending on the cultivation technology applied;
- Flower production and top quality flowerswere higher in Variant 5, where the substrate consisted of 70% peat, 5% beech leaves, 5% old manure, and 20% fir bark;
- In the case of Variant 6 - Culture in substrate consisting of 70% peat, 5% beech leaves, 5% old manure, and 20%, although fir bark was the same as in Variant 5, the larger amount of bark and the lack of beech leaves and old manure resulted in obtaining lower productions by 10 stems/m^2 and 6% fewer top-quality flowers asagainst Variant 5;
- The thermal regime and the supply of water and nutrients were more effective and beneficial in the case of substrate containing 70% peat, 5% beech leaves, 5% old manure, and 20% fir bark.
- The additional expenses incurred for the procurement of the constituents and the making of the substrate in Variant 5, were recovered and secure a profit of 789 lei/m^2 (i.e. 7890000 lei/ha).
- Scientific results we obtained in this study can be considered as a valuable contribution to gerbera flowers businesses.

REFERENCES

Borgeois, H. (1998) Nitrogen fertilizers: multiple choices. *P.H.M. Horticultural Journal*, April.

Budoi, G. (2001). *Agrochemistry II. Fertilizers. Technologies. Efficiency*. Didactic and Pedagogical Publishing House, Bucharest.

Burzo, I., Amariuței A., Vâșca Z. D. (2005). *Physiology of flowering plants*. Elisavaros Publishing House, Bucharest.

Davidescu, D. & Davidescu, V. (1999). *Horticultural Agrochemistry*. Romanian Academy Publishing House.

Frandin, P. et. al. (1990). Study of the commercial impact of florets and new flowers. *L'Horticulture Francaise*, October, 1990.

Garibaldi, A.E. (1993). Treatise on floriculture. Edagricole, Italy.

Gheorgieș, C. & Geamăn, I. (2003). *Diseases of horticultural plants*. Universitas Co. Publishing House, Bucharest.

Ivașcu, M. (2007). *Edible flowers. Health and color*. Cetatea de Scaun Publishing House, Târgoviște.

Leonardi, D. (1997). Organic amendments organic and organo-mineral fertilizers: who are they? *P.H.M.*, October 1997.

Șelaru, E. & Drăghici, M. (1989). Research on the development of culture technology in the greenhouse-solarium. *IANB Scientific papers, Series 13, Horticulture*, vol. XXXII.

Șelaru, E. & Petrescu, M. (1993). *Floriculture*. Agricultural Technical Publishing House, Bucharest.

Șelaru, E. (1994) *Instructor of practical floriculture works*. AMC Workshops of the U.S.A.M.V. Bucharest.

Șelaru, E. (2002). *Culture for cut flowers*. Ceres Publishing House, Bucharest.

Selaru, E. (1996). *Horticulture. Second Edition*, The Farmer Open University. Bucharest.

Țepordei, R. (2002). *Floriculture.* Agricultural Sciences Publishing House. Bucharest.

Viard, M. (2015). *The flowers of the world*. Publishing House Hatier, Paris.

Vlad, I. (2011). *Floriculture*. University Publishing House, Oradea.

Vidalie, H. (2020). *Floral productions*. France, 2020.

Vlad, I. (2000). *Practical work on floriculture*. Western Printing House, Oradea.

Zaharia, D. (1995). Floriculture. AMC lithographed course, Agronomic Institute Cluj-Napoca.

***Green Profit Plants flowers and the people who buy them. America in bloom, 2023.

****Great Encyclopedia of Garden Plants & Flowers*, Bordas, Paris 2020.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

FROM NEGLECT TO VITALITY: LANDSCAPE DESIGN APPROACHES FOR RECONVERTING LOST URBAN SPACES

Ina VLADIMIR (ASIMINEI), Mirela COJOCARIU

"Ion Ionescu de la Brad" Iaşi University of Life Sciences, 3 Mihail Sadoveanu Alley, Iaşi, Romania

Corresponding author email: mirela.cojocariu@iuls.ro

Abstract

A modern city`s landscape reflects the diversity and inconsistency of the processes that occur within it. The changing landscape of the city and its surroundings has become a common phenomenon as urban areas expand and technological capabilities for modeling nature develop. The interaction between the natural and artificial environments becomes more intense as urban development pressures the surrounding landscape. The city constantly expands its borders into neighboring territories, gradually diminishing natural potential and bringing more chaos than harmony to the landscape. In this chaos and rapid pace of modernization, attention has increasingly shifted to appearances at the expense of content. Urban spaces that did not align with external trends and influences were abandoned, leading to community fragmentation and a decline in authentic values and traditions. Using this concept as a starting point, the study aims to propose several landscape design approaches and appropriate solutions for rehabilitating degraded or abandoned urban spaces in Romania..

Key words*: urban renewal, sustainability, reconverting urban spaces, rehabilitation strategies.*

INTRODUCTION

Do people on Earth have enough space? If the answer is yes, then why does the modern world complain about the decreasing space allocated for housing, forests, water, and wildlife? If the answer is no, then why is the world blind to the possibility of using the spaces left in the collective forgetfulness of society?

The gap in current research on the reconversion of abandoned urban spaces is evident in the absence of design strategies that balance multiple aspects, such as ecological, social, and economic factors, as well as the lack of strategies and models for participatory design and the influence of the community on such urban spaces.

The positive influences and aspects of various nature-based methods and their role in urban design are well known; however, the long-term effects, whether positive or negative, of combining these methods in the restoration of abandoned urban spaces are not fully researched.

The main goal of this research is to visualize some of these methods that can be incorporated into a proposed design for an abandoned space in the city of Iași, and to determine whether this approach represents a better option for the use of these forgotten places by many others.

MATERIALS AND METHODS

For the chosen topic, a mixed approach has been applied to conduct research combining both qualitative and quantitative study methods.

To gain a better understanding of the studied topic, a qualitative approach was utilized, identifying case studies that present abandoned spaces or marginalized sites. At the same time, an analysis was conducted on nature-based solutions that could be put into practice for the reconversion of the spaces identified in the research.

As a quantitative method, a spatial analysis of the city of Iași was conducted to identify existing abandoned sites, followed by field visits to document, through photography, the state of degradation of the infrastructure, as well as the vegetation and the surrounding land. During the field visits, we discovered three potential locations that would be suitable for spatial reconversion.

The analysis of the existing literature led to the formulation of a series of criteria based on

which the three locations discovered during the field visits were selected.

Luciano Crespi, in his extensive work *Regeneration of Abandoned Spaces: A New Design Approach*, emphasizes the importance of principles such as community involvement in the design solution and maintenance of the revitalized site to ensure the long-term viability of the proposed solution. Sustainable practices, eco-friendly materials, reusing existing structures, and creating a framework that is more attractive from a functional rather than purely aesthetic standpoint, all represent principles that will ensure the long-term success of urban regeneration (Crespi, 2024).

An analysis of the condition of the built spaces proposed for revitalization is essential, assessing their potential to be transformed and reintegrated into public use, while also identifying the needs of the local community (Santos & Ramalhete, 2024).

The history of the site and the importance it has had over time for the community also represented a decisive factor in the selection of the spaces. Places that once represented an important center for the adjacent community, thus having strong historical roots, can be transformed into cultural hubs for the community, enhancing the identity and pride of future users (Ghida, 2024).

Following the ideas proposed by the mentioned authors, the following criteria were suggested for selecting possible urban spaces suitable for their reconstruction:

- to be located in neighborhoods with a high population percentage;
- to be easily accessible, ensuring a smooth connection with the surrounding community;
- the presence of one or more built structures on the site that could provide a starting point for reconversion, while also reducing the costs of building a new structure;
- the possibility of implementing nature-based solutions on the site;
- the existence of a sufficiently large space to create multifunctional areas;
- sites that would have a significant social impact in the community, and whose reconversion would offer an opportunity to involve local residents;
- sites that belong to the municipality or are owned by city authorities and could be used without depending on external investors.

The first identified site represents one of the currently non-functional wings of the Iași Clinical Hospital of Pneumoftiziology. The second site represents the abandoned building of the former Dermatology clinic and the adjacent land. The third site represents the unfinished structure of the Olympic swimming pool, the construction of which began 35 years ago and has remained in ruins for 30 years since the work was halted (Figure 1).



Figure 1. The three representative locations identified (OpenStreetMap)

Site number two was chosen to describe the proposed principles for reintroducing abandoned sites into the city's viable urban network, namely the former Dermatology hospital building and the adjacent green space, located in the northeastern part of the city.

The area of the site under study is 3,388 square meters, and the construction year is 1950. The initial function of the building was a kindergarten. Regarding property rights, it is a public space, owned by the City Hall of Iași. There are three existing buildings on the land: Building C1, C2, and an annex, and the current state of the space is abandoned (Figure 2).

Analyzing the site's location from a broader territorial perspective, it is notable that it is situated in the northeastern part of Iași, specifically in the Tătărași neighborhood, one of the oldest and most historically significant districts of the city.

Originally a vast green area of forests and hills before 1850, the neighborhood became home to a diverse range of nationalities, so that by the 1950s, it was one of the most developed areas of the city (Baciu G. et al., 2007) (Figure 2).

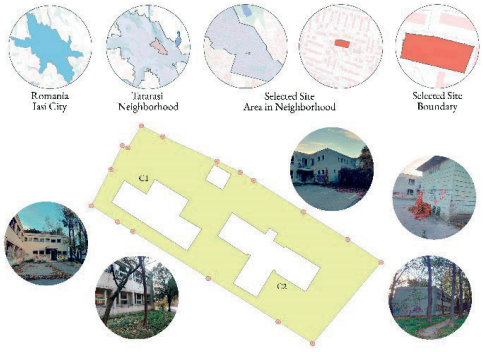


Figure 2. The physical context of the site and the current condition of the space

The proposed design solution was based on the following stages: defining the purpose and function of the rehabilitated site, site investigation and analysis, establishing the concept and implemented solutions, analyzing and justifying the proposed solutions for implementation, and evaluating the outcome.
For the digital development of the actual solution, specialized landscape design software such as AutoCAD, SketchUp, and Lumion was used.

RESULTS AND DISCUSSIONS

Defining the purpose and function of the rehabilitated site

The main approach to the identified issue is the implementation of a multilateral design, based on the needs of the community and the promotion of civic spirit.
By creating a space dedicated to the community and involving its members in managing and maintaining it, cultural identity can be strengthened, which is often absent in the present, where the cult of personality and focus on individual needs prevail. The participation of community members in implementing sustainable principles will contribute to preserving biodiversity and sustainability (Nijhuis S., 2022).

Site investigation and analysis

Being one of the oldest neighborhoods in the history of Iași, the area occupied by Tătărași was overtaken in the 1950s by numerous block constructions. Thus, through a spatial analysis of a 500-meter radius from the site, approximately 3,000 apartments were identified in the immediate vicinity of the site (Figure 3).

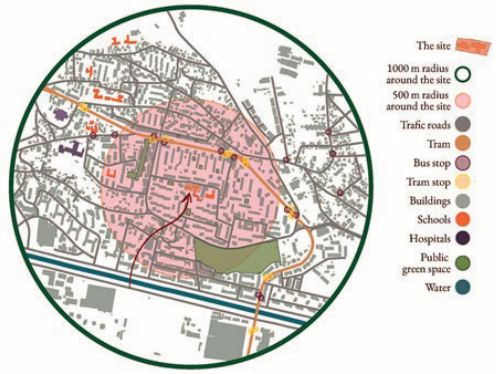


Figure 3. Spatial analysis of a 500-meter radius and 1000-meter radius from the site

Most of the blocks are 75 years old, indicating a diverse demographic range among the population living in this area, including elderly residents who have witnessed the area's evolution as well as young families with children who represent the community's next generation.
Due to the high percentage of the population in the area, there is also a large number of cars, and the poor road infrastructure and lack of adequate parking spaces have led to the transformation of the green spaces around the blocks into unofficial parking areas, further reducing the spaces intended for the community or activities for larger groups.
Due to its age, the neighborhood is considered one of the richest in mature vegetation. However, the lack of a green registry for the city and inadequate tree maintenance has led to the decline of many existing species. The state of the shrub vegetation is even more neglected, predominantly consisting of uncontrolled, spontaneous growth, which has led citizens to avoid the remaining green islands.

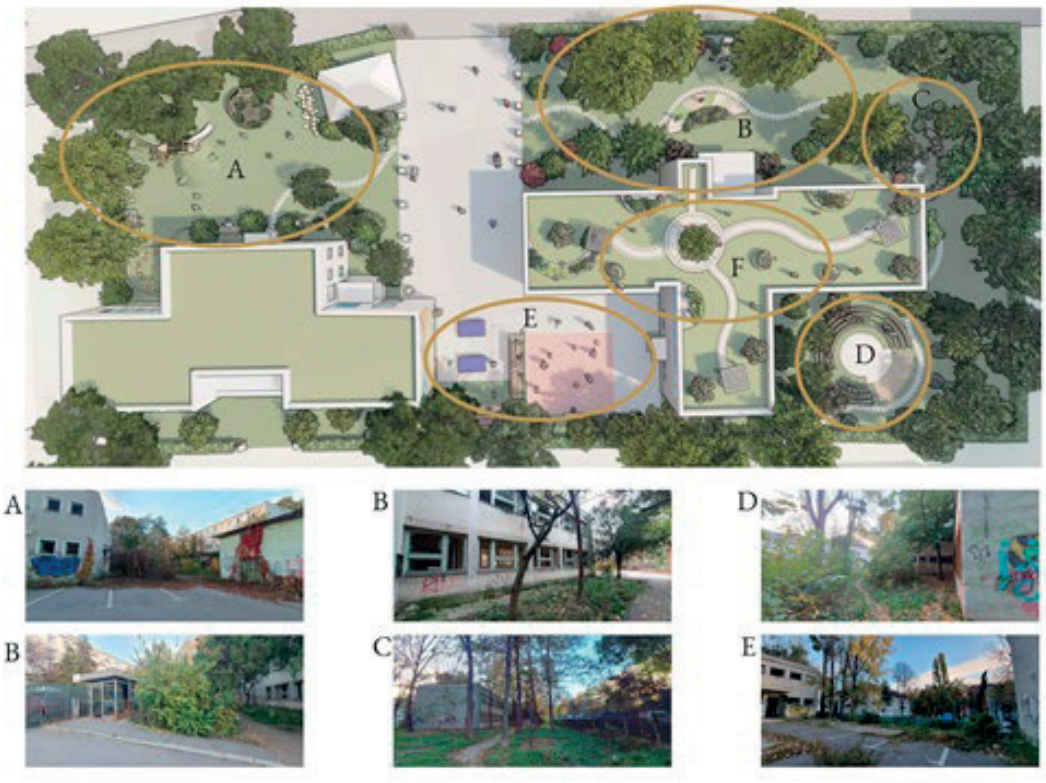


Figure 4. Representation of the existing areas and the proposed design

Analyzing and justifying the proposed solutions for implementation.

The design is structured around 6 major areas of interest (Figure 4).

The first area marked with an A on the plan is dedicated to activities for children and parents. The time children spend with their parents is very important for their psychological and social development. Among the long-term benefits of this type of interaction, the following can be listed: the development of better social adaptability, the ability to manage emotions and frustrations that may arise, and an increased level of resilience to problems that may later occur during the adolescent's development. The time spent together provides parents with the opportunity to model positive behaviors, strengthening values and laying the foundation for lasting connections (Waters et al., 2023).

The area is designed to meet the needs of a wider variety of ages, equipped with smaller subzones for both active and passive rest (Figure 5).



Figure 5. Representation of playspace

Area B (Figure 6) presents the implementation of two nature-based solutions, namely the integration of a sensory area with natural

materials and perennial plants and an area dedicated to insects and biodiversity, which also have an educational character, with learning opportunities, for both adults and children, about biodiversity conservation and how they can contribute to it.



Figure 6. The representation of the sensory path and the space dedicated to insects

One of the issues created by rapid urbanization is the involuntary development of spaces that can be harmful to mental health, highlighting the need for the implementation of solutions that would combat sensory overload caused by inadequate urban environment design. Thus, incorporating sensory pathways into the design would facilitate a temporary escape from the chaotic landscape of the built environment, offering community members a much-needed refuge (Lauv, 2024).

The incorporation of a sensory garden, regardless of its size, represents a high-potential model for urban environment development, aligned with the principles of smart and sustainable urban development (Zajadacz & Lubarska, 2023).

The analysis of an extensive study of 728 papers, described in the work *The Application of Rain Gardens in Urban Environments: A Bibliometric Review*, clearly demonstrates the growing interest in incorporating rain gardens into urban design. These are described as one of the nature-based solutions with a fundamental role in managing water within urban environments (Wang et al., 2024).

In this context, it is proposed to implement a rain garden space in area C, which will be beneficial both ecologically and aesthetically (Figure 7).



Figure 7. The representation of the rain garden

Zone D, with its circular-tiered seating design, serves as a gathering space for the community, providing an area for activities such as meetings, celebrations, performances, games, or outdoor evenings. The circle is a fundamental symbol, representing perfection, unity, and spiritual harmony. For this reason, the core community space was designed in a circular shape (Figure 8).

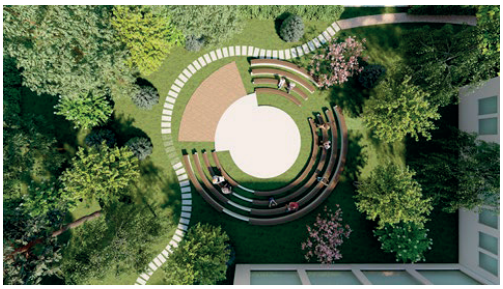


Figure 8. The representation of the space dedicated to community gatherings

Another area with a significant social character and impact is Zone E, dedicated to intergenerational play activities. Intergenerational play creates unique bonds by combining the energy and curiosity of youth with the wisdom and experience of older generations (Figure 9).



Figure 9. The space dedicated to intergenerational play

The last zone, but not the least important, is the one dedicated to the vegetable and herb garden, located on the roof of Building C2 (Figure 10). The concept of green roofs and vegetable gardens not only makes use of otherwise unused spaces but also provides an opportunity for educational activities for community members. By implementing this concept, the goal is to educate the community about healthy eating and the origins of food while fostering a connection with nature and a sense of environmental responsibility in younger generations. In this way, the rooftop garden is transformed into a living classroom and a symbol of green urban regeneration.



Figure 10. The rooftop garden

CONCLUSIONS

The proposed design for the study creates a connection between history and people, serving as a model where each proposed element contributes to a narrative advocating for renewal, inclusion, and shared identity.

To achieve the harmonious integration of design elements in the reconversion of an abandoned space, it is necessary to consider the neighboring residents of the site and their needs.

The implementation of design methods based on nature-inspired solutions is essential, as well as their appropriate selection, considering the physical characteristics of the space.

A history-based approach to the site can significantly contribute to creating a design rooted in the strong character of the local community's culture, thus refreshing the collective memory of the space.

The aim of the study was to create a community hub suitable for people of all ages, promoting inclusion and respect for the surrounding environment.

It was demonstrated that by repurposing an abandoned space, it can be reintegrated into the urban network, promoting biodiversity within the built environment.

REFERENCES

Baciu G., Bejenaru M., Lungu D. (2007). *Tătărași: Memoria unui cartier*, "Alexandru Ioan Cuza" University Press.

Crespi, L. (2024). *Regeneration of Abandoned Spaces: A New Design Approach.* Bentham Books

Ghida, D. B. (2024). Revitalizing urban spaces: Ten key lessons from the “Viaduc des arts” adaptive reuse and placemaking. *Frontiers of Architectural Research.* 13(5), 1095-1112.

Lauv, Ethan (2024). *Transitional Sensory-Scapes*. Open Access Te Herenga Waka-Victoria University of Wellington.Thesis.

Nijhuis, S. (2022). Landscape-Based Urbanism: Cultivating Urban Landscapes Through Design. In R. Roggema (Ed.), *Design for Regenerative Cities and Landscapes : Rebalancing Human Impact and Natural Environment* (pp. 249-277). (Contemporary Urban Design Thinking). Springer.

Santos, T., & Ramalhete, F. (2024). Urban Transformation: Analyzing the Combined Forces of Vacant Building Occupancy and Socio-Economic Dynamics. *Sustainability*, 16(11), 4351.

Wang, M., Zhuang, J., Sun, C., Wang, L., Zhang, M., Fan, C., & Li, J. (2024). The Application of Rain Gardens in Urban Environments: A Bibliometric Review. *Land*, 13(10), 1702.

Waters K.A., Salinas-Miranda A., Kirby R.S. (2023). The association between parent-child quality time and children's flourishing level. *J Pediatr Nurs*. Nov-Dec;73:e187-e196.

Zajadacz, A., & Lubarska, A. (2023). Sensory gardens as a new form of urban green space in smart sustainable cities. *Czasopismo Geograficzne*, 94(1), 125-145.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

GARDENS DESIGN AS OUTDOOR EVENT SPACES

Cristina ZLATI, Măriuca ȘTIRBAN, Alice CHIHAIA, Roxana PAȘCU

"Ion Ionescu de la Brad" Iași University of Life Sciences,
3 Mihail Sadoveanu Alley, 700490, Iași, Romania

Corresponding author email: roxana.pascu@iuls.ro

Abstract

Present research theme aims to design and implement an outdoor garden as event spaces. The subject is addressing the need for more attractive and functional environments, spaces for various social and cultural activities. In the context of a continuous growing demand for outdoor events, our project aims to provide new and functional plant compositions, plant selections well adapted to the specific needs of event spaces, integrating decorative elements and functional facilities (alleys, pots, etc.). Event gardens are spaces especially designed to host various social and cultural activities, such as weddings, parties, concerts or exhibitions. This type of gardens are carefully created to provide an inviting and relaxing atmosphere, combining all landscape design elements, functional facilities and pleasing aesthetics. The theme was chosen based on market research analysis, client requirements and principles of sustainable design. The expectations are linked to the opportunities, innovation and adaptation to ever-changing trends in the field of outdoor events design in particular and landscape architecture in general. These types of gardens are of interest not only because they provide a delightful natural setting for events, but also because promote the connection between people and environment, contributing to community general well-being and of course, environmental sustainability.

Key words*: outdoor events; design; environment; community.*

INTRODUCTION

The proposed theme of research aims to design and implement an outdoor garden as event space, addressing the need for more attractive and functional spaces for various social and cultural activities.

In the context of a continuous growing demand for outdoor events, this project aims to provide innovative solutions in plant compositions schemes and landscape design, integrating all necessary decorative elements, functional facilities and plant selections adapted to the specific needs of outdoor event spaces.

An event garden is a specific space, especially designed to host various social and cultural activities, such as weddings, parties, concerts or art events as exhibitions. These gardens are created to provide first an attractive and relaxing atmosphere while combining landscape design elements, the functional facilities and a pleasant aesthetic (Dumitras A. et al., 2010). They are places where people can meet and celebrate important moments, in a natural and inspiring setting, contributing to the creation of unforgettable memories (Bezerra A.F. et al., 2010).

The research theme was chosen based on market research analysis, client requirements and principles of sustainable design. The expectations are linked to the opportunities, innovation and adaptation to ever-changing trends in the field of outdoor events design in particular and landscape architecture in general. By approaching a design method which integrates as many factors involved, is expected to create such spaces that combine aesthetics and utility, comfort, sustainability and durability. Thus, the contribution will be, improving the experience of event participants and capitalizing on urban landscapes (Istrate A.M.R. et al., 2023).

Through this project, we aimed not only to create attractive and functional spaces, but also to promote a more conscious and interactive environment for the local community, strengthening the role of outdoor gardens as central elements of contemporary urban life. The choice to approach this theme is due to continuous fascination with how nature and landscape architecture can transform urban spaces into vibrant and lively places, suitable for celebrating special moments (Boangiu G., 2021).

These types of gardens not only provide a delightful natural setting for events, but also promote the connection between people and the environment, contributing to community well-

being and environmental sustainability (Yarushina K., 2021).

MATERIALS AND METHODS

The general purpose of this work is to design and arrange a pontoon on Lake Ciric, intended for matrimonial events, civil weddings in particular, or other unique events. This involves expanding the space on Lake Ciric, the Elysium event hall (Figures 1 and 2). Figure 3 present the current situation of the site.

By arranging the new space, it is proposed to create a special, unique, and romantic space, in a dream setting, a natural setting, suitable for holding ceremonies and other special activities.

Another goal is to offer participants a memorable and comfortable experience, in a natural and picturesque setting.

To achieve the general goal, the project follows a series of directions in the sense of:

- Creating a unique, romantic and relaxing atmosphere, by using ambient lighting, effects and floral decoration.
- Ensuring the necessary comfort and safety of participants, by including safe chairs and railings.
- Optimizing the space to allow the development of various types of events, adapting to the needs and preferences of customers.
- Harmonious integration into the natural environment, respecting and protecting nature, the aquatic ecosystem and the surrounding vegetation.

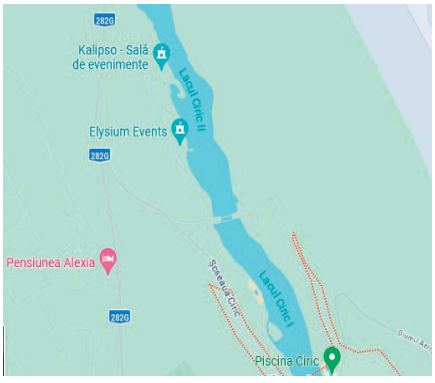


Figure 1. Location of Elysium Ciric Restaurant (https://www.google.ro/maps/)

The methods used to achieve the general goal are oriented towards collecting data and analyzing a wide range of examples, both positive and negative, regarding the creation and management of databases, in order to understand and identify the essential elements for achieving a favorable spatial structure. By carefully examining previous experiences, we are able to highlight effective practices and those that require improvement, thus providing a solid basis for the future development and implementation of these systems. Through all these efforts, the design and arrangement of the pontoon on the water will provide an unforgettable setting for ceremonies and events, where couples and participants can enjoy the beauty and tranquillity of nature (Galluzzi G. et al., 2010).



Figure 2. Current situation of the Elysium recreation area



Figure 3. Current situation – details

RESULTS AND DISCUSSIONS

The presented proposal integrates various geometric elements, a play of lines and irregular shapes. The plan presents two generous entrances, green spaces, a bar area, sumptuous terrace with dining area, intended for private events, but also two pontoons on the lake intended for photo shoots, civil weddings, private parties, etc. This design proposal implements and promotes outdoor events, especially on the lake (Figure 4).

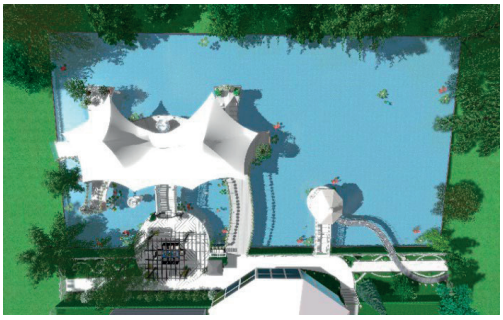


Figure 4. Planning proposal

Within the proposed development, four zones with distinct functions are identified, as shown in figure 5, all of these zones are connected by a main alley. Each zone is designed to offer a unique and memorable experience, transforming each event into an unforgettable moment.

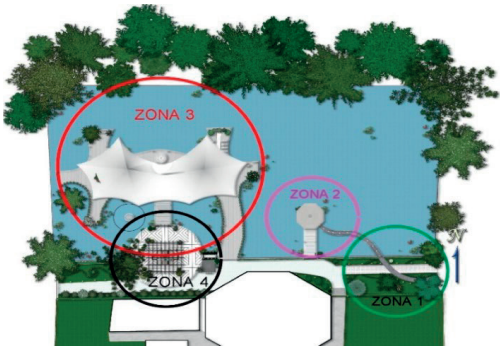


Figure 5. Zoning plan

Zone 1 and zone 2 are directly connected by the same alley, the main one, but also a secondary one, while zones 3 and 4 are connected only by the main alley. Also, around these main zones there are various design elements, which complete the overall development.

Original alleys were modified, with the aim of modernizing and facilitating access, emphasizing the points of interest created, and providing an elegant and festive touch, while offering a relaxing and intuitive navigation. They bring an aesthetic, but also functional addition to our gardens, elegantly and harmoniously connecting the various areas of the space.
Zone 1, includes the main alley to the hall and the two pontoons, up to the exit (Figure 6).



Figure 6. Arrangement of alleys

This is essential for creating a positive first impression and for facilitating access to the complex. The entrance alley is decorated with decorative vegetation (*Alyssum*, *Rosa*, *Buxus*, *Abies, Magnolia*, *Quercus*) attractive paving and ambient lighting, offering a welcoming, elegant atmosphere but also increased accessibility. The small pontoon is an area designed for events, especially intended for photos and civil weddings. It offers a fairytale setting, perfect for unforgettable moments. It can be decorated with refined flowers and elegant floral arrangements, according to the style and preferences of the bride and groom (Bernardis R. et al., 2023). This pontoon exudes romance and elegance. The high-quality paving, combined with the delicate railings, ensures both safety and a special aesthetic appearance. The large main pontoon is decorated with sumptuous elegance. It is a very versatile and refined space, perfect for various special and spectacular events (Figure 7). It includes multiple areas dedicated to photos, civil weddings, select parties and sumptuous weddings.



Figure 7. The view of the two pontoons

It can be decorated with floral arrangements, which can be changed depending on the style of each event, but also furniture and different materials of the highest quality. Glass railings could also be adorned with bouquets of *Gypsophila.*

The special, irregular shape, give the pontoon a special uniqueness, which distinguishes it from any other structure in the city. With an innovative design and impressive architecture it attracts attention, turning it into a point of reference and attraction. It is the only one of this kind in the city, thus it offers not only a place for events, but also a unique visual aesthetic and sensory experience, which leaves a lasting impression on all participants. Potted flowers carefully chosen to be well adapted will be strategically placed in order to add an extra touch of beauty, attraction and naturalness to the pontoon, placing the event in a bohemian setting. The variety of vegetation including flowering plants brings color and fragrance, contributing to a lively and elegant atmosphere (Table 1).

In addition, the pontoon allows for the customization of the decor for each event. Whether it is a romantic wedding, a sophisticated party or an intimate civil ceremony, organizers can decorate the space according to their preferences, using various decorative elements. Fine draperies, bright lights, themed floral arrangements and elegant furniture are just a few of the options available, ensuring that each event is unique and perfectly reflect the wishes and style of the hosts. Thus, the pontoon becomes a fairytale place, adaptable and charming, ideal for any special occasion.

Table 1. The list of proposed species

No. crt.	Species name
1.	*Abies concolor* (Gordon & Glend.) Lindl. ex Hildebrand
2.	*Betula nigra* L.
3.	*Buxus sempervirens* L.
4.	*Choisya grandiflora* Regel
5.	*Cornus alba* L.
6.	*Cornus florida* L.
7.	*Cupressus sempervirens* L.
8.	*Cyperus alternifolius* Rottb.
9.	*Gypsophila elegans* M.Bieb
10.	*Hibiscus rosa-sinensis* L.
11.	*Hibiscus syriacus* L.
12.	*Hydrangea* sp.
13.	*Hedeoma incana* Torr.
14.	*Juniperus chinensis* L.
15.	*Juniperus horizontalis* Moench
16.	*Leucothoe fontanesiana 'Zeblid'*
17.	*Lobularia maritima* (L.) Desv.
18.	*Magnolia grandiflora* L.
19.	*Matthiola* sp.
20.	*Nymphaea* sp.
21.	*Pinus thunbergii* Parl.
22.	*Podophyllum peltatum* L.
23.	*Quercus douglasii* Hook. & Arn.
24.	*Rhododendron augustinii* Hemsl.
25.	*Rosa* sp.
26.	*Salix caprea* L.
27.	*Salix nigra* Marshall
28.	*Verbena hybrida* L.

Area 4 presents the arrangement of an intimate, suspended, and refined terrace. The setting is intended for private parties, anniversaries and other small events, offering a refined and comfortable setting for special occasions. Decorated with elegant and high-quality outdoor furniture, terrace has comfortable chairs and tables, placed under a pergola adorned with plants, lights and other accessories. The floral arrangements and pots use carefully chosen plant compositions (for example *Poliomintha* sp., *Juniperus* sp., *Rosmarinus* sp., *Abelia* sp.) to add a touch of freshness and color, creating pleasant and relaxing context (Figure 8). Near the exit area, next to the main alley, a floral arabesque is placed, using *Alyssum* sp., which will delight viewer's eyes and in the same time will add a distinct element of beauty and elegance to the environment.



Figure 8. Details of the interest areas

CONCLUSIONS

The design solution of the outdoor event space belonging to Elysium Event Hall, Iași, exemplifies a remarkable interconnection between elegance and innovation, skillfully framed into the natural landscape of Ciric Lake. The modern pontoon on the lake is a splendid and innovative setting, ideal for scenic weddings and other memorable events. The environment can be enriched with delicate floral arrangements and sophisticated ambient lighting, elements that will contribute to a fairytale atmosphere. The entire layout, exclusive in its complexity, combine elegance and refinement in a private almost intimate setting, harmonizing the multiple areas. This way of approach not only that amplifies the aesthetics and functionality of the space, but also offers a distinct and unforgettable experience for all participants at the events.

In conclusion, the Bloom Hall area from Elysium complex design, manages to merge nature with modern elegance, establishing an ideal setting for any special event, meeting standards of excellence in the field of event organization.

The outdoor event space design with its unique and refined layout, with multiple areas intended for various private events and photo shoots sessions, offers an intimate and spectacular setting on the modern pontoon on Lake Ciric. This interfluent combination of aesthetics and functionality not only emphasize the experience of participants, but also sets a new standard of excellence in the field of event planning. All these will ensure unforgettable memories for all those who will visit this extraordinary place.

REFERENCES

Bernardis, R., Sandu, Tatiana, Dascălu, M., Zlati, Cristina, Cojocariu Mirela, Poșta Daniela (2023). Aspects regarding the ornamental value of some *Teahibrida* and *Floribunda Rose* varieties from "Tudor Neculai" nursery collection – Iași County. *U.S.V. Scientific papers, vol. 66, nr. 2, seria Horticulture*, pp. 75-80, ISSN 1454-7376. http://www.uaiasi.ro/revista_horti/files/Nr2_2023/vol%2066_2_2023%20(11).pdf

Bezerra, A.F. and Rocha, Y.T. (2010). Public open spaces of Baeta Neves district, São Bernardo Do Campo city, state of São Paulo, Brazil. *Acta Hortic*.

2010, 881, 359-362, DOI: 10.17660/ActaHortic.2010.881.53 https://doi.org/10.17660/ActaHortic.2010.881.53.

Boangiu, G. (2021). The symbolic imaginary specific to the wedding in Oltenia. *Current Issues of Social Sciences and History of Medicine*, 2021, 30, 68–72. https://doi.org/10.24061/2411-6181.2.2021.271.

Dumitras, A., Ilca-Suciu, T., Pop, P., Damian, A., Mazăre, G., Zaharia, D., Oroian, I., Singureanu, V. and Sabo, G. (2010). Romanian urban landscape, antagonism or cooperation between public and private green areas. *Acta Hortic*. 2010, 881, 351-354, DOI: 10.17660/ActaHortic.2010.881.51 https://doi.org/10.17660/ActaHortic.2010.881.51.

Galluzzi, G. and Negri, V. (2010). Contribution of backyard gardens to conservation of biological and cultural diversity and to human well-being. *Acta* Hortic. 2010, 881, 179-183, DOI: 10.17660/ActaHortic.2010.881.22 https://doi.org/10.17660/ActaHortic.2010.881.22.

Istrate A.M.R, Cojocariu M., Grecu C., Stoleru V. (2023). Landscape design process of a private events venue Garden in Iasi county, Romania. *Scientific Papers. Series B, Horticulture.* 2023, Vol. LXVII, No. 1, 404-412, Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653.

Yarushina, K. (2021). Anthropological description of a modern urban wedding. *Ideas and Ideals*, 2021, 13, 373–388. https://doi.org/10.17212/2075-0862-2021-13.3.2-373-388.

https://www.google.ro/maps/

MISCELLANEOUS


EX·TERRA·AVRVM

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

CHARACTERIZATION OF FOREST TREE SEEDS USING VIBRATIONAL SPECTROSCOPY

Luisa ANDRONIE[1], Ioana POP[1], Florica MATEI[1], Iulia COROIAN[1], Adina TRUȚĂ[1], Aurelia COROIAN[2], Ștefania ANDRONIE[2]

[1]University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Faculty of Forestry and Cadastre, 3-5 Calea Mănăştur Street, Cluj-Napoca, Romania
[2]University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Faculty of Agriculture, 3-5 Calea Mănăştur Street, Cluj-Napoca, Romania

Corresponding author email: andronie_luisa@yahoo.com

Abstract

The biological activity and the pharmaceutical properties of plants are strongly dependent on their structure.
In the current study, the FT-IR spectroscopy technique was applied to detect molecular components in forest tree seeds samples. We used FTIR (Fourier transform infrared) spectroscopy because this tehnique gives information on specific functional chemical groups present in organic matter. However, the whole spectrum may also contain information that relates to more general chemical or biological properties. The FT-IR results revealed the presence of different bio-active compounds in pine such as flavonoids, tannins, sugars, acids, proanthocyanidins, carotenoids, citric metabolites and others. The comparison of the antioxidant activity of the studied forest tree emphasized a hierarchy of the antioxidant capacity in pineseeds and beechseeds. The purpose of the study was to investigate the antioxidant capacity and nutritional value of pineseeds and beechseeds. Antioxidant capacity was evaluated with photochemical assay as well as humidity, protein, fibre, lipid and carbohydrate content. The highest antioxidant capacity was observed in beechseeds 1292.79 ± 5.71 in comparation whit pineseeds 1.78 ± 0.39 (μg/mg equivalent ascorbic acid).
These results can provide useful information providing a research interest for the identification of new molecular compounds from forest tree seeds.

Key words*: pineseed (Pinus sylvestris), beechseed (Fagus sylvatica), FT-IR spectroscopy (Fourier transform infrared), antioxidants.*

INTRODUCTION

Conifers, including Scots pine (*Pinus sylvestris* L.), are a major source of wood products for forest industry.
The various species of pine are distributed throughout the northern hemisphere, found in Asia, Europe and North America, both in the wild (in coniferous forests) and in pine plantations that exist in several countries. The main pine seed producing countries are China, North Korea, Russia, Pakistan, Afghanistan, Italy, Spain, Portugal, Turkey. (Sharashkin and Gold, 2004).
This type of seeds contains a large amount of vitamin A, as well as lutein, an essential antioxidant for preventing macular degeneration. The significant magnesium content in pine seeds makes them ideal, through consumption, for eliminating fatigue and stress.
Pinolenic acid is a polyunsaturated fatty acid found exclusively in pine nut oil. It may help lower LDL (bad) cholesterol levels in the blood (Lee et al., 2004). Selective increase in pinolenic acid (all-cis-5, 9, 12-18:3) in Korean pine (*Pinus koraiensis*) nut oil by crystallization and its effect on LDL-receptor activity.
Beech (*Fagus sylvatica* L.) species are largely represented in the European, Asia and North America flora and various parts of the tree are used in traditional medicine.
The non-extractable components in bark include polysaccharides (cellulose, hemicellulose and pectic substances), phenolic polymers (lignin and high-molecular-weight tannins) and cross-linked polyesters (cutin and suberin (El-Bahy, 2005).
Some of the most important health benefits of beech include its ability to stimulate hair growth, protect the immune system, improve the health of the skin, boost newborn health, reduce respiratory distress, prevent infections, relieve pain, detoxify the kidneys, and protect against chronic diseases (Takala et al., 2023).

The beech acorn contains Omega 6 and Omega 3 which have been shown to have medicinal properties. Nuts of beech and pine are tasty nutrient-dense nuts produced in the fall by beech and pine trees (*Fagus* s., *Pinus* s.).
The biological activity and the pharmaceutical properties of plants are strongly dependent on their structure.
In the last year, FT-IR spectroscopy has been introduced as a very efficient and nondestructive analytical tool for the reliable way to determine the functional groups of tree components.
IR (infrared) spectroscopy is one such method which may be applied to dried samples and gives information on the broad chemical groups present depending upon the absorption peaks present. However, the whole spectrum may contain information that may relate to more general physical, chemical or biological properties. NIR (Near infrared reflectance) spectroscopy has been used extensively to assess changes during litter decomposition (McLellan et al., 1991), composting (Ben Dor et al., 1997) and for the prediction of soil (Couillard et al., 1997) and peat properties (HaÈmaÈlaÈinen and Albano, 1992; McTiernan et al., 1998).
FTIR is widely used in quantitative and qualitative analysis of wood because of its capacity to give information on the presence of functional groups, on composition and on some specific structural features (Esteves et al., 2013).
Fourier Transform Infrared Spectroscopy (FT-IR) techniques were used by Feria-Reyes et al., (2023) to evaluate the tannin content in the bark of five pine species.
Also, FT-IR spectroscopy has also been used to study the chemical and biological properties of peat in pine forests (Chapman et al., 2003).
The use of the FT-IR technique has also been reported for the analysis of food matrices (Andronie et al. 2016, Bebu et al. 2020), but literature mentions lots of works that involve the use of this technique and its great potential to be used in a large variety of other research fields (Andronie et. al. 2019; Keseru et al., 2016; Bebu et al., 2019, Andronie et al., 2022).
In this work, we employed FT-IR spectroscopy to characterize the spectra of the pine seeds *(Pinus sylvestris*) and beech seeds (*Fagus sylvatica*). Also, we analyzed the antioxidant capacity of the two types of seeds.

MATERIALS AND METHODS

In this research, we analyzed two different varieties of forest trees seeds (pine - *Pinus sylvestris* var. *hamata*) and beech *Fagus sylvatica tree* from the forests of Romania) according to the analytical information obtained from dried seeds by means of Fourier transform infrared spectroscopy (FT-IR). One common varieties of seeds from forest tress plants, pine seed *(Pinus sylvestris*), beech seed (*Fagus sylvatica*) was bought from a naturist store. The seeds samples were crushed using a commercial blender (Figure 1).

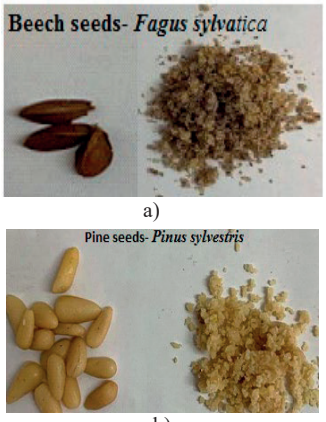


Figure 1. The forest trees seeds: beech - *Fagus sylvatica* (a) and pine - *Pinus sylvestris* (b)

Moisture determination
The determination of moisture content was evaluated by the air-oven method, according to the methodology previously described by Kumar and Balasubrahmanyam (1986) with slightly modifications. The weighted sample was introduced into a vial containing lid and conditioned at 105 °C for at least 45 minutes until a constant mass was obtained and therefore cooled in air-oven. Then 5-10 g of sample were spread uniformly on the entire surface of the vial and weighted with an accuracy of 0.001 g.
The humidity content was expressed in percentages.

FT-IR spectroscopy
The sample was prepared using calcinated potassium bromide as a matrix material and was mixed at a proportion of 3 mg of the sample (powder of pine seeds and beech seeds)

to 200 mg KBr. Then the mixture was condensed in 15 mm dies at a pressure equal to 10 t till 2 min. Same procedure was applied for all samples (Crișan et al., 2019)
Measurements were carried out on the infrared scale of 350-4000 cm^{-1} and a spectral resolution was set at 4 cm^{-1} using a Jasco FT-IR-4100 spectrophotometer (Oklahoma City, OK United States) using KBr pellet technique. All spectra were acquired over 256 scans.
The spectral data were analysed using Origin 6.0 software (Figures 2 and 3).
Measurements were carried out on infrared scale of 500-4000 cm^{-1}.

Photochemical assay

The ACL (Antioxidant Capacity of the Liposoluble Compounds) method previously described by (Hegedus et al., 2010), was used for estimating the antioxidant capacity. Photochem® equipment (Analytik Jena AG, Jena, Germany) was used for measuring the antioxidant capacity.
The hydrophilic and lipophilic radical-scavenging activities were measured using the ACL kits, provided by the manufacturer.
The extractions for ACL measurements were also carried out according to the manufacturer's protocol using Kit and methanol.
For these measurements we used 1 g of extract of the sample with 20 ml of methanol, then centrifuge the sample for 5 minutes (10,000 rpm).
The equipment calibration and measurement of samples were based on the inhibition of free radicals. The values of antioxidant capacity were achieved by establishing measurement curves that were compared to the measurement curves obtained for the standard solution.
The results were expressed in ascorbic acid equivalents (μg/mg). Data was calculated automatically using a software program called PCL soft.

Statistical analysis

The IBM SPSS v.19.0 for Windows, was used for statistical analysis. Basic statistics were implemented in order to emphasize the arithmetic mean (X) ± standard deviations (SD) of the content of humidity and antioxidant capacity of the studied forest seeds.
The mean concentration of humidity and antioxidant capacity were compared across the various forest plants using ANOVA followed by Tukey's test. Differences of the means were considered to be significant when p-value < 0.05 (Paunovic et al., 2019). ANOVA analysis was followed by Tukey's test in order to perform multiple comparisons regarding the antioxidant capacity of considered forest tree seeds.

RESULTS AND DISCUSSIONS

FT-IR analysis

The FT-IR spectra were used to identify the functional groups of the macronutrients bades on the IR absorption in typical spectral regions.
The characteristics of pine and beech seeds were the bands obtained from proteins, lipids and carbohydrates and these were clearly highlighted in the spectra obtained by the FT-IR technique.
Carbohydrates possess characteristic IR absorptions between 1200 and 750 cm relevant to coupling and combination of stretching and deformation or vibrational modes of individual bonds in the molecular skeleton.

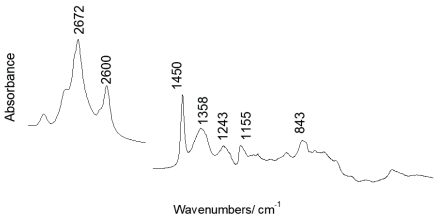


Figure 2. FT- IR spectrum of pine seeds (*Pinus sylvestris*)

The absorption bands for rocking of CH_2 may be found at 721 cm^{-1}.

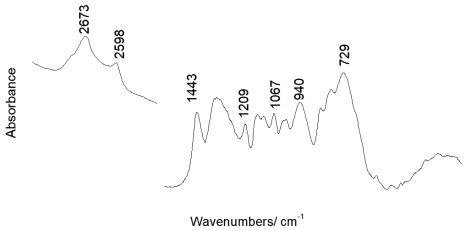


Figure 3. FT- IR spectrum of beech seeds (*Fagus sylvatica*)

The nutritional quality of the studied

The nutritional values for 100 g of two types of forest plants and the characteristic differences among them were present in Table 1.

Table 1. Nutritional parameters of the investigate seeds (100 g/sample)

Samples	Calories (kcal)	Lipids (g)	Protein (g)	Carbohydrates (g)	Fiber (g)
Pine	673±3.08	68.40±0.89	13.7±0.73	13.1±0.69	3.7± 0.89
Beech	619±2.05	51±1.39	17.21±1.24	20.59±0.34	11.5± 0.48

Note: *The mean difference at significant p< 0.05.

In pine seeds was identified the highest concentration in lipids and calories.

The beech seeds have the highest carbohydrates and fibers content in comparison with their content in pine. In addition, the beech showed an content of protein in comparison with pine.

Statistical analysis for the humidity content and antioxidant capacity

The test revealed significant differences between the mean amounts of calories, lipids and protein contained respectively in *Pinus sylvestris* and *Fagus sylvatica* (Table 1). Also, the amount of fiber contained in beech is significantly higher than that reported in pine seeds.

The Turkey test revealed significant differences between the mean amount of antioxidants contained respectively in the two categories of forest trees considered. The results of Turkey‘s test are displayed in Table 2.

Table 2. The content of humidity and antioxidant capacity in tested seeds

Samples	Humidity	Antioxidant Capacity (µg/mg equivalent ascorbic acid)
Pine (Pinus sylvestris)	0.39± 0.04	1.78 ± 0.08
Beech (*Fagus sylvatica*)	0.51 ± 0.07	1292.79 ± 5.71

Note: *The mean difference at significant p< 0.05.

The significance was determined at $p < 0.05$.

The results were expressed or plotted as the mean values ± standard deviation.

Relationships among antioxidant capacity and humidity content of pine and beech seeds are presented in Table 3. We obtained a strong significant positive correlation at $p<0.05$ level between antioxidant capacity and humidity content of considered seeds.

Table 3. Linear correlation coefficients between antioxidant capacity and humidity content of tested seeds

Samples	Antioxidant Capacity (µg/mg equivalent ascorbic acid) - *Pinus sylvestris*	Antioxidant Capacity (µg/mg equivalent ascorbic acid) - *Fagus sylvatica*)
Humidity - pine *(Pinus sylvestris)* %	0.62*	-
Humidity - beech (*Fagus sylvatica*) %	-	0.93*

Note: *Correlation is significant at p< 0.05

CONCLUSIONS

The data revealed that the beech have the highest antioxidant capacity when compared with pine samples. Beech samples from natural shop exhibit antioxidant capacity and more investigations should be performed in order to describe the complex interactions between antioxidants and the human body. It is promising to observe that molecular compounds, such as those in pine and beech seeds, may provide a potential interest in food and pharmaceutical research.

The comparison of the antioxidant activity of the studied forest tree seeds emphasized a hierarchy of the antioxidant capacity in pine seeds and beech seeds.

The resulted data revealed that the beech seeds have the highest antioxidant capacity. We obtained a strong significant positive correlation at $p<0.05$ level between antioxidant capacity and humidity content of considered seeds.FT-IR spectroscopy could provide quantitative information concerning the functional groups of treem components

REFERENCES

Andronie, L., Coroian, A., Miresan,V., Pop, I., Raducu, C., Rotaru, A., OLAR L. (2016). Results obtained by investigating saffron using FT-IR spectroscopy. *Bulletin UASVM Animal Science and Biotechnologies*, 73(2). 238-239.

Andronie, L., Holonec, L., Pop, I., Truta, A. M., Odagiu, A., Salagean, T., Sobolu, R., Coroian, A., Balta, I., & Șuba, E. E. (2019). Antioxidant Capacity of Several Romanian Forest Fruits (*Rosa canina* L., *Prunus spinosa* L., *Vaccinium vitis-idaea* L. and *Cornus mas* L.*). Notulae Botanicae Horti Agrobotanic,* Cluj-Napoca, 47(4), 1178-1184.

Andronie, L., Pop, I., Matei, F., Coroian, A., Rotaru, A., Sobolu. R., Maties, A., Roman, V., Bebu, A. (2022). Results obtained by investigating pumpkin (*Cucurbita maxima* L.) using ft-ir spectroscopy, *Scientific Papers. Series B, Horticulture*. Vol. LXVI, No. 2, Bucuresti.

Bebu, A., Andronie, L., Odagiu, A. (2019). Vibtational study for citrus fruits using Fourier Transform Infrared, *Scientific Papers, Series B, Horticulture*, Volume: LXIII, Issue: 1 Pages: 565-568.

Bebu, A., Andronie, L., Maties, A., Svetlana Micle, S., Darjan, S., Culcear, O. (2020). Comparative analysis between mushrooms *Lactus piperatus* and *Agaricus bisporus* (champignon) using FT-IR spectroscopy, *Scientific Papers. Series B, Horticulture*. Vol. LXIV, No. 1, 633-637.

BenDor, E., Inbar, Y., Chen, Y. (1997). The reflectance spectra of organic matter in the visible near-infrared and short wave infrared region (400± 2500 nm) during a controlled decomposition process. *Remote Sensing of Environment* , 61, 115.

Chapman, S.J., Campbell, C.D., Fraser, A.R., Puri, G. (2001). FTIR spectroscopy of peat in and bordering Scots pine woodland: relationship with chemical and biological properties, *Soil Biology & Biochemistry*, 33, 1193-1200.

Couillard, A., Turgeon, A.J., Shenk, J.S., Westerhaus, M.O. (1997). Near infrared refectance spectroscopy for analysis of turf soil profile. *Crop Science,* 37(5) 1554-1559.

Crișan, I., Vidican, R., Olar, L., Stoian, V., Morea, A., Ștefan, R. (2019). Screening for Changes on Iris germanica L. Rhizomes Following Inoculation with Arbuscular Mycorrhiza Using Fourier Transform Infrared Spectroscopy. *Agronomy*, 9:815. doi:10.3390/agronomy9120815.

E-Bahy, G.M.S. (2005). FTIR and Raman spectroscopic study of Fenugreek (*Trigonella foenum graecum* L.) seeds, *Journal of Applied Spectroscopy*, Vol. 72, No. 1.

Esteves, B., Marques, A., Domingos1, I,. Pereira, H. (2013). Chemical changes of heart treated pine and eucalypt wood monitored by FTIR, Maderas. *Ciencia y tecnología,* 15(2): 245-258.

Feria-Reyes, R., Ramírez-Cruz, S.O., Ruiz-Aquino, F., Robledo-Taboada, L.H., Sánchez-Medina, M.A., Mijangos-Ricárdez, O.F., Gabriel-Parra, R., Suárez-Mota, M.E., Puc-Kauil, R., Porcallo-Vargas, J. (2023).Pine Bark as a Potential Source of Condensed Tannin: Analysis through Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM), and Energy Dispersive X-ray (EDX). *Forests*, 14(7):1433.

HaÈmaÈlaÈinen, M., Albano, C. (1992). Principal variations in the chemical composition of peat: predictive peat scales based on multivariate strategies. *Journal of Chemometrics,* 6, 41-56.

Hegedus, A., Engel, R., Abrankó, L., Balogh, E., Blázovics, A., Hermán, R., Halász, J., Ercisli, S., Pedryc, A., Sefanovits Banyai, E. (2010). Antioxidant and Antiradical Capacities in Apricot (*Prunus armeniaca* L.) Fruits: Variations from Genotypes, Years, and Analytical Methods. *Journal of Food Science*,75(9):722-730.

Jin-Won, L., Kwang-Won, L., Seog-Won L., In-Hwan, K., Chul, R. (2004). Selective increase in pinolenic acid (all-cis-5,9,12-18:3) in Korean pine nut oil by crystallization and its effect on LDL-receptor activity. *Lipids*, 39(4):383-7.

Kesezu, A., Andronie, L., Maniutiu, D., Pop, I., Rotaru, A. (2016). Characterization *Momordica charantia* using FT-IR spectroscopy**.** *Bulletin UASVM Holticulture and Forestry*, 73(2).

Kumar, K.R., Balasubrahmanyam, N. (1986). Moisture sorption and the applicability of the Brunauer-Emmett-Teller equation for some dry food products. *Journal of Stored Products Research* 22(4):205-209.

McLellan, T.M., Aber, J.D., Martin, M.E., Melillo, J.M., Nadelhoffer, K.J. (1991). Determination of nitrogen, lignin, and cellulose content of decomposing leaf material by near-infrared reŽectance spectroscopy. *Canadian Journal of Forest Research*, 21, 1684-1688.

McTiernan, K.B., Garnett, M.H., Mauquoy, D., Ineson, P., Couteaux, M.M. (1998). Use of near-infrared re¯ectance spectroscopy (NIRS) in palaeoecological studies of peat. *Holocene*, 8, 729-740.

Paunovic, D., Kalusevic, A., Petrovic, T., Urosevic, T., Djinovic, D., Nedovic, V., Popovic-Djordjevic, J. (2019). Assessment of Chemical and Antioxidant Properties of Fresh and Dried Rosehip (*Rosa canina* L.) *Notulae Botanicae Horti Agrobotanici,* Cluj-Napoca 47(1)108-113.

Sharashkin, L., Gold, M. (2004). Pine nuts: species, products, markets, and potential for U.S. production. In: Northern Nut Growers Association 95th Annual Report. Proceeding for the 95th annual meeting, Columbia, Missouri, August 16-19.

Takala, R., Ramji, D.P., Choy, E.(2023). The Beneficial Effects of Pine Nuts and Its Major Fatty Acid, Pinolenic Acid, on Inflammation and Metabolic Perturbations in Inflammatory Disorders. *Int J Mol Sci*. Jan 6;24(2):1171 **.**

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

COMPARATIVE RESEARCH ON THE SITUATION, STRUCTURE AND ECOLOGICAL INDICES OF ARTHROPOD SPECIES ENCOUNTERED IN SOME FOREST AND APPLE ORCHARDS IN THE NECHIT AREA, NEAMȚ COUNTY

Vasile BARDAN, Nela TĂLMACIU, Ionela MOCANU, Renata SIPOS, Mihai TĂLMACIU, Monica HEREA

"Ion Ionescu de la Brad" Iași University of Life Sciences,
3 Mihail Sadoveanu Alley, Iași, Romania

Corresponding author email: monica.herea@iuls.ro

Abstract

The research was carried out in an apple orchard, located in the Nechit area, Neamț County and in a forestry plantation managed by the forestry district in the same area. For the collection of the material, pitfall traps were used during 2024. The samples were collected from both the apple orchard and the forestry plantation on the same dates, as follows: Harvest I: July 20 Harvest II: July 22 Harvest III: July 31 Harvest IV: August 14 Harvest V: August 28 Harvest VI: September 11 In the apple orchards, 8 material harvests were carried out on the same dates as in the forest plantations. The largest number of specimens was collected at the first harvest, 1,066 specimens, followed by the 6th harvest, on September 11, with 458 specimens. Then, 420 specimens were collected at the 7th harvest, on September 25, and 410 specimens at the 8th harvest, on October 16. The most frequently collected species, which also recorded the largest number of specimens, were Armadillidium vulgare, Pterostichus cupreus, Dermestes laniarius and arachnids.

Key words*: arthropod species, forest, apple orchards.*

INTRODUCTION

Entomofauna is an essential element of forest and orchard ecosystems, playing a fundamental role in the dynamics of insect populations, pollination, trophic cycles, and plant health. The study of the diversity and structure of the entomofauna in forests and apple orchards provides valuable information about ecological balance and the impact of agricultural practices on biodiversity (Bacal and Mihailov, 2020).

In forests, insect communities are influenced by factors such as tree species composition, humidity, temperature, and the presence of invasive species. On the other hand, apple orchards, being anthropogenically managed agroecosystems, are affected by phytosanitary treatments, monoculture, and soil management, which can significantly alter the structure of the entomofauna (Petrescu, 2008).

Coniferous and broadleaf forests are grouped into two layers.

Altitudinally, they are differentiated, with the transition from one layer to another occurring through plant groupings typical for each. The spruce forest layer, found between 1300 and 1700 m, is much more developed on the northern slopes. It is mainly composed of pure spruce stands (*Picea abies*).

At the source of rivers, it appears as a thin band at the upper limit of the forest, with silver fir (*Abies alba*) and, rarely, specimens of Swiss pine (*Pinus cembra*).

The broadleaf forest layer consists of beech forests mixed with spruce, fir, beech forests, and beech forests mixed with sessile oak.

Mixed beech and coniferous forests occur at altitudes between 900 and 1400 m.

They consist of European beech (*Fagus sylvatica*), spruce (*Picea abies*), silver fir *(Abies alba)*, mountain maple (*Acer pseudoplatanus*), mountain elm (*Ulmus glabra*), and rowan (*Sorbus aucuparia*). Beech forests surround the mountain ranges on the northern and western sides, occupying areas between 500 m and 1050 m, and sometimes reaching up to 1500 m in valleys. Typically, at the lower limit, beech appears in association with sessile oak (*Quercus petraea*).

The apple tree (*Malus domestica*) is one of the most cultivated fruit species worldwide, valued for its fruits rich in vitamins, antioxidants, and fiber. The apple cultivation is of significant economic and ecological importance, being essential for the food industry and for maintaining biodiversity through its role in supporting pollinators and beneficial entomofauna.

MATERIALS AND METHODS

Entomofauna plays an essential role in both natural forests and apple orchards, influencing the ecological balance and productivity of these ecosystems. In forests, insects contribute to the decomposition of organic matter, pollination, and regulation of pest populations, thereby maintaining the health of the habitat (Bârdan, 2023).
In apple orchards, beneficial entomofauna, such as pollinators and natural predators of pests, is crucial for achieving sustainable yields and reducing the need for chemical interventions (Malschi and Stan, 2006).
The main objective of this study is to compare the diversity and abundance of insect species in natural forests and apple orchards, highlighting the differences between these habitats and the potential effects of anthropogenic interventions on the entomological balance. The study aims to contribute to a better understanding of the role of insects in maintaining ecosystem health and to provide recommendations for the conservation of beneficial entomofauna in orchards.
To collect the epigeic fauna from both ecosystems (forest and orchard), Barber-type soil traps were installed (Andron, 2019). A 800 ml box was used as a soil trap, which was buried in the soil to its level, allowing insects to move freely without being obstructed by obstacles. A 2.5% NaCl solution was used as a fixative liquid. A metal lid was placed on top of the jar, supported by two stands, to protect the trap from precipitation.
The number of traps installed in each biotope was 6 or more. Talmaciu (2019) demonstrated that by using 5 traps, the dominant, subdominant, rare, and sub-rare species can be captured, while using more than 12 traps significantly alters the percentage ratios.
The traps can be arranged in a line, with equal distances between them, or uniformly across the entire area. The samples were collected from both study sites between April and September at intervals of approximately 7-10 days.
The experimental plots were established in both a forest plot and an intensive apple orchard, both belonging to the Nechit Monastery in Neamț County.



Figure 1. Installation of traps in the experimental plots

At each collection, the contents of the box were placed in a plastic container with a lid, gently cleaned, and then filled with a diluted medicinal alcohol solution. Each sample was labeled with the collection date, trap number, and variant.
In the laboratory, the material was cleaned of plant residues, then washed under a water jet, and the process of identification and species determination followed.

RESULTS AND DISCUSSIONS

The epigeic fauna collected in the Nechit station differs between the forest and the apple orchard, reflecting the characteristics of each ecosystem. In the forest, species diversity is greater, including beetles, millipedes, and other arthropods adapted to the forest environment. In contrast, the orchard is dominated by ants, spiders, and other invertebrates influenced by agricultural practices and soil structure. These differences highlight the impact of the habitat on the composition of the epigeic fauna.
Throughout the year 2024, from May to September, data collected from six sampling events of epigeic arthropod fauna in both the forest plot and the apple orchard provided the basis for determining the structure, dynamics, and ecological impact of the fauna.

Table 1. Species composition of arthropods collected in soil traps in the forest area from Nechit

No.	Name of species	Harvested								No. of traps	Total samples
		I	II	III	IV	V	VI	VII	VI		
1.	*Geotrupes vernalis*	11	16	2	22	3	3	8	33	31	98
2.	*Phalangium opilo*	28	13	9	8	16	10	12	0	32	96
3.	*Armadilidium vulgare*	6	3	16	0	3	3	2	3	9	36
4.	*Cychrus caraoides*	29	0	0	0	1	0	0	0	1	30
5.	*Necrophorus vespillo*	14	7	1	7	1	0	0	0	10	30
6.	*Panorpa comunnis*	5	11	0	0	0	0	0	0	9	16
7.	*Chromatoulus unilineatus*	3	1	0	0	2	3	3	3	12	15
8.	*Messor structor*	10	2	1	1	0	0	0	0	9	14
9.	*Aptinus bombarda*	8	2	0	3	0	0	0	0	8	13
10.	*Atomaria turgida*	0	9	0	0	0	0	0	0	1	9
11.	*Staphilinus olens*	3	4	0	0	0	0	0	0	5	7
12.	*Dolycoris baccarum*	0	0	1	0	0	0	5	1	5	7
13.	*Carabus coriaceus*	0	3	0	0	0	1	1	1	4	6
14.	*Dadobia immersa*	0	5	0	0	0	0	0	0	1	5
15.	*Poecilus cupreus*	0	1	3	0	0	0	0	0	2	4
16.	*Pterostichus niger*	0	0	0	3	1	0	0	0	4	4
17.	*Carabus convexus*	3	0	0	0	0	0	0	0	1	3
18.	*Ectobius lapponicus*	2	0	0	0	0	0	0	1	2	3
19.	*Dociostaurus marocanus*	0	0	0	2	1	0	0	0	3	3
20.	*Cychrus attenuatus*	0	0	0	0	0	0	3	0	3	3
21.	*Quedius unicolor*	0	0	0	0	0	0	0	3	2	3
22.	*Carabus hungaricus*	2	0	0	0	0	0	0	0	2	2
23.	*Petrobius brevistylis*	0	2	0	0	0	0	0	0	2	2
24.	*Oiceoptoma thoracica*	0	2	0	0	0	0	0	0	1	2
25.	*Silpha carinata*	0	1	1	0	0	0	0	0	2	2
26.	*Pterostichus vulgaris*	0	0	0	0	0	0	0	2	1	2
27.	*Ceruchus chrysomelinus*	1	0	0	0	0	0	0	0	1	1
28.	*Hister sinuatus*	1	0	0	0	0	0	0	0	1	1
29.	*Poecilus cupreus*	1	0	0	0	0	0	0	0	1	1
30.	*Brachynus crepitans*	0	1	0	0	0	0	0	0	1	1
31.	*Cychrus semigranosus*	0	1	0	0	0	0	0	0	1	1
32.	*Pterostichus cylindricus*	0	1	0	0	0	0	0	0	1	1
33.	*Staphylinus caesareus*	0	1	0	0	0	0	0	0	1	1
34.	*Meligethes aeneus*	0	0	0	0	0	1	0	0	1	1
35.	*Psyllobora vigintiduopunctata*	0	0	0	0	0	0	1	0	1	1
36.	*Carabus intricatus*	0	0	0	0	0	0	0	1	1	1
37.	*Leistus rufomarginatus*	0	0	0	0	0	0	0	1	1	1
	Total 37 species	127	86	34	46	28	21	35	49	48	412

The data summarized in Table 1 present the diversity and abundance of the epigeic fauna collected in the 48 traps in the Nechit station, located in the forest plot. A total of 37 species were identified, comprising 412 individuals.

The most abundant species are *Geotrupes vernalis* (98 individuals) and *Phalangium opilo* (96 individuals), indicating their dominant presence in the habitat. Some species, such as *Ceruchus chrysomelinus* and *Hister sinuatus*, as well as 11 other species, are very rare, represented by only a single specimen.

The distribution across collections varies widely, suggesting differences in microhabitat and the ecological preferences of the species.

Table 2. The situation regarding the calculation of ecological factors for each species during the period in forest area from Nechit

No.	Name of species	No. of traps	Abundance	Constancy		Dominance		W	
				%	***	%	***	%	***
1.	*Geotrupes vernalis*	31	98	64,6	C3	23,8	D3	15,375	W5
2.	*Phalangium opilo*	32	96	66,7	C3	23,3	D5	15,541	W5
3.	*Armadilidium vulgare*	9	36	18,7	C1	8,7	D4	1,627	W3
4.	*Cychrus caraoides*	1	30	2,1	C1	7,3	D1	0,153	W2
5.	*Necrophorus vespillo*	10	30	20,8	C1	7,3	D4	1,518	W3
6.	*Panorpa comunnis*	9	16	18,7	C1	3,9	D3	0,729	W2
7.	*Chromatoulus unilineatus*	12	15	25,0	C1	3,6	D3	0,900	W2
8.	*Messor structor*	9	14	18,7	C1	3,4	D1	0,636	W2
9.	*Aptinus bombarda*	8	13	16,7	C1	3,1	D3	0,518	W2
10.	*Atomaria turgida*	1	9	2,1	C1	2,2	D3	0,046	W1
11.	*Staphilinus olens*	5	7	10,4	C1	1,7	D2	0,176	W2
12.	*Dolycoris baccarum*	5	7	10,4	C1	1,7	D2	0,176	W2
13.	*Carabus coriaceus*	4	6	8,3	C1	1,5	D2	0,124	W2

No.	Name of species	No. of traps	Abundance	Constancy		Dominance		W	
				%	***	%			
14.	*Dadobia immersa*	1	5	2,1	C1	1,2	D2	0,025	W1
15.	*Poecilus cupreus*	2	4	4,2	C1	1,0	D1	0,042	W1
16.	*Pterostichus niger*	4	4	8,3	C1	1,0	D1	0,083	W1
17.	*Carabus convexus*	1	3	2,1	C1	0,7	D1	0,015	W1
18.	*Ectobius lapponicus*	2	3	4,2	C1	0,7	D4	0,029	W1
19.	*Dociostaurus marocanus*	3	3	6,2	C1	0,7	D1	0,043	W1
20.	*Cychrus attenuatus*	3	3	6,2	C1	0,7	D1	0,043	W1
21.	*Quedius unicolor*	2	3	4,2	C1	0,7	D1	0,029	W1
22.	*Carabus hungaricus*	2	2	4,2	C1	0,5	D1	0,021	W1
23.	*Petrobius brevistylis*	2	2	4,2	C1	0,5	D1	0,021	W1
24.	*Oiceoptoma thoracica*	1	2	2,1	C1	0,5	D1	0,010	W1
25.	*Silpha carinata*	2	2	4,2	C1	0,5	D1	0,021	W1
26.	*Pterostichus vulgaris*	1	2	2,1	C1	0,5	D1	0,010	W1
27.	*Ceruchus chrysomelinus*	1	1	2,1	C1	0,2	D1	0,004	W1
28.	*Hister sinuatus*	1	1	2,1	C1	0,2	D5	0,004	W1
29.	*Poecilus cupreus*	1	1	2,1	C1	0,2	D1	0,004	W1
30.	*Brachynus crepitans*	1	1	2,1	C1	0,2	D1	0,004	W1
31.	*Cychrus semigranosus*	1	1	2,1	C1	0,2	D1	0,004	W1
32.	*Pterostichus cylindricus*	1	1	2,1	C1	0,2	D1	0,004	W1
33.	*Staphylinus caesareus*	1	1	2,1	C1	0,2	D1	0,004	W1
34.	*Meligethes aeneus*	1	1	2,1	C1	0,2	D1	0,004	W1
35.	*Psyllobora vigintiduopunctata*	1	1	2,1	C1	0,2	D1	0,004	W1
36.	*Carabus intricatus*	1	1	2,1	C1	0,2	D1	0,004	W1
37.	*Leistus rufomarginatus*	1	1	2,1	C1	0,2	D1	0,004	W1
	Total 37 species	48	412						

As a result of the calculation of the ecological indices, the distribution and importance of species in the epigeic fauna collected are highlighted (Table 2). *Geotrupes vernalis* (C3, D3, W5) and *Phalangium opilo* (C3, D5, W5) are constant and edifice species, having the highest values of constancy and dominance, which gives them an essential role in the community structure. *Armadilidium vulgare* (C1, D4, W3) and *Necrophorus vespillo* (C1, D4, W3) are dominant accessory species, with a significant presence, but lower compared to the edifice species. In contrast, species like *Cychrus caraboides* (C1, D1, W2) and *Panorpa communis* (C1, D3, W2) are subdominant and occasional, with low constancy, indicating a sporadic presence in the habitat.

The majority of species (e.g., *Staphilinus olens*, *Carabus coriaceus*, *Dociostaurus marocanus*) are classified as accidental species (C1, D1-D2, W1), having very low frequency and ecological influence.

This analysis suggests an ecosystem in which only a few species are dominant, while most species have a sporadic presence, influenced by environmental factors, possibly even by the zoophagous nature of predatory species.

Table 3. Species composition of arthropods collected in soil traps in apple orchards from Nechit

No.	Name of species	Harvest								Number of traps	Total samples
		I	II	III	IV	V	VI	VII	VIII		
1.	*Absidia pilosa*	1	0	0	0	0	0	0	0	1	1
2.	*Aclypea alpicola*	0	3	1	0	7	15	1	0	3	3
3.	*Acylophorus glaberimus*	0	8	0	0	0	0	0	0	5	8
4.	*Acylophorus glaberrimus*	0	0	1	0	0	0	0	0	1	1
5.	*Agriotes aterrimus*	7	0	0	0	0	0	0	0	4	7
6.	*Agriotes lineatus*	0	0	0	0	0	0	1	0	1	1
7.	*Agriotes ustulatus*	15	1	0	0	0	0	0	0	10	16
8.	*Aleochara moerens*	0	0	0	0	1	0	0	0	1	1
9.	*Amara aenea*	6	0	0	0	0	0	0	0	4	6
10.	*Anisodactylus binotatus*	0	0	1	0	1	0	0	0	2	2
11.	*Anisodactylus signatus*	0	0	0	7	0	0	0	0	2	7
12.	*Aphanisticus pusillus*	0	1	0	0	0	0	0	0	1	1
13.	*Aptinus bombarda*	0	0	0	0	1	0	0	0	1	1
14.	*Armadilidium vulgare*	389	194	108	42	74	287	329	339	81	1762
15.	*Athous haemorrhoidalis*	0	2	0	0	0	0	0	0	1	2
16.	*Barynotus obscurus*	0	0	0	0	0	1	0	0	1	1
17.	*Bembidion varium*	1	0	0	0	0	0	0	0	1	1
18.	*Brachinus crepitans*	4	0	0	0	0	0	0	0	3	4
19.	*Byrrhus pustulatus*	0	0	0	0	0	0	0	1	1	1
20.	*Calathus fuscipes*	0	0	0	0	0	1	0	0	1	1
21.	*Carabus coriaceus*	2	0	0	0	0	0	0	0	2	2

No.	Name of species	No. of traps								Abundance	Constancy
		I	II	III	IV	V	VI	VII	VIII		
22.	*Chromatiulus unilineatus*	0	1	1	0	1	0	1	11	14	15
23.	*Chrysomela geminata*	1	1	0	0	0	0	0	0	2	2
24.	*Chrysomela rufa*	0	0	0	0	0	0	0	1	1	1
25.	*Cicindela campestris*	1	0	0	0	0	0	0	0	1	1
26.	*Cicindela germanica*	0	1	0	0	0	0	0	0	1	1
27.	*Cleonis punctiger*	1	0	0	0	0	0	0	0	1	1
28.	*Clivina fossor*	1	0	0	0	0	0	0	0	1	1
29.	*Clytra humeralis*	1	0	0	0	0	0	0	0	1	1
30.	*Coccinella septempunctata*	0	0	0	0	0	0	0	1	1	1
31.	*Dermestes laniarius*	15	6	1	1	1	1	0	0	12	25
32.	*Drypta dentata*	1	0	0	0	0	0	0	0	1	1
33.	*Elater nigrinus*	0	0	0	0	0	0	0	1	1	1
34.	*Forficula auricularia*	0	0	0	0	0	2	0	1	3	2
35.	*Galeruca pomonae*	0	0	1	0	0	0	0	1	2	2
36.	*Galeruca tanaceti*	0	0	0	0	0	0	1	1	1	1
37.	*Gryllotalpa gryllotalpa*	0	2	0	0	0	0	0	0	1	2
38.	*Harpalus calceatus*	0	0	0	0	0	1	1	0	1	2
39.	*Harpalus distinguendus*	0	0	0	0	0	0	0	1	1	1
40.	*Harpalus marginella*	0	0	0	0	0	0	0	1	1	1
41.	*Harpalus tardus*	1	0	0	1	0	0	0	0	2	2
42.	*Hister funestus*	1	0	0	0	0	0	0	0	1	1
43.	*Hister neglectus*	3	0	0	0	0	0	0	0	2	3
44.	*Hister ruficornis*	0	2	0	0	0	0	0	0	1	2
45.	*Hister vernalis*	0	1	0	0	0	0	0	0	1	1
46.	*Hypera nigriostris*	0	1	0	0	0	0	0	0	1	1
47.	*Licinus granulatus*	1	0	0	0	0	0	0	0	1	1
48.	*Liophloeus tessulatus*	0	0	0	0	0	0	1	0	1	1
49.	*Lixus cardui*	0	0	0	0	0	0	1	0	1	1
50.	*Lygaeus aequestris*	47	7	3	0	0	0	0	0	12	57
51.	*Messor structor*	15	0	0	0	22	92	2	0	9	131
52.	*Mordellistena parvula*	0	1	0	0	0	0	0	0	1	1
53.	*Nebria brevicollis*	0	0	0	0	0	0	0	2	1	2
54.	*Necrophorus vespillo*	1	6	2	0	0	0	0	0	7	9
55.	*Notaris bimaculatus*	0	0	0	0	0	1	0	0	1	1
56.	*Oiceoptoma thoracium*	1	0	0	0	0	0	0	0	1	1
57.	*Onthophagus ovatus*	20	2	1	0	0	0	6	0	11	29
58.	*Onthophagus vacca*	5	0	0	0	0	0	0	0	2	5
59.	*(Dociostaurus maroccanus)*	0	0	0	0	0	0	0	1	1	1
60.	*Panorpa communis*	1	1	1	0	0	0		0	10	14
61.	*Phalangium opilio*	206	71	29	49	49	49	44	23	87	520
62.	*Philonthus coruscus*	0	0	0	0	0	1	0	0	1	1
63.	*Plagiodera versicolora*	0	0	0	0	0	1	0	0	1	1
64.	*Platynus glacialis*	0	0	0	0	0	0	1	0	1	1
65.	*Pseudophonus griseus*	0	0	0	1	0	0	0	0	1	1
66.	*Pseudophonus pubescens*	4	4	0	0	2	0	0	0	9	10
67.	*Pterostichus cupreus*	286	74	30	19	6	11	17	22	53	485
68.	*Pterostichus lepidus*	6	0	0	0	0	0	0	0	3	6
69.	*Pterostichus niger*	0	0	0	0	0	2	0	0	1	2
70.	*Pterostichus nigrita*	1	0	0	0	0	0	0	0	1	1
71.	*Pterostichus vulgaris*	0	0	0	0	0	0	0	2	1	1
72.	*Pyrrhocoris apterus*	0	6	0	6	6	2	0	0	15	20
73.	*Quedius cruentus*	5	0	0	0	0	0	0	0	2	5
74.	*Quedius unicolor*	0	0	0	0	1	1	0	1	3	3
75.	*Silpha carinata*	1	1	0	0	0	0	0	0	2	2
76.	*Silpha obscura*	5	0	0	1	0	0	1	0	5	7
77.	*Sitona lineatus*	0	0	0	0	0	0	1	1	2	2
78.	*Sphenophorus piceus*	1	0	0	0	0	0	0	0	1	1
79.	*Staphylinus caesareus*	13	1	0	0	0	0	1	0	7	15
80.	*Staphylinus olens*	5	1	0	0	0	0	0	0	4	6
81.	*Tachinus elongatus*	0	0	0	0	0	0	1	0	1	1
82.	*Typocerus attenuatus*	0	1	0	0	0	0	0	0	1	1
Total 82 species		1075	400	180	127	172	468	409	411	96	3247

The study of epigeic arthropods (Table 3) collected through soil traps in the apple orchard at Nechit from May to September highlights significant diversity, with 82 identified species and a total of 3247 individuals collected. The data suggest the presence of dominant species, as well as a variable seasonal distribution. The dominant species include *Armadilidium vulgare* (1762 individuals), *Phalangium opilio* (520 individuals), and *Pterostichus cupreus* (485 individuals), indicating a major presence of detritivorous and predatory species, which are

essential for the ecological balance of the soil. In contrast, some species such as *Brachinus crepitans* or *Cicindela campestris* are represented by a single specimen, suggesting a sporadic presence or more restricted habitat. Additionally, the significant variation between collections suggests a heterogeneous distribution of species, possibly influenced by microclimatic factors.

Table 4. The situation regarding the calculation of ecological factors for each species during the period in apple orchards from Nechit

No.	Name of species	Number of traps	Abundance	Constancy		Dominance		W	
				%	***	%	***	%	***
1.	*Armadilidium vulgare*	81	1762	84,4	C4	54,3	D5	45,829	W5
2.	*Phalangium opilio*	87	520	90,6	C4	16,0	D5	14,496	W5
3.	*Pterostichus cupreus*	53	485	55,2	C3	14,9	D5	8,224	W4
4.	*Messor structor*	9	131	9,3	C1	4,0	D3	0,372	W2
5.	*Lygaeus aequestris*	12	57	12,5	C1	1,7	D2	0,212	W2
6.	*Onthophagus ovatus*	11	29	11,5	C1	0,9	D1	0,103	W2
7.	*Dermestes laniarius*	12	25	12,5	C1	0,8	D1	0,100	W1
8.	*Pyrrhocoris apterus*	15	20	15,6	C1	0,6	D1	0,094	W1
9.	*Agriotes ustulatus*	10	16	10,4	C1	0,5	D1	0,052	W1
10.	*Chromatiulus unilineatus*	14	15	14,6	C1	0,5	D1	0,073	W1
11.	*Staphylinus caesareus*	7	15	7,2	C1	0,5	D1	0,036	W1
12.	*Panorpa communis*	10	14	10,4	C1	0,4	D1	0,042	W1
13.	*Pseudophonus pubescens*	9	10	9,3	C1	0,3	D1	0,028	W1
14.	*Necrophorus vespillo*	7	9	7,2	C1	0,3	D1	0,022	W1
15.	*Acylophorus glaberimus*	5	8	5,2	C1	0,2	D1	0,010	W1
16.	*Agriotes aterrimus*	4	7	4,2	C1	0,2	D1	0,008	W1
17.	*Anisodactylus signatus*	2	7	2,1	C1	0,2	D1	0,004	W1
18.	*Silpha obscura*	5	7	5,2	C1	0,2	D1	0,010	W1
19.	*Amara aenea*	4	6	4,2	C1	0,2	D1	0,008	W1
20.	*Pterostichus lepidus*	3	6	3,1	C1	0,2	D1	0,006	W1
21.	*Staphylinus olens*	4	6	4,2	C1	0,2	D1	0,008	W1
22.	*Onthophagus vacca*	2	5	2,1	C1	0,1	D1	0,002	W1
23.	*Quedius cruentus*	2	5	2,1	C1	0,1	D1	0,002	W1
24.	*Brachinus crepitans*	3	4	3,1	C1	0,1	D1	0,003	W1
25.	*Aclypea alpicola*	3	3	3,1	C1	0,1	D1	0,003	W1
26.	*Hister neglectus*	2	3	2,1	C1	0,1	D1	0,002	W1
27.	*Quedius unicolor*	3	3	3,1	C1	0,1	D1	0,0003	W1
28.	*Anisodactylus binotatus*	2	2	2,1	C1	0,1	D1	0,002	W1
29.	*Athous haemorrhoidalis*	1	2	1,0	C1	0,1	D1	0,001	W1
30.	*Carabus coriaceus*	2	2	2,1	C1	0,1	D1	0,002	W1
31.	*Chrysomela geminata*	2	2	2,1	C1	0,1	D1	0,002	W1
32.	*Forficula auricularia*	3	2	3,1	C1	0,1	D1	0,003	W1
33.	*Galeruca pomonae*	2	2	2,1	C1	0,1	D1	0,002	W1
34.	*Gryllotalpa gryllotalpa*	1	2	1,0	C1	0,1	D1	0,001	W1
35.	*Harpalus calceatus*	1	2	1,0	C1	0,1	D1	0,001	W1
36.	*Harpalus tardus*	2	2	2,1	C1	0,1	D1	0,002	W1
37.	*Hister ruficornis*	1	2	1,0	C1	0,1	D1	0,001	W1
38.	*Nebria brevicollis*	1	2	1,0	C1	0,1	D1	0,001	W1
39.	*Pterostichus niger*	1	2	1,0	C1	0,1	D1	0,001	W1
40.	*Silpha carinata*	2	2	2,1	C1	0,1	D1	0,002	W1
41.	*Sitona lineatus*	2	2	2,1	C1	0,1	D1	0,002	W1
42.	*Absidia pilosa*	1	1	1,0	C1	0,03	D1	<0,001	W1
43.	*Acylophorus glaberrimus*	1	1	1,0	C1	0,03	D1	<0,001	W1
44.	*Agriotes lineatus*	1	1	1,0	C1	0,03	D1	<0,001	W1
45.	*Aleochara moerens*	1	1	1,0	C1	0,03	D1	<0,001	W1
46.	*Aphanisticus pusillus*	1	1	1,0	C1	0,03	D1	<0,001	W1
47.	*Aptinus bombarda*	1	1	1,0	C1	0,03	D1	<0,001	W1
48.	*Barynotus obscurus*	1	1	1,0	C1	0,03	D1	<0,001	W1
49.	*Bembidion varium*	1	1	1,0	C1	0,03	D1	<0,001	W1
50.	*Byrrhus pustulatus*	1	1	1,0	C1	0,03	D1	<0,001	W1
51.	*Calathus fuscipes*	1	1	1,0	C1	0,03	D1	<0,001	W1
52.	*Chrysomela rufa*	1	1	1,0	C1	0,03	D1	<0,001	W1
53.	*Cicindela campestris*	1	1	1,0	C1	0,03	D1	<0,001	W1
54.	*Cicindela germanica*	1	1	1,0	C1	0,03	D1	<0,001	W1
55.	*Cleonis punctiger*	1	1	1,0	C1	0,03	D1	<0,001	W1
56.	*Clivina fossor*	1	1	1,0	C1	0,03	D1	<0,001	W1
57.	*Clytra humeralis*	1	1	1,0	C1	0,03	D1	<0,001	W1
58.	*Coccinella septempunctata*	1	1	1,0	C1	0,03	D1	<0,001	W1
59.	*Drypta dentata*	1	1	1,0	C1	0,03	D1	<0,001	W1
60.	*Elater nigrinus*	1	1	1,0	C1	0,03	D1	<0,001	W1
61.	*Galeruca tanaceti*	1	1	1,0	C1	0,03	D1	<0,001	W1

No.	Name of species	Number of traps	Abundance	Constancy		Dominance		W	
				%	***	%	***	%	***
62.	*Harpalus distinguendus*	1	1	1,0	C1	0,03	D1	<0,001	W1
63.	*Harpalus marginella*	1	1	1,0	C1	0,03	D1	<0,001	W1
64.	*Hister funestus*	1	1	1,0	C1	0,03	D1	<0,001	W1
65.	*Hister vernalis*	1	1	1,0	C1	0,03	D1	<0,001	W1
66.	*Hypera nigriostris*	1	1	1,0	C1	0,03	D1	<0,001	W1
67.	*Licinus granulatus*	1	1	1,0	C1	0,03	D1	<0,001	W1
68.	*Liophloeus tessulatus*	1	1	1,0	C1	0,03	D1	<0,001	W1
69.	*Lixus cardui*	1	1	1,0	C1	0,03	D1	<0,001	W1
70.	*Mordellistena parvula*	1	1	1,0	C1	0,03	D1	<0,001	W1
71.	*Notaris bimaculatus*	1	1	1,0	C1	0,03	D1	<0,001	W1
72.	*Oiceoptoma thoracium*	1	1	1,0	C1	0,03	D1	<0,001	W1
73.	*Ortoptere (Dociostaurus maroccanus)*	1	1	1,0	C1	0,03	D1	<0,001	W1
74.	*Philonthus coruscus*	1	1	1,0	C1	0,03	D1	<0,001	W1
75.	*Plagiodera versicolora*	1	1	1,0	C1	0,03	D1	<0,001	W1
76.	*Platynus glacialis*	1	1	1,0	C1	0,03	D1	<0,001	W1
77.	*Pseudophonus griseus*	1	1	1,0	C1	0,03	D1	<0,001	W1
78.	*Pterostichus nigrita*	1	1	1,0	C1	0,03	D1	<0,001	W1
79.	*Pterostichus vulgaris*	1	1	1,0	C1	0,03	D1	<0,001	W1
80.	*Sphenophorus piceus*	1	1	1,0	C1	0,03	D1	<0,001	W1
81.	*Tachinus elongatus*	1	1	1,0	C1	0,03	D1	<0,001	W1
82.	*Typocerus attenuatus*	1	1	1,0	C1	0,03	D1	<0,001	W1
Total 82 species		96	3247						

As a result of the determinations and data compilation obtained from the apple orchard in Nechit, the epigeic arthropod fauna is dominated by a few species with constant presence and major ecological impact, while the majority of species are accidental and have reduced influence. The species *Armadilidium vulgare* (C4, D5, W5) and *Phalangium opilio* (C4, D5, W5) are the most frequent and influential, with high constancy and significant ecological weight, while the species *Pterostichus cupreus* (C3, D5, W4) joins them as a constant species, playing an important role in the ecosystem.

In contrast, most species are accidental (C1), with a presence below 25% and low dominance (D1-D3). Among these, *Messor structor* (C1, D3, W2) and *Lygaeus aequestris* (C1, D2, W2) are worth mentioning. Over 90% of these species have a very low ecological significance index (W1 <0.1%).

Overall, the structure of the fauna suggests a relatively stable ecosystem, dominated by a few species adapted to the orchard environment, while the rest appear sporadically, without a major impact.

Table 5. Centralization of common species collected in both ecosystems during the observation period

No.	Name of species	Forest area		Apple orchards		Total samples	Type of food***
		No. of traps	Abundance	No. of traps	Abundance		
1.	*Aptinus bombarda*	8	**13**	1	**1**	**14**	P
2.	*Armadilidium vulgare*	9	**36**	81	**1762**	**1798**	I
3.	*Brachinus crepitans*	1	**1**	4	**5**	**6**	P
4.	*Carabus coriaceus*	4	**6**	2	**2**	**8**	P
5.	*Chromatiulus unilineatus*	12	**15**	14	**15**	**30**	
6.	*Messor structor*	9	**14**	9	**131**	**145**	P
7.	*Necrophorus vespillo*	10	**30**	7	**9**	**39**	I
8.	*Oiceoptoma thoracium*	1	**2**	1	**1**	**3**	I
9.	*Panorpa communis*	9	**16**	10	**14**	**30**	P
10.	*Phalangium opilio*	32	**96**	87	**520**	**616**	P
11.	*Pterostichus niger*	4	**4**	1	**2**	**6**	P
12.	*Quedius unicolor*	2	**3**	3	**3**	**6**	P
13.	*Silpha carinata*	2	**2**	2	**2**	**4**	P
14.	*Staphylinus caesareus*	1	**1**	7	**15**	**16**	P
Total			**239**		**2482**	**2721**	

***P=pradatory species, D=harmful species, I= indifferent species

Table 5 reflects the distribution and abundance of 14 common insect species in the two ecosystems, forest and apple orchard, in Nechit village, Neamț County.

Species such as *Armadilidium vulgare* and *Phalangium opilio* are much more abundant in the apple orchard plantation, being present in 81 and 87 traps, respectively, in this location,

indicating their preference for the conditions in the orchard. Other species, such as *Necrophorus vespillo* and *Messor structor*, have a more balanced distribution between the two experimental plots (forest and apple orchard), suggesting greater adaptability to both environments.
When analyzing the data for species such as *Brachinus crepitans* and *Oiceoptoma thoracium*, these are much rarer, with only a few individuals captured in both ecosystems.
In total, there is a significantly greater abundance in the apple orchard, with 2482 individuals collected, compared to 239 individuals from the traps placed in the forest plot, suggesting a higher ecological diversity in this habitat.

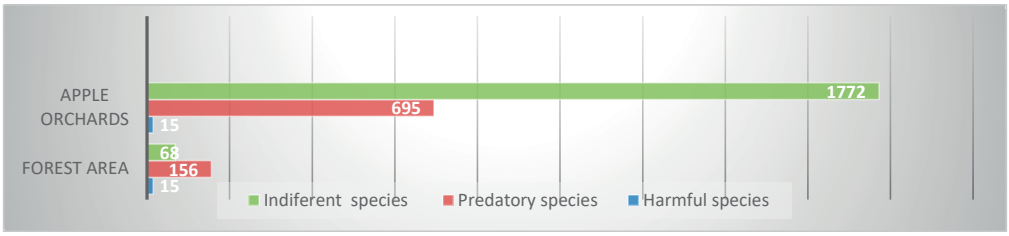


Figure 2. Abundance of common species collected in the two stations and their feeding type

In the graphical representation, it can be observed that the common species collected in the two ecosystems are strongly influenced by the habitat conditions provided. Thus, following the collections from May to September 2024, the common arthropod species totaled 239 individuals in the traps from the forest plot and 2482 individuals in the station located in the apple orchard plantation. It is evident that in the orchard plantation, the number of indifferent individuals is the highest, reaching a value of 1772, followed by the predatory species, while the number of herbivorous individuals is balanced in both plots, totaling 15 individuals each.

CONCLUSIONS

Diversity and abundance of fauna are influenced by the type of ecosystem, which reflects the fact that in the forest plot, the epigeic fauna is characterized by low abundance and a sporadic presence of most species, whereas in the apple orchard plantation, a much greater diversity and significantly higher abundance were observed (2482 individuals compared to 239 in the forest). This suggests that apple orchards provide much more favorable conditions for the development
The dominant species are constant in both ecosystems, and species such as *Geotrupes vernalis* and *Phalangium opilio* are the most abundant and constantly present in both locations, having a significant ecological impact. These species are considered "edifices," with a large proportion in the faunal structure of the studied habitats.
Accidental species play a minor ecological role, as many of the species collected in both stations, such as *Staphilinus olens* and *Carabus coriaceus*, have low frequency and minor ecological influence, which places them in the category of accidental species, with a sporadic presence and low weight in the faunal structure.
The significant variability in the collections from May to September indicates the influence of microclimatic factors on species distribution, especially in the case of species with sporadic presence (e.g., *Brachinus crepitans*, *Cicindela campestris*).
The different abundance between the two ecosystems suggests specific ecological preferences. Therefore, detritivorous and predatory species, such as *Armadilidium vulgare* and *Pterostichus cupreus*, are much more abundant in apple orchards, indicating a preference for the ecological conditions of this type of habitat, in contrast to species with a lower presence in the forest.

REFERENCES

Andron G., Tălmaciu M., Herea Monica, Manole Liliana, Tălmaciu Nela (2019). Observations on the epigeous coleopters fauna in some fruit apple orchards, *Annals of the University of Craiova - Agriculture, Montanology, Cadastre Series*, XLIX, 329-336.
Bacal Svetlana, Mihailov Irina (2020). Contribuții la cunoașterea coleopterelor epigee din unele ecosisteme forestiere din regiunea de nord a Republicii Moldova,

Provocări și tendințe actuale în cercetarea componentelor naturale și socio-economice ale ecosistemelor urbane și rurale, Chișinău, ISBN 978-9975-89-160-8.

Bârdan M.V., Tălmaciu M. (2023). Integrated pest management in deciduous and resinous forests in Neamț Country, *Natural sciences in the dialogue of generations*, Moldova State University, Chisinau, 24-29.

Malschi Dana, Stan Ghe. (2006). Reconstrucția ecologică - principii, noi orientări, perspective. *In Environment & Progress*, 7, 257-266

Petrescu I., Rusu T., Oroian I. (2008). Educatie si cercetare in protectia mediului. *Univ. Babeş-Bolyai, Facultatea de ştiinţa mediului, ED. Fundatia pentru Studii Europene*, Cluj-Napoca, p. 49-56.

Talmaciu M., Talmaciu Nela, Mocanu Ionela, Herea Monica (2020). Research on the entomofauna structure of coleopters from apple tree plantations according to the growing area and in the climate change context, *Scientific Papers. Series B, Horticulture*. LXIV, No. 2, 417-423.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

RESEARCH ON THE DEVELOPMENT OF A LOW CARB MINI-BAGUETTE FORTIFIED WITH *SPIRULINA* POWDER, INTENDED FOR DIABETICS AND OBESE INDIVIDUALS

Luminița CATANĂ[1], Monica CATANĂ[1], Anda-Grațiela BURNETE[1], Monica-Alexandra DÂRĂ[1], Florica CONSTANTINESCU[1], Gabriela CRIVEANU[1], Adrian ASĂNICĂ[2]

[1]National Research & Development Institute for Food Bioresources, IBA Bucharest, 6 Dinu Vintilă Street, District 2, 021102, Bucharest, Romania
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Horticulture, 59 Mărăşti Blvd, District 1, 011464, Bucharest, Romania

Corresponding author email: mcatana1965@gmail.com

Abstract

Diabetes mellitus is a condition with a high incidence both nationally and internationally. In Romania, according to data provided by the Romanian Society of Diabetes, Nutrition, and Metabolic Diseases, 1 in 10 romanians suffer from diabetes mellitus. In diabetes and obesity, diet is an essential therapeutic element, and therefore, the development of low-carb bakery products is of real interest. This paper presents research on the development of a mini-baguette fortified with Spirulina powder (fortification levels 1-4%), intended for diabetics and obese individuals. The low-carb mini-baguette fortified with 4% Spirulina powder exhibits superior sensory qualities and has the lowest total carbohydrate content (39.47%) and available carbohydrates (30.57%). It stands out due to its protein content (13.98%), total fiber (8.90%), mineral elements (K =315.67 mg/100 g; Mg =92.85 mg/100 g; Ca =68.47 mg/100 g; Fe = 3.576 mg/100 g; Zn = 2.387 mg/100 g), total polyphenols (158.75 mg GAE/100 g) and demonstrates antioxidant capacity (182.45 mg Trolox Equivalents/100 g).

Key words*: fortification, mini-baguette, Spirulina, diabetics, obese.*

INTRODUCTION

Significant increases in the prevalence of physiological dysfunctions are caused by a sedentary lifestyle, along with an unbalanced diet and contaminants resulting from soil and environment pollution (d'Angelo et al., 2019).
According to data from the World Health Organization (WHO), approximately 200 million men and 300 million women worldwide are classified as obese, while around 1.5 billion people are classified as overweight (WHO, 2024).
Excess weight and obesity are contributing factors to the development of metabolic syndrome, which increases the risk of diabetes, atherosclerosis, liver diseases, brain disorders, and cancer. All these disorders share a predominant feature: low-grade inflammation caused by overnutrition, which can be diagnosed at the molecular level (Hatch-McChesney & Smith, 2023).
According to statistics, in 2021, 537 million adults aged 20–79 worldwide were affected by diabetes.
In Europe, 61 million people were affected by diabetes in the same year. By 2030, the global number of people with diabetes is expected to rise to around 643 million, and in Europe to about 67 million (IDF Diabetes Atlas 10th edition, 2021; http://www.idf.org/EU-diabetes-policy-audit). In Romania, 2021 recorded a record number of diagnosed diabetes cases: approximately 1.5 million.
Of these, the majority are cases of type 2 diabetes, specifically 83.9% of the total cases reported by diabetes specialists.
In terms of prevalence, Romania is close to the European average, slightly below it: in Romania, 1 in 12 adults has diabetes, compared to 1 in 11 adults in Europe (https://www.rodiabet.ro/barometrul-datelor-privind-diabetul-zaharat-in-romania-1-din-12-romani-are-diabet-zaharat/).

The prevalence of diet-related chronic diseases, such as obesity and type 2 diabetes, has led to the development of starch-rich foods with added functional ingredients, aimed at controlling starch digestibility and providing health benefits (Torres et al., 2024).

Thus, bread formulation can be modified by adding functional ingredients such as phytochemicals and dietary fiber (Onacik-Gür et al., 2022). Dietary fibers are biopolymers resistant to digestion in the small intestine but can be fermented in the large intestine (Zhou et al., 2021). Depending on their water solubility, dietary fibers are classified as soluble and insoluble fibers. In bread formulations, the addition of soluble fibers improves dough texture, lowers cholesterol levels, and reduces postprandial blood glucose (Ferreira et al., 2021). Inulin and methylcellulose are often used in the composition of starch-rich products (Papagianni et al., 2024). Catană et al. (2022) developed and qualitatively characterized a product called "Mini baguette fortified with Jerusalem artichoke flour" intended for people with diabetes and obesity. This mini baguette has a reduced carbohydrate content (48.64-49.17%) and available carbohydrates (43.93-44.37%), but a high content of proteins (11.60-12.15%) and fiber (4.43-4.99%).

This paper presents research on the development of a mini-baguette fortified with Spirulina powder (fortification levels 1-4%), intended for diabetics and obese individuals.

MATERIALS AND METHODS

Materials

To obtain the product "Low carb mini-baguette fortified with *Spirulina* powder", natural sourdough fortified with Spirulina powder, whole wheat flour, white wheat flour type 650, butter 65% fat, milk 1.5% fat, ground Pecan nut, freeze-dried fruits (cranberries, raspberries, etc.), *Spirulina* powder, white sesame seeds, sea salt, and other ingredients rich in proteins and dietary fiber were used.

For the control sample ("White mini baguette"), the following materials were used: natural sourdough, white wheat flour, butter 65% fat, milk 1.5% fat, white sesame and black sesame seeds, and sea salt. Figure 1 shows the natural sourdough fortified with *Spirulina* powder, along with the yeast and lactic acid bacteria present in its composition.

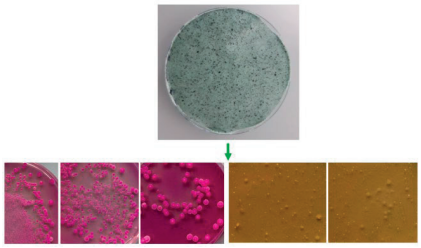


Figure 1. "Natural sourdough fortified with *Spirulina* powder" and its composition with yeasts and lactic acid bacteria

it was developed and tested at the Human Nutrition Laboratory in IBA-Bucharest. The Spirulina powder was produced and provided by Arborvitae S.R.L.

Mini baguette-making

For the development of the "Low carb mini-baguette fortified with *Spirulina* powder," four experimental variants were created (Figures 2-6), corresponding to the levels of fortification with *Spirulina* powder: 1%, 2%, 3%, and 4%. In parallel, a control variant was used ("White mini baguette"- C) (Figure 2).



Figure 2. The "White mini baguette" (Control sample)

For the development of the bakery products, a biphasic method was applied. This method ensures superior sensory qualities (appearance, taste and aroma), good protein digestibility, high bioavailability of minerals (Fe, Ca, Mg, Zn, etc.) and bioactive compounds, as well as a reduced glycemic impact after the consumption and digestion of the mini-baguettes (Catană et al., 2022; Ribet et al., 2022).

Methods

Statistical Analysis

"Low carb mini-baguette fortified with Spirulina powder" (V1, V2, V3, V4) and "White mini baguette" (Control sample) were

analyzed in triplicate, and the obtained results are presented as the arithmetic mean and standard deviation.



Figure 3. "Low carb mini-baguette fortified with *Spirulina* powder" - V1



Figure 4. "Low carb mini-baguette fortified with *Spirulina* powder" - V2



Figure 5. "Low carb mini-baguette fortified with *Spirulina* powder" - V3



Figure 6. "Low carb mini-baguette fortified with *Spirulina* powder" - V4

Sensory analysis

The sensory analysis of the "Low carb mini-baguette fortified with *Spirulina* powder" (V1, V2, V3, V4) and "White mini baguette" (Control sample) it was done by evaluating the sensory characteristics (appearance, color, taste, and smell) and by "Comparison method with unitary score scales" method (Burnete et al., 2020).

Instrumental analysis of color (L*, a* and b*) was performed with CM-5 colorimeter (Konica Minolta, Japan), using SpectraMagic NX software.

Instrumental texture analysis was performed with Instron Texture Analyzer (model 5944, Illinois Tool Works Inc., USA) equipped with Bluehill 3.13 software.

Physico-chemical analysis

Nutritional composition of the mini-baguette samples it was determined by the method AOAC: 979.09 (protein content), 963.15 (fat content), 923.03 (ash content) and 985.29 (total dietary fiber). Moisture content of the mini-baguette samples was determined by Halogen Moisture Analyzer HE53 (Mettler Toledo). Total carbohydrates were calculated with the formula: 100 (%) - moisture (%) - protein (%) - fat (%) - total dietary fiber (%). The energy value is calculated using the conversion factors of 9 kcal/g for fat, 4 kcal/g for carbohydrates and protein, and 2 kcal/g for fiber, in accordance with the provisions of Commission Regulation no. 1169/2011 (European Commission, 2011).

Determination of iron, copper, manganese and zinc was performed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS, model NexION300Q, Perkin Elmer) after dry digestion of the samples. The determination of sodium, potassium, calcium and magnesium was carried out by High-Resolution Continuum Source Atomic Absorption Spectrometry (Analytik Jena, model contrAA 700 - High-Resolution Continuum Source Atomic Absorption Spectrometer), flame technique, after dry digestion of the samples.

Bioactive compounds content

The determination of the total polyphenol content was carried out using the Folin-Ciocalteau spectrophotometric method, according to Horszwald and Andlauer (2011), with some modifications (extraction medium, extraction mode and time, volume of extract used in analysis) using a UV-VIS Jasco V 550 spectrophotometer, at a wavelength of $\lambda = 755$ nm. For quantification, a calibration curve of gallic acid in the concentration range of 0-0.20 mg/mL was used, and the results were expressed as mg of Gallic Acid Equivalents (GAE) per g of product. The content of total carotenoids was determined using a spectrophotometric method (Chinnadurai et al., 2013).

Antioxidant capacity

Antioxidant capacity was assessed using the DPPH (1,1-diphenyl-2-picryl hydrazyl) method, according to Horszwald and Andlauer (2011), with some modifications (extraction medium, extraction mode and time, volume of extract used in analysis), using a UV-VIS Jasco V 550 spectrophotometer at a wavelength of λ = 517 nm. For quantification, a calibration curve of Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic Acid) in the concentration range of 0-0.4375 mmol/L was used, and the results were expressed as µmol Trolox Equivalents per g of cauliflower stalks powder.

Microbiological analysis

The microbiological analysis of cauliflower stalks powder was carried out using the following methods: SR ISO 21527-1:2009 (Yeasts and molds), SR EN ISO 21528-1:2017 method (Enterobacteriaceae), ISO 21807:2004 (Water activity). Water activity was determined using Aquaspector AQS 31 equipment.

RESULTS AND DISCUSSIONS

Sensory analysis

As a result of the sensory analysis of the "Low carb mini-baguette fortified with *Spirulina* powder," it was found that the addition of Spirulina powder (fortification levels of 1%, 2%, 3%, and 4%) in its composition does not affect the taste and smell/aroma. Thus, the mini-baguettes fortified with this microalga, at the specified concentrations, have a pleasant taste and smell/aroma, characteristic of a well-baked bakery product with added *Spirulina* powder. Moreover, the "Low carb mini-baguette fortified with *Spirulina* powder" has an elastic, dense crumb, with uniform pores, brown in color with a purplish tint, and without clumps.

The addition of ingredients necessary for lowering the glycemic index and increasing the antioxidant potential causes the product "Low carb mini-baguette fortified with *Spirulina* powder" to have a darker color compared to the "White mini baguette" (Control sample), which is reflected in the decrease in L* brightness by 1.40-1.47 times (Figure 7).

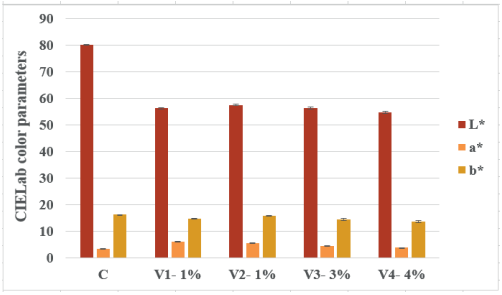


Figure 7. Color parameters of the product "Low carb mini-baguette fortified with *Spirulina* powder" (V1, V2, V3, V4) and of the Control sample (C)

The instrumental texture analysis revealed that, after 7 days from the production date, the firmness of the "Low carb mini-baguette fortified with *Spirulina* powder" product is similar to that of the "White mini baguette" (Control sample):

- "White mini baguette" (Control sample) - Firmness = 23.48 N;
- "Low carb mini-baguette fortified with Spirulina powder" (V1) - Firmness = 23.30 N;
- "Low carb mini-baguette fortified with Spirulina powder" (V2) - Firmness = 18.24 N;
- "Low carb mini-baguette fortified with Spirulina powder" (V3) - Firmness = 20.43 N;
- "Low carb mini-baguette fortified with Spirulina powder" (V4) - Firmness = 23.48 N.

Figures 8-12 present the compression curves obtained for the "Low carb mini-baguette fortified with *Spirulina* powder" product, following the instrumental texture analysis, 7 days after the production date.

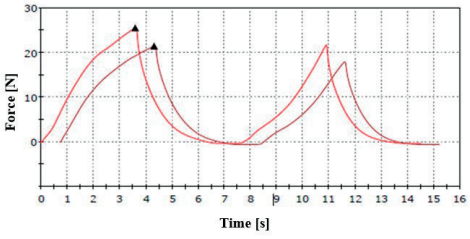


Figure 8. Compression curves for the "Control sample"

Firmness is sensorily correlated with the softness of the crumb: a lower firmness represents a crumb with a soft texture, while a higher firmness represents a denser, firmer crumb. Therefore, firmness is an important

sensory characteristic that defines the sensory quality of bakery products.

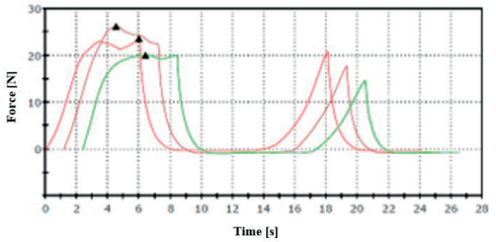


Figure 9. Compression curves for the product "Low carb mini-baguette fortified with *Spirulina* powder" (V1)

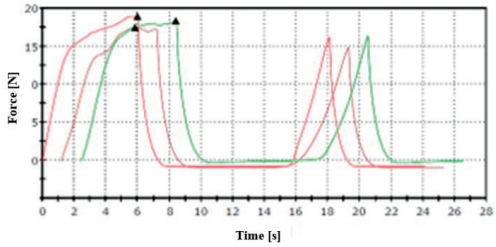


Figure 10. Compression curves for the product "Low carb mini-baguette fortified with *Spirulina* powder" V2)

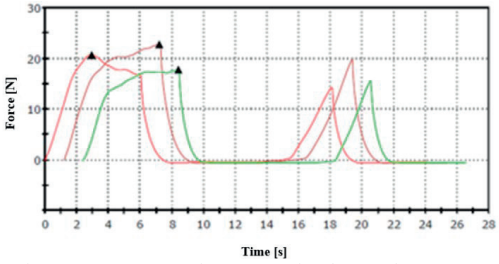


Figure 11. Compression curves for the product "Low carb mini-baguette fortified with *Spirulina* powder" (V3)

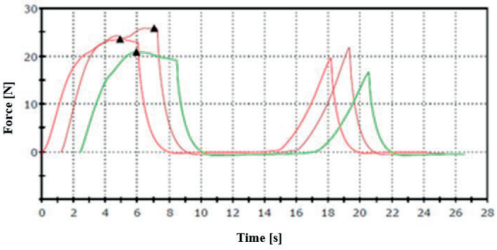


Figure 12. Compression curves for the product "Low carb mini-baguette fortified with *Spirulina* powder" (V4)

As a result of the sensory evaluation, using the "Method of comparison with unitary scoring scales," the products "White mini baguette" (Control sample) and "Low carb mini-baguette fortified with *Spirulina* powder" (V1, V2, V3, V4) received the rating "very good," with the following scores (Figure 13):

- Control sample - 20 points
- V1 - 19.52 points
- V2 - 19.44 points
- V3 - 19.28 points
- V4 - 19.04 points.

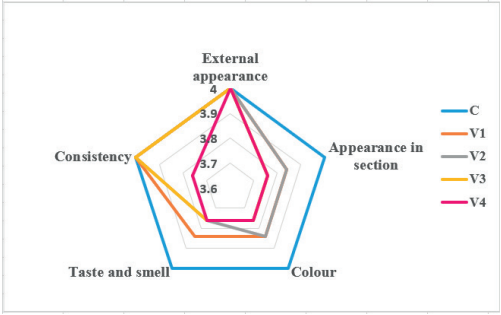


Figure 13. Sensory evaluation of the product "Low carb mini-baguette fortified with *Spirulina* powder" and of the Control sample

Physico-chemical analysis

The physico-chemical composition of the product "Low carb mini-baguette fortified with *Spirulina* powder" and of the Control sample is presented in Table 1.

Table 1. Physico-chemical composition of the product "Low carb mini-baguette fortified with *Spirulina* powder" and of the Control sample

Component	C	V1	V2	V3	V4
Acidity (degrees)	3.7±0.03	3.10±0.03	3.20±0.03	3.30±0.03	3.4±0.03
Moisture (%)	34.09±0.82	35.17±0.84	35.42±0.85	35.85±0.86	36.45±0.87
Ash (%)	0.84±0.01	2.11±0.04	2.19±0.04	2.28±0.04	2.35±0.04
Protein (%)	9.65±0.12	12.87±0.18	13.23±0.19	13.61±0.19	13.98±0.20
Fat (%)	1.88±0.02	7.56±0.08	7.63±0.08	7.70±0.08	7.75±0.08
Carbohydrates (%)	53.54±0.04	42.29±0.03	41.53±0.03	40.56±0.03	39.47±0.39
Available carbohydrates (%)	52.73±0.04	33.61±0.02	32.77±0.02	31.72±0.02	30.57±0.25
Total dietary fiber (%)	0.81±0.01	8.68±0.16	8.76±0.16	8.84±0.16	8.90±0.16
Salt (NaCl) (%)	0.70±0.004	0.74±0.005	0.68±0.004	0.72±0.005	0.69±0.004
Energy value (kcal/100g)	268	271	270	268	266
Energy value (kJ/100g)	1137	1139	1134	1126	1115

According to the results, the product "Low carb mini-baguette fortified with Spirulina powder" has a reduced content of carbohydrates, specifically available carbohydrates, but a high content of protein, ash, and total fibers compared to the Control sample. The highest values for protein, ash, and total fiber content were recorded in the experimental variant V4, which corresponds to the maximum level of fortification with *Spirulina* powder, namely 4%. The protein content of the "Low carb mini-baguette fortified with *Spirulina* powder" (V4) is comparable to the one reported by Fratelli et al. (2023) for wheat bread fortified with

Spirulina (fortification level of Spirulina powder in wheat flour: 3%): 14.96 g/100 dry matter. The "Low carb mini-baguette fortified with *Spirulina* powder" is a source of protein, as, according to the provisions of Regulation (EC) No 1924/2006 of the European Parliament and of the Council, at least 12% (in this case 19.06-21.02%) of its energy value comes from proteins. The ash content of the "Low carb mini-baguette fortified with *Spirulina* powder" is 1.36-1.52 times higher compared to the one reported by Tătaru and Iatco (2024) for the "Mini baguette fortified with *Spirulina*" (1.55%). At the same time, the total dietary fiber content of the "Low carb mini-baguette fortified with *Spirulina* powder" is about 2.3 times higher, comparable to the value reported by Tătaru and Iatco (2024) for the "Mini baguette fortified with *Spirulina*" (3.75%).

It is noteworthy that the product "Low carb mini-baguette fortified with *Spirulina* powder" is rich in fiber, in accordance with the provisions of Regulation (EC) No. 1924/2006 of the European Parliament and of the Council, having a fiber content greater than 6 g/100 g. The carbohydrate content, specifically available carbohydrates, of the product "Low carb mini-baguette fortified with *Spirulina* powder" varied between 39.47% and 42.29%, and 30.57%–33.61%, respectively, which are lower than those reported by Burnete et al. (2020) for the product "Hypoglycemic bread with antioxidant potential" (carbohydrates=42.64%; available carbohydrates=39.49%) and those reported by Fratelli et al. (2023) for wheat bread fortified with *Spirulina* (fortification level of *Spirulina* powder in wheat flour: 3%) (carbohydrates = 57.30 %). Due to its high content of proteins and fibers and its low content of carbohydrates and available carbohydrates, the product "Low carb mini-baguette fortified with *Spirulina* powder" is suitable for the diet of diabetics and obese individuals, as it ensures quick and stable satiety and, at the same time, has a low glycemic impact after consumption and digestion.

The product "Low carb mini-baguette fortified with *Spirulina* powder" is an important source of minerals (K, Ca, Mg, Zn, and Fe). The mineral element content of this product is presented in Figures 13 and 14.

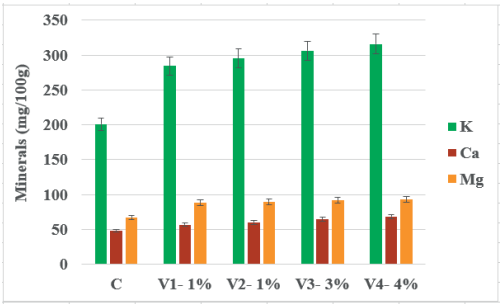


Figure 13. Mineral content (K, Ca and Mg) of the product "Low carb mini-baguette fortified with *Spirulina* powder" (V1, V2, V3, V4) and of the Control sample

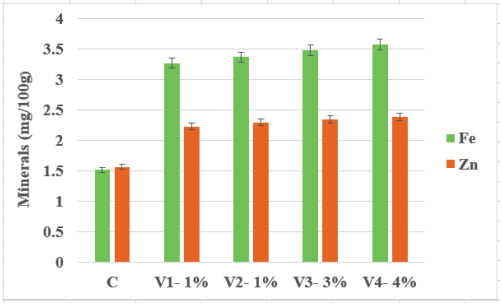


Figure 14. Mineral content (Fe and Zn) of the product "Low carb mini-baguette fortified with *Spirulina* powder" (V1, V2, V3, V4) and of the Control sample

The potassium content of the product "Low carb mini-baguette fortified with *Spirulina* powder" ranged between 284.48-315.67 mg/100 g, with the highest value recorded in the experimental variant V4 (fortification level with *Spirulina* powder: 4%). The potassium content of this Spirulina-fortified mini-baguette is 1.59-1.76 times higher compared to the one reported by Fratelli et al. (2023) for wheat bread fortified with *Spirulina* (fortification level of *Spirulina* powder in wheat flour: 3%) which was 178.85 mg/100 dry matter. Additionally, the potassium content of this mini-baguette is higher compared to that reported by Catană et al. (2022) for the product "Mini baguette fortified with Jerusalem artichoke flour," which was 260.85 mg/100 g.

The calcium content of the product "Low carb mini-baguette fortified with *Spirulina* powder" ranged from 56.60 to 68.47 mg/100 g, being significantly higher (7.09-8.58 times) compared to the calcium content reported by Fratelli et al. (2023) for formulated bread with *Spirulina*

residual biomass, which was 7.98 mg/100 dry matter.

The magnesium content of the product "Low carb mini-baguette fortified with *Spirulina* powder" is 1.36-1.56 times higher than its calcium content.

The iron (Fe) content of the product "Low carb mini-baguette fortified with *Spirulina* powder" is 1.53-1.63 times higher than that reported by Fratelli et al. (2023) for wheat bread fortified with *Spirulina* (fortification level with Spirulina powder in wheat flour: 3%), which was 2.20 mg/100 dry matter.

The zinc content of the product "Low carb mini-baguette fortified with *Spirulina* powder" is lower compared to its iron content, ranging between 2.23-2.39 mg/100 g. Bread is a staple food in countries around the world and is therefore important in nutrition. The metabolic effect of bread depends on several qualitative aspects, from the type of cereal, its carbohydrate content, and other nutrients, to its chemical structure and processing. The qualitative aspects of bread are best captured by its content of dietary fiber, whole grains, sugars, etc. (Ludwig et al., 2018; Pagliai et al., 2021). Therefore, it is very important for bread and bakery products (mini-baguettes, croissants, rolls, etc.) to have a high content of mineral elements.

Bioactive compounds content

Due to the valuable ingredients used in the composition of the fortified mini-baguettes (Spirulina powder, freeze-dried cranberries and raspberries, etc.), the product "Low carb mini-baguette fortified with *Spirulina* powder" stands out for its content of total polyphenols and total carotenoids. The total polyphenol content of this product ranged from 131.87 to 148.93 mg/100 g, with the highest value recorded in the experimental variant V4 (fortification level with Spirulina powder: 4%) (Figure 15). The polyphenol content of this Spirulina-fortified mini-baguette is 1.49-3.3 times higher compared to that reported by Hernández-López et al. (2023) for *Spirulina*-fortified bread (fortification levels: 1.5% and 2.5%): 40-100 mg GAE/100 dry weight).

In recent years, dietary polyphenols have gained significant interest among researchers due to their chemopreventive/protective potential in maintaining human health and preventing diseases.

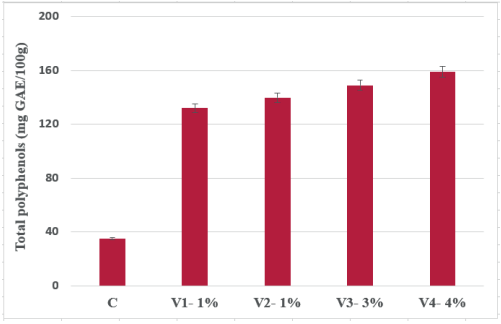


Figure 15. Total polyphenols of the product "Low carb mini-baguette fortified with *Spirulina* powder" (V1, V2, V3, V4) and of the Control sample

It is believed that dietary polyphenols/ flavonoids exert a strong antioxidant action to protect against reactive species and cellular oxidative stress, aiming to prevent pathological conditions or diseases associated with oxidative stress. Preclinical and clinical evidence suggests that following long-term polyphenol-rich diets provides protection against the development of various chronic diseases (neurodegenerative diseases, cardiovascular diseases, cancer, diabetes, etc.) (Rudrapal et al., 2022).

Figure 16 shows the total carotenoid content of the *Spirulina*-fortified mini-baguette.

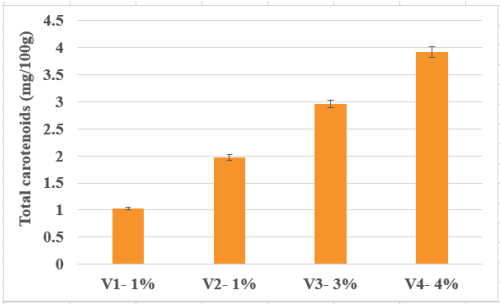


Figure 16. Total carotenoids of the product "Low carb mini-baguette fortified with *Spirulina* powder" (V1, V2, V3, V4)

The product "Low carb mini-baguette fortified with *Spirulina* powder" stands out due to its total carotenoid content: 1.03-3.92 mg/100 g (the highest value was recorded in the experimental variant V4).

The carotenoid content of the Spirulina-fortified mini-baguette (with a fortification level of 4%) is comparable to that reported by Dipra et al. (2020) for gluten-free bread fortified with *Chlorella sorokiniana* powder (fortification level 2.5%): 4.72 mg/100 g. The total carotenoid content of the "Low carb mini-baguette fortified with *Spirulina* powder" (V3 and V4) is higher compared to that reported by Chikpah et al. (2025) for bread fortified with pumpkin flour (wheat flour 85%; pumpkin flour 15%): 2.46 mg/100 g d.b.

Carotenoids have been associated with a range of health benefits. Their dietary intake and circulating levels have been linked to a reduced incidence of obesity, diabetes, certain types of cancer, and even lower overall mortality (Eroglu et al., 2023).

Considering the beneficial effects of antioxidants (polyphenols, carotenoids, etc.) on the human body, the fortification of bread and bakery products with functional ingredients rich in antioxidants is very important.

Antioxidant capacity

Due to its content in antioxidants (phenolic compounds, carotenoids, the product "Low carb mini-baguette fortified with *Spirulina* powder" has antioxidant capacity (Figure 17).

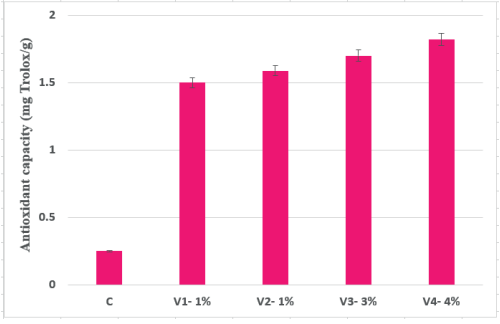


Figure 17. Antioxidant capacity of the product "Low carb mini-baguette fortified with *Spirulina* powder" (V1, V2, V3, V4) and of the Control sample

The highest value of antioxidant capacity was recorded in the experimental variant V4: 1.82 mg Trolox/g. Due to the ingredients used to reduce the carbohydrate content, increase the nutritional value, and enhance the content of bioactive compounds (polyphenols, carotenoids), the product "Low carb mini-baguette fortified with *Spirulina* powder" has an antioxidant capacity 6-7.28 times higher compared to the Control sample. Moreover, this type of mini-baguette has an antioxidant capacity comparable to that reported by Burnete et al. (2020) for the product "Hypoglycemic bread with antioxidant potential": 1.25 mg Trolox/g.

Considering the results obtained from the sensory analysis, physicochemical properties, content of bioactive compounds, and antioxidant capacity, the experimental variant V4 of the product "Low carb mini-baguette fortified with *Spirulina* powder" was selected as the optimal variant.

Microbiological analysis

The microbiological indicators of the "Low carb mini-baguette fortified with *Spirulina* powder" and of the Control sample are presented in Table 2. The results of the microbiological analysis confirmed the fact that these products fall within the provisions of the legislation in force.

Table 2. Microbiological analysis of the product "Low carb mini-baguette fortified with *Spirulina* powder" and of the Control sample

Microbiological indicators	Control sample	"Low carb mini-baguette fortified with *Spirulina* powder"			
		V1	V2	V3	V4
Yeast and molds (CFU/g)	< 10	< 10	< 10	< 10	< 10
Enterobacteriaceae (CFU/g)	< 10	< 10	< 10	< 10	< 10
Water activity	0.908	0.915	0.920	0.925	0.936

The water activity of the products ranged from 0.908 to 0.936. Based on the results of the microbiological analyses, sensory evaluation, and instrumental texture analysis, the shelf life of the product "Low carb mini-baguette fortified with *Spirulina* powder" was set at 3 days.

CONCLUSIONS

The product "Low carb mini-baguette fortified with *Spirulina* powder" has corresponding sensory qualities, high nutritional value, and presents antioxidant capacity, making it suitable for the diet of diabetics and obese individuals. Thus, this mini-baguette features a low content of carbohydrates (39.47-42.29%) and available carbohydrates (30.57-33.61%) and stands out with its protein content (12.87-13.98%), total dietary fiber (8.68-8.90%), and minerals (K: 284.48-315.67 mg/100 g; Ca:

56.60-68.47 mg/100 g; Mg: 88.01-92.85 mg/100 g; Fe: 3.27-3.58 mg/100 g; Zn: 2.23-2.39 mg/100 g). Additionally, the "Low carb mini-baguette fortified with *Spirulina* powder" is remarkable for its content of bioactive compounds: total polyphenols (131.87-148.93 mg/100 g) and total carotenoids (1.03-3.92 mg/100 g). Due to its antioxidant content, this product exhibits antioxidant capacity (1.50 - 1.82 mg Trolox/g). The shelf life of the product "Low carb mini-baguette fortified with *Spirulina* powder" was set at 3 days.

ACKNOWLEDGEMENTS

This work was carried out through the Core Program within the National Research Development and Innovation Plan 2022-2027, carried out with the support of MCID, project no. PN 23 01 01 01.

REFERENCES

AOAC International. (2016). Official Methods of Analysis of AOAC International, 20th ed.; Association of Official Analytical Chemists: Gaithersburg, MD, USA.

Burnete, A.G., Catană, L., Catană, M., Lazăr, A. M., Teodorescu, R.I., Asănică, A. C., Belc, N. (2020). Use of vegetable functional ingredients to achieve hypoglycemic bread with antioxidant potential, for diabetics. *Scientific Papers. Series B, Horticulture, Vol. LXIV, No. 2*, p. 367-374.

Chikpah, S.K., Korese, J.K., Sturm, B., Hensel, O. (2025). Evaluation of nutrients, antioxidants and sensory characteristics of optimized wheat-orange-fleshed sweet potato and pumpkin composite bread and storage stability in three packaging materials. *Discover Food, 5*:36

Catană L., Catană M., Burnete, A.G., Constantinescu, F. Asănică A.C. (2022). Organic sourdough mini baguette fortified with Jerusalem artichoke flour, for diabetics. *Scientific Papers. Series B, Horticulture. Vol. LXVI, No. 2,* 362-368.

Chinnadurai, S., Karthik, G., Chermapandi, P., Hemalatha, A., Anantharaman (2013). Estimation of major pigment content in seaweeds collected from Pondicherry coast. *The Experiment, Vol. 9*(1), 522-525.

D'Angelo, M., Castelli, V., Tupone, M. G., Catanesi, M., Antonosante, A., Dominguez-Benot, R., & Benedetti, E. (2019). Lifestyle and food habits impact on Chronic diseases: Roles of PPARs. *International Journal of Molecular Sciences, 20*(21), 5422.

Diprat, A.B., Cruz Silveira Thys, R., Rodrigues, E., Rech, R. (2020). Chlorella sorokiniana: A new alternative source of carotenoids and proteins for gluten-free bread. *LWT - Food Science and Technology*, *134*, 109974.

Eroglu, A., Al'Abri, I.S., Kopec, R.E., Crook, N., Bohn, T. (2023). Carotenoids and Their Health Benefits as Derived via Their Interactions with Gut Microbiota-*Review. Advances in Nutrition 14*, 238–255.

European Commission. Regulation (EC) No. 1169/2011 of 25/10/2011, on the Provision of Food Information to Consumers. *Off. J. Eur. Union* 2011, L304, 18–63.

European Commission. Regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods. *Off. J. Eur. Union* 2011, L404, 9–25.

Ferreira, S. M., Capriles, V. D., & Conti-Silva, A. C. (2021). Inulin as an ingredient for the improvement of glycemic response and sensory acceptance of breakfast cereals. *Food Hydrocolloids*, *114*, Article 106582.

Fratelli, C., Nunes, M.C., De Rosso, V.V., Raymundo, A., and Braga, A.R.C. (2023). *Spirulina* and its residual biomass as alternative sustainable ingredients: impact on the rheological and nutritional features of wheat bread manufacture. *Front. Food. Sci. Technol., 3*:1258219.

Hatch-McChesney, A., & Smith, T. J. (2023). Nutrition, immune function, and infectious disease in military personnel: A narrative review. *Nutrients, 15*(23), 4999.

Hernández-López, I., Alamprese, C., Cappa, C., Prieto-Santiago, V., Abadias, M., Aguiló-Aguayo, I. (2023). Effect of *Spirulina* in Bread Formulated with Wheat Flours of Different Alveograph Strength. *Foods, 12*, 3724.

Horszwald, A., & Andlauer, W. (2011). Characterisation of bioactive compounds in berry juices by traditionalphotometric and modern microplate methods. *Journal of Berry Research*, *1,* 189–199.

International Diabetes Federation. IDF Diabetes Atlas, 10th edn. Brussels, Belgium: International Diabetes Federation, 2021.

ISO 21807:2004. Microbiology of food and animal feeding stuffs - Determination of water activity.

Ludwig, D.S., Hu, F.B., Tappy, L., Brand-Miller, J. (2018). Dietary carbohydrates: role of quality and quantity in chronic disease. *BMJ* 361, k2340.

Onacik-Gür, S., Szafrańska, A., Roszko, M., & Stępniewska, S. (2022). Interaction of dough preparation method, green tea extract and baking temperature on the quality of rye bread and acrylamide content. *LWT - Food Science and Technology*, *154*, Article 112759.

Pagliai, G., Dinu, M., Madarena, M.P., Bonaccio, M., Iacoviello, L., Sofi, F. (2021). Consumption of ultra-processed foods and health status: a systematic review and meta-analysis. *Br J Nutr*, *125*, 308–318.

Papagianni, E., Kotsiou, K., Matsakidou, A., Biliaderis, C. G., & Lazaridou, A. (2024). Development of "clean label" gluten-free breads fortified with flaxseed slurry and sesame cake: Implications on batter rheology, bread quality and shelf life. *Food Hydrocolloids*, *150*, Article 109734.

Ribet, L., Dessalles, R,. Lesens, C., Brusselaers, N., Durand-Dubief, M. (2023). Nutritional benefits of sourdoughs: A systematic review. *Adv Nutr.*, 14(1), 22-29.

Rudrapal, M, Khairnar, S.J., Khan, J., Dukhyil, A.B., Ansari, M.A., Alomary, M.N., Alshabrmi, F.M., Palai, S., Deb, P.K. and Devi, R. (2022) Dietary Polyphenols and Their Role in Oxidative Stress-Induced Human Diseases: Insights Into Protective Effects, Antioxidant
Potentials and Mechanism(s) of Action. *Front. Pharmacol.*, *13*:806470.

Tătaru, E. and Iatco, I. (2024). Research on the fortification of a bakery product with *Spirulina*, to increase its nutritional value and antioxidant capacity. Volume International Symposium, ISB-INMA TEH, Agricultural and Mechanical Engineering, Bucharest 31 October - 1 November 2024.

Torres, J.D., Dueik, V., Carré, D., Contardo, I., Bouchon, P. (2024). Non-invasive microstructural characterization and *in vivo* glycemic response of white bread formulated with soluble dietary fiber. *Food Bioscience* 61, 104505.

WHO. (2024). *int/en/news-room/fact-sheets/detail/obesity-and-overweight*.

http://www.idf.org/EU-diabetes-policy-audit

https://www.rodiabet.ro/barometrul-datelor-privind-diabetul-zaharat-in-romania-1-din-12-romani-are-diabet-zaharat/

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

THE CHEMICAL COMPOSITION OF THE ESSENTIAL OIL AND ASSOCIATED HYDROSOL OBTAINED FROM TWO PLANTS OF THE ASTERACEAE FAMILY, *SANTOLINA CHAMAECYPARISSUS* L. AND *TANACETUM VULGARE* L.

Raluca CIUBOTĂRAȘU[1], Monica Luminița BADEA[1], Oana LIVADARIU[1], Mihaela PARASCHIV[2], Dorel HOZA[1]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest,
59 Mărăști Blvd, District 1, Bucharest, Romania
[2]Research-Development Institute for Vegetable and Floriculture Vidra,
22 Calea București Street, Vidra, 077185, Ilfov, Romania

Corresponding author email: raluca.ciubotarasu@doctorat.usamv.ro

Abstract

The use of plants throughout the centuries has been essential for the survival and development of human civilizations. Plants have been used in multiple fields, such as food, medicine, construction, religion, and even in art and culture. In recent years, natural compounds have attracted considerable attention in various fields due to their wide-ranging biological activities. Essential oils and their associated hydrosols, in particular, are being explored for their ability to control plant pests, as well as their, antimycotic and antiparasitic effects. These substances are typically faster and more cost-effective to produce, and are generally considered safer for the environment and non-target organisms than conventional pesticides. Earlier studies have demonstrated that the primary components found in the essential oil of Tanacetum vulgare L. were oxygenated monoterpenes, including compounds like camphor, trans-chrysantenyl acetate, cis-verbenol, thujone, eucalyptol, and α-campholenal. For Santolina chamaecyparissus L., the primary components identified were artemisia ketone, camphor, and beta-phellandrene.
This study highlights the chemical composition of the essential oil and associated hydrosol obtained from two plants of the Asteraceae family, Santolina chamaecyparissus L. and Tanacetum vulgare L .

Key words*: chemical composition , essential oil, hydrosol, Santolina chamaecyparissus, Tanacetum vulgare.*

INTRODUCTION

The use of plants has been pivotal for the survival and advancement of human civilizations over centuries. They have served diverse purposes, including food, medicine, construction, religion, art, and culture. In recent years, natural compounds have garnered significant attention across various fields due to their broad biological activities. Essential oils and their corresponding hydrosols, in particular, are being investigated for their potential to control plant pests and their antimycotic and antiparasitic properties. These natural substances are generally faster and more cost-effective to produce and are considered safer for both the environment and non-target organisms compared to conventional pesticides.

This study focuses on analyzing the chemical composition of the essential oil and hydrosol extracted from two Asteraceae family plants: *Santolina chamaecyparissus* L. and *Tanacetum vulgare* L..

The genus *Santolina* (family Asteraceae) is a taxonomically intricate group of plant species found extensively throughout the Mediterranean region. *Santolina chamaecyparissus* L., commonly known as cotton lavender, is a resilient, aromatic, evergreen shrub characterized by its dense mound of appealing grayish-silver foliage. Renowned for its biological properties, it has been utilized in traditional medicine since ancient times.

Some species of *Santolina chamaecyparissus* L. has been studied for its oil constituents (Senatore F., 1994; Perez-Alonso M. J. and Negueruela, 1988). Research indicates that its chemical composition varies depending on factors such as origin and season (Derbesy M., 1982; Brunke E.J., 1992; Perez-Alonso M. J., 1992; Giner R. M., 1993; Lawrence B. M., 1995; Vernin G., 1991).

Several subspecies of *Santolina chamaecyparissus* L. originating from Spain have been reported to primarily contain 1,8-cineole (2-18%), artemisia ketone (0.1-28%), camphor (trace - 43%), borneol (1-28%), copaenol (trace - 15%), cubenol (1-17%), and alloaromadendrene (19%) as their major constituents (Villar A., 1986; Perez-Alonso M. J., 1992).

The essential oil of *Santolina chamaecyparissus* L. from French origin primarily contained artemisia ketone, myrcene, and β-phellandrene as its major constituents (Derbesy M., 1982; Giner R. M., 1993; Lawrence B. M., 1995). In contrast, the oil from a cultivar of *Santolina chamaecyparissus* L. was characterized by longiverbenone (9-18%) in addition to artemisia ketone (8-34%) and β-phellandrene (8-18%) as key components. *Santolina chamaecyparissus* L. is notable for its intriguing fragrance, making it valuable to the perfume industry, and its oil holds commercial importance in France.

In Turkey studies revealed that the major compounds were : artemisia ketone (38.1%), camphor (11.7%), β-phellandrene (9.2%), α-bisabolol (6.6%) and myrcene (4.3%) (Demirci B., 2000).

Tanacetum vulgare L. is a perennial, herbaceous flowering plant belonging to the genus *Tanacetum* in the Asteraceae family. Native to temperate regions of Europe and Asia, it has also been introduced to other parts of the world, including North America, where it has become invasive in certain areas. Commonly referred to as common tansy, bitter buttons, cow bitter or golden buttons.

Numerous species of *Tanacetum* L. have been studied for the biological activities of their metabolites, including anti-inflammatory (Kwok BHB, 2001; Dornelles VCM. 1998), anti-migraine (Dornelles VCM., 1998), anti-ulcerogenic (Tournier H., 1998), cytotoxic (Goren N., 1996), antifeedant (Goren N., 1994) and antibacterial properties (Goren N., 1992). The genus comprises approximately 200 species, primarily distributed across Europe and western Asia (Heywood VH., 1976). Some species, such as tansy (*T. vulgare*), Dalmatian insect flower (*T. cinerariifolium*), and feverfew (*T. parthenium*), are particularly well-known.

The roots, leaves, and flowers of the two most extensively studied species, *Tanacetum vulgare* L. and *Tanacetum parthenium* L. Sch. Bip. ex Oliv., are abundant in sesquiterpene lactones (such as parthenolide, tanacetin, and tanacetols), tannins, and flavonoids (Bruni A., 2009).

Research into the chemical composition of essential oils from various *Tanacetum* species has revealed significant variability (Mockute D., 2004; Rohloff J., 2004; Keskitalo M., 2001; Chanotiya CS., 2007). These studies identified camphor, α- and β-thujone, carvone, pinocarvone, borneol, bornyl acetate, and 1,8-cineole as major constituents of the oils. A comprehensive review by Lawrence (Lawrence BM., 2000) of studies on *T. vulgare* oil composition published up to 1996 classified tansy oils into 23 chemotypes based on their primary components. Additionally, it was noted that commercial tansy oils are predominantly of the thujone type. Thujone is recognized as a bioactive compound with medicinal properties but exhibits toxicity at high concentrations. The essential oils of 40 *T. vulgare* samples from Lithuania were analyzed and grouped into four categories, with 1,8-cineole and camphor chemotypes being the most prevalent (Judzentiene A., 2005).

MATERIALS AND METHODS

Plant material from *Santolina chamaecyparissus* L. was harvested from a garden in Constanța, Romania (44°10′24″N 28°38′18″E), in August 2024.

For *Tanacetum vulgare* L., the plant material was collected from the wild flora in the Viscri area, Brașov, Romania (46°3′24″N 25°5′49″E), in August 2024. Both the essential oil and hydrosol were extracted from dried plant material.

For both species, the entire plant, including the stems, leaves, and inflorescences, was used.

Various methods can be used to obtain essential oils and hydrosols, such as water distillation techniques, cold pressing, supercritical CO_2 extraction, Soxhlet extraction, and newer approaches like microwave- and ultrasonic-assisted extractions. The goal is to extract volatile compounds from plants, which are present in smaller amounts but provide significant value. Water distillation remains the traditional method for essential oils and hydrosols production, representing 93% of the

total production. Two main techniques can be used, depending on how water interacts with the aromatic plants during extraction: hydro distillation or steam distillation . This process results in two immiscible phases during condensation, with the aqueous phase byproduct referred to as "hydrosol" (Degirmenci, H., 2020; de Elguea-Culebras, G.O., 2022; Tang, M., 2021; Timung, R., 2016; Traka, C.K., 2018).

Oil and hydrosol samples were analyzed without any other preparation or dilution. 1 µL of sample was injected in the gas-chromatographic system coupled with mass spectrometer (GC-MS, Perkin Elmer Clarus 680/SQ 8T, Perkin Elmer, CT, USA) equipped with an Elite-5MS capillary column (30 m x 0.25 x 0.25 µm (Perkin Elmer, CT, USA), using helium as carrier gas (flow rate of 1.0 mL/min). The temperature program was as follows: initial temperature: 40°C, initial hold time 2min, increase by 10°C/min to 300°C, hold time 2 min. The injection was performed in split mode (split ratio 50:1 for hydrosol and 200:1 for oil sample), and the injector temperature was set to 200°C. The MS operating conditions were: source temperature 210°C, transfer line temperature 310°C, electron impact ionization EI+ at 70eV and a solvent delay of 3 min. The qualitative analysis was performed using NIST Library, version 2017 and Wiley Library, version 9.0.

For each sample, 30 most abundant peak areas were included in the library search. A threshold for spectra match of 700 (70% probability) was chosen to determine which compounds are present in the sample analyzed.

RESULTS AND DISCUSSIONS

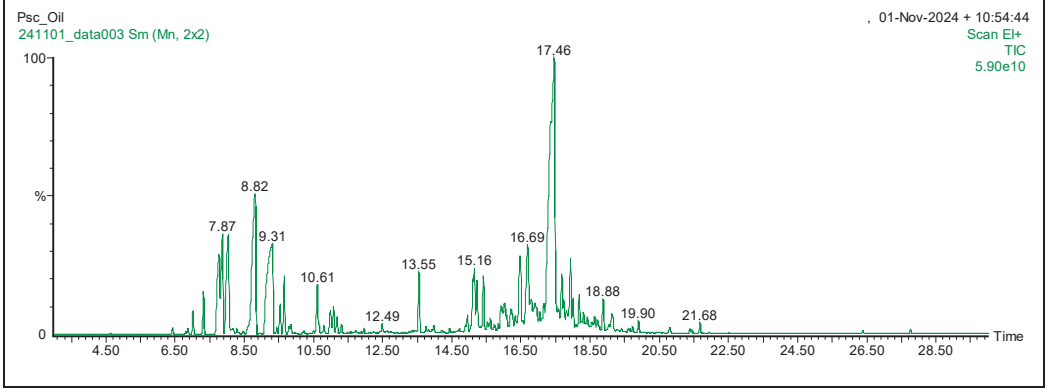
The chemical composition of *Santolina chamaecyparissus* L. essential oil was analyzed, revealing its complexity, with variations in the ratio (Table1, Figure1).

Table 1. *Santolina chamaecyparissus* L. essential oil compounds

	Compound name	Retention time (min)	Probability (%)	CAS #	Area	% from the sum of areas
1	à-PINENE (-)-	7.00	92.4	80-56-8	91444856	0.88
2	Camphene	7.31	93.2	79-92-5	182800224	1.76
3	à-PINENE	7.72	80.6	80-56-8	656930816	6.33
4	2-a-Pinene	7.82	84.4	127-91-3	670512192	6.46
5	a-Myrcene	7.99	68.9	123-35-3	776015936	7.48
6	Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methylethyl)-, (1à,2à,5à)-	8.75	72.8	17699-16-0	1536558464	14.81
7	3-Butyn-1-ol	9.16	62.3	927-74-2	1637742208	15.79
8	1,5-Heptadien-4-ol, 3,3,6-trimethyl-	9.47	86.0	27644-04-8	85404568	0.82
9	Cyclohexene, 1-methyl-4-(1-methylethylidene)-	9.59	90.6	586-62-9	149126032	1.44
10	Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1S)-	10.57	93.1	464-48-2	150287424	1.45
11	endo-Borneol	10.95	92	507-70-0	62799712	0.61
12	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)- (CAS)	11.05	81.4	562-74-3	66607784	0.64
13	2-Cyclohexen-1-one, 4-(1-methylethyl)-	11.15	91.2	500-02-7	50927672	0.49
14	1-Cyclohexene-1-carboxaldehyde, 4-(1-methylethyl)-	12.47	84	21391-98-0	11364010	0.11
15	Tricyclo[5.4.0.0(2,8)]undec-9-ene, 2,6,6,9-tetramethyl-, (1R,2S,7R,8R)-	13.53	91	5989-08-2	208374032	2.01
16	AROMADENDRENE	14.95	88.7	489-39-4	43160140	0.42
17	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl- (CAS)	15.12	90	644-30-4	189657248	1.83
18	GERMACRENE-D	15.20	90.8	23986-74-5	185693184	1.79

	Compound name	Retention time (min)	Probability (%)	CAS #	Area	% from the sum of areas
19	(1S,2E,6E,10R)-3,7,11,11-Tetramethylbicyclo[8.1.0]undeca-2,6-diene	15.38	89.6	24703-35-3	169193424	1.63
20	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, [1ar-(1aà,4aà,7á,7aá,7bà)]-	16.39	85.3	6750-60-3	145673600	1.40
21	(-)-Caryophyllene oxide	16.45	86.8	1139-30-6	220750816	2.13
22	8-Isopropenyl-1,3,3,7-tetramethyl-bicyclo[5.1.0]oct-5-en-2-one	16.63	76.1	-	304379168	2.93
23	Longiverbenone	17.31	77.8	64180-68-3	2491115264	24.01
24	((4aS,8S,8aR)-8-Isopropyl-5-methyl-3,4,4a,7,8,8a-hexahydronaphthalen-2-yl)methano	17.86	79.3	135118-51-3	130993424	1.26
25	3-(1-Naphthyl)pentane	18.83	82.2	3042-56-6	86161184	0.83
26	2(1H)-Naphthalenone, octahydro-4a-methyl-7-(1-methylethyl)-, (4aà,7á,8aá)-	20.78	68.3	54594-42-2	20530308	0.20
27	Kaur-16-ene (CAS	21.37	91.3	562-28-7	11904855	0.11
28	1-Octadecanol	21.44	88.5	112-92-5	6803466	0.07
29	Phytol	21.67	87.9	150-86-7	26131014	0.25
30	Pentatriacontane (CAS)	26.39	84.9	630-07-9	5995508	0.06

Total ion chromatogram recorded (TOC)



*Psc oil - Plant *Santolina chamaecyparissus* L. essential oil

Figure 1. *Santolina chamaecyparissus* L. essential oil TOC

The compound longiverbenone is the most abundant, contributing 24.01% of the total area. This suggests that longiverbenone is the dominant compound in this sample and could significantly influence the oil's aroma and bioactivity.

Other significant compounds include:3-butyn-1-ol (15.79%), bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methylethyl)- (14.81%), bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, (1S)- (15.43%), (1S,2E,6E,10R)-3,7,11,11, tetramethylbicyclo[8.1.0]undeca-2,6-diene (1.63%).

These compounds are major contributors to the chemical composition of the oil and could play a role in its fragrance and therapeutic effects.

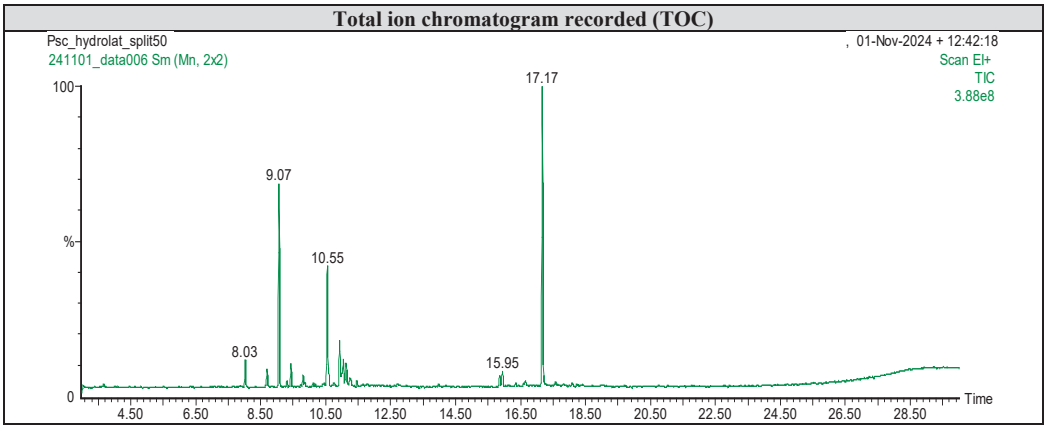
A significant presence of terpenes and sesquiterpenes, such as α-pinene, camphene, myrcene, and caryophyllene oxide, indicates that the oil likely has strong aromatic properties and may have biological activities such as anti-inflammatory or antimicrobial effects.

The oil contains a wide variety of functional groups, including alcohols, ketones, aldehydes, and hydrocarbons, which suggests that the oil may have complex chemical interactions. These components may contribute to its bioactive properties, such as potential antimicrobial, anti-inflammatory, or antioxidant effects.

Analyses of *Santolina chamaecyparissus* L. hydrosol revealed that longiverbenone is the most abundant, accounting for 40.88% of the total area (Table 2, Figure 2).

Table 2. *Santolina chamaecyparissus* L. hydrosol compounds

	Compound name	Retention time (min)	Probability (%)	CAS #	Area	% from the sum of areas
1	2,5,5-trimethyl-3,6-heptadien-2-ol	8.03	87.9	26127-98-0	1308652	3.66
2	1,5-Heptadien-4-one, 3,3,6-trimethyl- (CAS)	9.07	92.3	546-49-6	9340579	26.11
3	1,5-Heptadien-4-ol, 3,3,6-trimethyl-	9.44	84.1	27644-04-8	1178120	3.29
4	Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1S)-	10.55	94.1	464-48-2	5520606	15.43
5	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, (1S-endo)-	10.94	90.9	464-45-9	1869845	5.23
6	Terpinen-4-ol	11.05	85.5	562-74-3	1050179	2.94
7	2-Cyclohexen-1-one, 4-(1-methylethyl)-	11.14	80.9	500-02-7	879902	2.46
8	Longiverbenone	17.18	82.9	64180-68-3	14623996	40.88



*Psc hydrosol – Plant *Santolina chamaecyparissus* L. hydrosol

Figure 2. *Santolina chamaecyparissus L. hydrosol* TOC

This suggests that longiverbenone is the major contributor to the *Santolina chamaecyparissus* L. hydrosol's chemical profile and may have a significant influence on its aroma and bioactivity.

Several important terpenoid compounds are identified, such as bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl- (15.43%) and bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl- (5.23%). These compounds contribute to the distinctive aroma and may have antimicrobial or other bioactive properties, which are common for compounds found in essential oils and hydorsols.

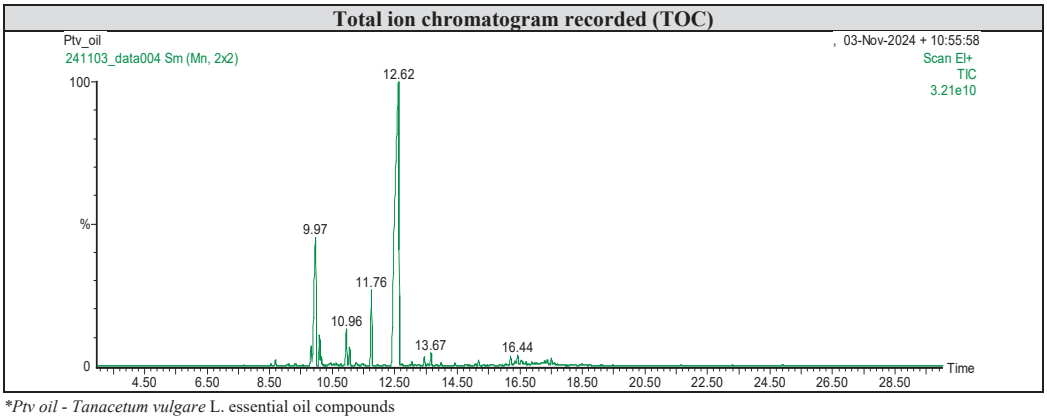
The presence of oxygenated compounds like terpinen-4-ol (2.94%) and 2-cyclohexen-1-one, 4-(1-methylethyl)- (2.46%) indicates that the hydrosol may also have therapeutic properties, including anti-inflammatory, antimicrobial, or antifungal effects.

Other compounds, such as 2,5,5-trimethyl-3,6-heptadien-2-ol (3.66%) and 1,5-heptadien-4-ol, 3,3,6-trimethyl- (3.29%), while present in smaller quantities, add complexity to the overall chemical profile, possibly contributing to the scent or synergistic biological effects of the hydrosol.

For *Tanacetum vulgare* L. *essential oil*, the analyses indicate that the compound linalool oxide is the most abundant in the sample, accounting for 68.61% of the total area (Table 3, Figure 3).

Table 3. *Tanacetum vulgare* L. essential oil compounds

	Compound name	Retention time (min)	Probability (%)	CAS #	Area	% from the sum of areas
1	p-Cymene	8.55	93	99-87-6	8973151	0.13
2	1,8-Cineole	8.69	88.8	470-82-6	24311886	0.36
3	ç-Terpinene	9.10	90.9	99-85-4	7410893	0.11
4	Linalool	9.83	88.6	78-70-6	83461944	1.22
5	BICYCLO[3.1.0]HEXAN-3-ONE, 4-METHYL-1-(1-METHYLETHYL)-	9.95	98	1125-12-8	1071773248	15.72
6	á-THUJONE	10.10	90.9	471-15-8	128314760	1.88
7	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, (1S-endo)-	10.96	89.3	464-45-9	186864848	2.74
8	Bicyclo[3.1.1]hept-2-en-6-ol, 2,7,7-trimethyl-, acetate, [1S-(1à,5à,6á)]-	11.77	71.8	50764-55-1	379507232	5.57
9	LINALOOL OXIDE	12.64	66.4	5989-33-3	4677715968	68.61
10	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl- (CAS)	15.09	88.2	644-30-4	7365104	0.11
11	GERMACRENE-D	15.19	86.5	23986-74-5	23497632	0.34
12	2-(4a,8-Dimethyl-1,2,3,4,4a,5,6,7-octahydro-naphthalen-2-yl)-prop-2-en-1-ol	16.05	75.5	-	10966000	0.16
13	GERANYL PENTANOATE	16.21	86.7	-	36780804	0.54
14	Caryophyllene oxide	16.44	85.9	1139-30-6	50738384	0.74
15	salvial-4(14)-en-1-one	16.54	79.1	-	20121556	0.30
16	trans-Sesquisabinene hydrate	16.70	84.1	145512-84-1	14278482	0.21
17	Neointermedeol	17.29	82.8	5945-72-2	10663753	0.16
18	á-Guaiene	17.38	82	88-84-6	19988978	0.29
19	(R)-1-Methyl-4-(6-methylhept-5-en-2-yl)cyclohexa-1,4-diene	17.51	83.4	28976-67-2	32698910	0.48
20	Lanceol, cis	17.71	75.2	10067-28-4	3732649	0.05
21	Spiro[4.5]decan-7-one, 1,8-dimethyl-8,9-epoxy-4-isopropyl-	18.49	73.8	61050-91-7	6015779	0.09
22	Phytol	21.66	85.9	150-86-7	5517628	0.08
23	Tricosane (CAS)	23.30	89.3	638-67-5	2018076	0.03
24	Tetracosane	24.90	87.7	646-31-1	3690392	0.05
25	Pentatriacontane (CAS)	26.38	84.7	630-07-9	1259301	0.02



**Ptv oil - Tanacetum vulgare* L. essential oil compounds

Figure 3. *Tanacetum vulgare* L. essential oil TOC

This compound is highly prevalent and likely contributes significantly to the aroma and therapeutic properties of the oil, given its common use in fragrances and as a bioactive compound. Several terpenes are present, including α-thujone (1.88%), bicyclo[3.1.0]hexan-3-one, 4-methyl-1-(1-methylethyl)- (15.72%), and bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-(2.74%).

These compounds contribute to the essential oil's fragrance profile and may have various biological effects, such as antimicrobial or calming properties. Other compounds such as geranyl pentanoate (0.54%), caryophyllene oxide (0.74%), and germacrene-D (0.34%) are present in lower quantities but still add to the complexity of the chemical profile. These compounds are often linked to earthy or spicy aromas and could contribute to the oil's overall effects.

Compounds such as p-cymene, 1,8-cineole, and geranyl pentanoate suggest a potential for aromatic, citrus-like, or herbal scents, which are common in essential oils used in perfumery, aromatherapy, and other applications.

In a 2004 study, it is mentioned that four chemotypes of *Tanacetum* were identified in Lithuania, each with different major components (Mockute D., 2004):

Chemotype 1: camphor and 1,8-cineole;
Chemotype 2: á-thujone and 1,8-cineole;
Chemotype 3 : 1,8-cineole;
Chemotype 4: artemisia ketone and camphor .

The analyzed sample is part of chemotype 2.

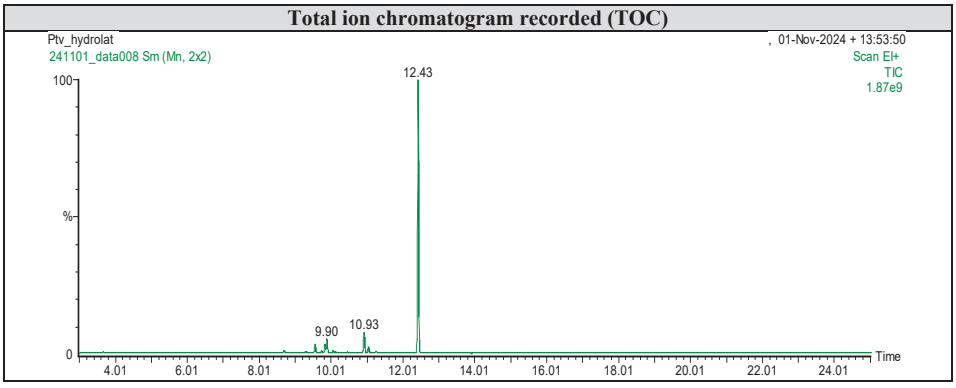
The analyses of *Tanacetum* hydrosol revealed its distinctive chemical composition (Table 4, Figure 4). The compound 2H-Pyran-3-ol, 6-ethenyltetrahydro-2,2,6-trimethyl-, acetate, trans- is the most abundant in the sample, comprising 89.76% of the total area. This suggests that it plays a significant role in the overall chemical composition and likely contributes the most to the hydrodol's or fragrance and potential biological effects.

Other important compounds like thujone (4.33%) and trans-linalool oxide (3.24%) are also present, indicating the presence of bioactive terpenes known for their aromatic and therapeutic properties. Thujone, in particular, is known for its neurotoxic and medicinal effects in small doses, while linalool oxide is linked to calming and antimicrobial properties.

The compound 2H-Pyran-3(4H)-one, 6-ethenyldihydro-2,2,6-trimethyl- (2.67%) contributes to the complexity of the hydrosol's or overall profile, likely adding to the fragrance and biological activity.

Table 4. *Tanacetum vulgare* L. hydrolat compounds

	Compound name	Retention time (min)	Probability (%)	CAS #	Area	% from the sum of areas
1	trans-Linalool oxide	9.56	89.4	34995-77-2	2448745	3.24
2	2H-Pyran-3(4H)-one, 6-ethenyldihydro-2,2,6-trimethyl-	9.84	87.6	33933-72-1	2022886	2.67
3	Thujone	9.90	89.5	546-80-5	3274052	4.33
4	2H-Pyran-3-ol, 6-ethenyltetrahydro-2,2,6-trimethyl-, acetate, trans-	12.43	88.9	56752-50-2	67910624	89.76



*Ptv hydrosol – Plant *Tanacetum vulgare* L. hydrosol

Figure 4. *Tanacetum vulgare* L. hydrolat TOC

CONCLUSIONS

The *Santolina chamaecyparissus* L.essential oil is characterized by a complex blend of terpenes, sesquiterpenes, and oxygenated compounds, with longiverbenone being the most abundant. This diverse composition suggests that the oil may have both aromatic and therapeutic applications, with the potential for use in the fragrance industry as well as for its bioactive properties, including antimicrobial, anti-inflammatory, and antioxidant effects. The high percentage of terpene compounds further underscores the potential commercial value of the oil.

The *Santolina chamaecyparissus* L. hydrosol appears to have a diverse and potentially beneficial composition, with longiverbenone being the most abundant compound, likely contributing significantly to its aroma and bioactivity. The high concentration of other terpenes and oxygenated compounds further supports its potential in various commercial and therapeutic applications.

The *Tanacetum vulgare* L. essential oil sample, with linalool oxide (68.61%), key terpenes like α-thujone (1.88%) and diverse minor compounds, shows potential for applications in perfumery, therapeutics, and personal care due to its complex aromatic and bioactive properties.

The *Tanacetum vulgare* L. hydrolat sample's composition, dominated by 2H-Pyran-3-ol acetate (89.76%) and supported by bioactive compounds like thujone and trans-linalool oxide, indicates strong potential for applications in perfumery, therapeutics, and antimicrobial products.

REFERENCES

Bruni A, Nicoletti M. (2003) Dizionario ragionato di Erboristeria e di Fitoterapia. Piccin, Padova.

Brunke E.J., Hammerschmidt F. J. and Schmaus G. (1992), The essential oil of *Santolina chamaecyparissus* L., Dragoco Report 39, 3-31.

Chanotiya CS, Mathela CS. (2007) Two distinct essential oil bearing races of *Tanacetum nubigenum Wallich* ex DC from Kumaon Himalaya. Natural Product Communications, 2, 785-788.

Davis P.H., (1982), Flora of Turkey and the East Aegean Islands, Vol.5, p 252, Edinburgh , University Press

Deg˘irmenci, H.; Erkurt, H. (2020). Relationship between Volatile Components, Antimicrobial and Antioxidant Properties of the Essential Oil, Hydrosol and Extracts of Citrus aurantium L. Flowers. J. Infect. Public Health, 13, 58–67.

Derbesy M. , Touche J. and Zola A. (1989). The essential oil of *Santolina chamaecyparissus* L., J. Essent. Oil Res.1, 269-275.

Demirci B. Ozek T. and Baser K.H.C. (2000). Chemical composition of *Santolina chamaecyparissus* L. Essential Oil, J.Essent. Oil Res., 12.

Dornelles VCM, De Paris F, Fiegenbaum M, Lino von Poser G. (1998). The use of *Tanacetum parthenium* in treatment of migraine and rheumatoid arthritis. Revista Brasileira de Farmacia, 79, 42-44.

Giner R. M., Manez S. and Rios J.L. (1993). Seasonal variations in the essential oil of *Santolina chamaecyparissus* L., Sci,Pharm.,61, 169-173.

Goren N, Woerdenbag HJ, Bozok-Johansson C. (1996). Cytotoxic and antibacterial activities of sesquiterpene lactones isolated from *Tanacetum praeteritum*. Planta Medica, 62, 419-422.

Goren N, Tahtasakal E, Pezzuto JM, Cordell GA, Schwarz B, Proksch P. (1994). Sesquiterpene lactones from *Tanacetum argenteum*. Phytochemistry, 36, 389-395.

Goren N, Bozok-Johansson C, Jakupovic J, Lin LJ, Shieh HL, Cordell GA, Celik N. (1992). Sesquiterpene lactones with antibacterial activity from *Tanacetum densum* subsp. *sivasicum*. Phytochemistry, 31, 101-104.

de Elungea-Culebras, G.O.; Bravo, E.M.; Sánchez-Vioque, R. (2022). Potential Sources and Methodologies for the Recovery of Phenolic Compounds from Distillation Residues of Mediterranean Aromatic Plants. An Approach to the Valuation of by-Products of the Essential Oil Market - A Review. *Ind. Crops Prod.*, *175*, 114261.

Heywood VH. (1976). *Tanacetum* L. In: Flora Europaea, Plantaginaceae to Compositae (and Rubiaceae), Tutin, TG, Heywood VH, Burges NA, Moore DM, Valentine DH, Walters SM, Webb DA (Eds), Vol. 4, pp. 169-171. Cambridge: Cambridge University Press.

Judzentiene A, Mockute D. (2005). The inflorescence and leaf essential oils of *Tanacetum vulgare* L. var. vulgare growing wild in Lithuania. Biochemical. Systematic and Ecology, 33, 487-498.

Keskitalo M, Pehu E, Simon JE. (2001). Variation in volatile compounds from tansy (*Tanacetum vulgare* L.) related to genetic and morphological differences of genotypes Biochemical. Systematic and Ecology, 29, 267-285.

Kwok BHB, Koh B, Ndubuisi MI, Elofsson M, Crews CM. (2001). The anti-inflammatory natural product parthenolide from the medicinal herb Feverfew directly binds to and inhibits IkB kinase. Chemistry and Biology, 8, 759-766.

Lawrence B. M. (1995). *Santolina* oil, In: Essential oils, 1992-1994. Carol Stream, USA, Allured Publ.Corp., pp 57-59.

Lawrence BM. (2000). Progress in essential oils: tansy oil. Perfumer and Flavorist, 25, 33-47.

Mockute D, Judzentiene A. (2004). Composition of the essential oils of *Tanacetum vulgare* L. growing wild in Vilnius district (Lithuania). Journal of Essential Oil Research, 16, 550-553.

Perez-Alonso M. J. and Negueruela (1988), The Essential oils of four Santolina species, Flav.Fragr. J.3, 37-42.

Perez-Alonso M. J. and Negueruela (1992). Essential oil components of *Santolina chamaecyparissus* L., Flav.Fragr. J.7, 37-41.

Rohloff J, Mordal R, Dragland S. (2004). Chemotypical variation of tansy (*Tanacetum vulgare* L.) from 40 different locations in Norway. Journal of Agricultural and Food Chemistry, 52, 1742 -1748.

Senatore F. and De Feo V. (1994). Composition of the essential oil of *Santolina neapolitana* Jordan et Fourr, Flav.Fragr. J.9.

Tang, M.; Song, F.; Huang, L.; Qin, W.; Chen, H.; Liu, L. (2021). Solvent-Free Microwave-Assisted Extraction of Essential Oil and Hydrosol from Piper Longum Linn. Fruit and Evaluation of Antioxidant Activities. *J. Essent. Oil-Bear. Plants*, *24*, 753-771.

Timung, R.; Barik, C.R.; Purohit, S.; Goud, V.V. (2016). Composition and Anti-Bacterial Activity Analysis of Citronella Oil Obtained by Hydrodistillation: Process Optimization Study. *Ind. Crops Prod.*, *94*, 178-188.

Tournier H, Schinella G, De Balsa EM, Buschiazzo H, Manez S, De Buschiazzo PM. (1999). Effect of the chloroform extract of *Tanacetum vulgare* and one of its active principles, parthenolide, on experimental gastric ulcer in rats. Journal of Pharmacy and Pharmacology, 51, 215-219.

Traka, C.K.; Petrakis, E.A.; Kimbaris, A.C.; Polissiou, M.G.; Perdikis, D.C. (2018). Effects of *Ocimum basilicum* and Ruta chalepensis hydrosols on Aphis gossypii and Tetranychus urticae. *J. Appl. Entomol.*, *142*, 413-420.

Vernin G. (1991). Volatile constituents of the oil of *Santolina chamaecyparissus* L., J. Essent. Oil Res.3, 49-53.

Villar A., Giner R.M. and RIOS J.L. (1986). Chemical composition of *Santolina chamaecyparissus* ssp. *squarrosa* essential oil, J. Nat.Prod., 1143-1145.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

EFFECTIVENESS OF META-TOPOLIN IN *IN VITRO* PROPAGATION AND GENETIC STABILITY ASSESSMENT OF MEDICINAL AND AROMATIC PLANTS

Doina CLAPA[1], Monica HÂRŢA[1], Ana Maria RADOMIR[2], Adrian PETICILĂ[3], Dorin Ioan SUMEDREA[2]

[1]Faculty of Horticulture and Business in Rural Development,
University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca,
3-5 Calea Mănăştur Street, 400372, Cluj-Napoca, Romania
[2]National Research and Development Institute for Biotechnology in Horticulture Ştefăneşti-Argeş,
37 Bucharest-Piteşti Road, 117715, Ştefăneşti, Argeş County, Romania
[3]University of Agronomic Sciences and Veterinary Medicine of Bucharest,
59 Mărăşti Blvd, District 1, 011464, Bucharest, Romania

Corresponding author email: monica.harta@usamvcluj.ro

Abstract

Meta-Topolin (mT), a compound from the cytokinin class that promotes plant growth and development, is characterized by lower toxicity than other cytokinins. This study explores the effect of mT on the in vitro multiplication of Hypericum perforatum L., Mentha x piperita L., and Stevia rebaudiana Bertoni. After six subcultures on Driver and Kuniyuki Walnut (DKW) medium supplemented with 2 mg/L mT, growth parameters were evaluated, including the number of shoots per explant, shoot length, the number of roots per explant, and root length. In the presence of mT, S. rebaudiana produced the highest number of shoots per explant (4.2 ± 0.12), while the longest shoots were recorded in M. piperita (6.2 ± 0.68 cm). The highest in vitro rooting percentage was observed in M. piperita, reaching 80%. The rooted shoots were acclimatized in perlite. Molecular analysis using Start Codon Targeted (SCoT) and Inter simple sequence repeat (ISSR) markers confirmed the genetic fidelity of the acclimatized plants compared to the mother plants, affirming the stability of in vitro cultures using mT as a cytokinin source.

Key words*: Hypericum perforatum, Mentha x piperita, ISSR, SCoT, Stevia rebaudiana.*

INTRODUCTION

Natural compounds derived from medicinal and aromatic plants (MAPs) have gained recognition as respected alternatives to synthetic drugs and as valuable resources for various industries, including cosmetics, food, feed, and environmental protection (Fierascu et al., 2021).
According to the World Health Organization, approximately 80% of the global population relies on traditional herbal medicines, driving a significant increase in the demand for medicinal plants worldwide (Radomir et al., 2023).
Hypericum perforatum L., commonly known as St. John's Wort, belongs to the *Hypericum* genus, which includes over 484 species (Kapoor et al., 2023). It is a herbaceous perennial plant native to Europe, Western Asia, and Northern Africa (Mohagheghzadeh et al., 2023). This species demonstrates various medicinal properties, such as antibacterial, antiviral, anti-inflammatory, and anticancer activities, attributed to its bioactive compounds, including hypericin, pseudohypericin, hyperforin, flavonoids, and phenolic compounds (Shasmita et al., 2023).
Mentha x piperita L., commonly referred to as peppermint, is a perennial herbaceous plant from the Lamiaceae family. It holds significant global importance as one of the leading essential oil crops. This species is highly valued for its rich content of secondary metabolites, which contribute to its distinctive aroma, flavor, and therapeutic properties. The secondary metabolites of peppermint are associated with various medicinal properties, including antioxidant, antimicrobial, anti-inflammatory, and anticancer activities (Hudz et al., 2023; Haddou et al., 2023; Afkar, 2024).
Stevia rebaudiana Bertoni, a perennial shrub belonging to the Asteraceae family, is renowned for its high content of steviol glycosides, which serve as a non-sucrose, calorie-free sweetener widely used in food products.

Beyond these sweet compounds, stevia leaves are rich in carbohydrates, lipids, dietary fibers, essential oils, water-soluble vitamins, minerals, and phenolic compounds.

Due to its diverse phytoconstituents, stevia exhibits a wide range of biological activities, including antidiabetic, antihypertensive, antimicrobial, anti-inflammatory, antitumor, and antioxidant properties (Chakma et al., 2023; Papaefthimiou et al., 2023; Śniegowska et al., 2024).

With the growing demand for herbal products, plant tissue culture emerges as a vital tool, offering an efficient alternative for the production of bioactive compounds and secondary metabolites. Consequently, it represents a promising strategy for conserving biodiversity while addressing the increasing global demand for medicinal plants and their derivatives (Akın, 2020).

Plant growth regulators (PGRs) and the multiple subcultures involved in the micropropagation process can lead to somaclonal variation. Therefore, the correct selection of PGRs and the assessment of genetic homogeneity are crucial aspects of micropropagation methods (Chirumamilla et al., 2021). Meta-Topolin [mT 6-(3-hydroxybenzylamino) purine], an aromatic cytokinin isolated from poplar leaves, stands out for its ability to promote shoot regeneration, delay senescence, and prevent necrosis, making it a valuable tool in micropropagation due to its beneficial effects on plant growth and proliferation *in vitro* (Gantait & Mitra, 2021).

The objectives of this study were to investigate the effects of mT on the *in vitro* growth and development of *H. perforatum*, *M. piperita*, and *S. rebaudiana*, as well as to assess the genetic variation that may have arisen among the clones of these three species using Start Codon Targeted (SCoT) and Inter simple sequence repeat (ISSR) molecular markers.

MATERIALS AND METHODS

Micropropagation

The source of plant material was potted plants of *H. perforatum*, *M. piperita*, and *S. rebaudiana*.

The young shoots for *in vitro* culture initiation were harvested from the mother plant and cut into fragments, each containing 2-3 nodes. The cuttings were thoroughly washed, first under running tap water and then with distilled water using a magnetic stirrer to remove impurities. Next, the shoot fragments were disinfected with a 20% bleach solution prepared from ACE (Procter and Gamble, Bucharest, Romania; <5% active ingredient) for 15 minutes, followed by triple rinsing with sterile distilled water. Single-node explants were inoculated onto Murashige and Skoog (MS) medium (Murashige & Skoog, 1962) without plant growth regulators (PGRs) and solidified with 5 g/L (w/v) plant agar in glass test tubes (11.5 × 2 cm ø) containing 5 mL of sterile medium. During the initiation phase, 30 explants per species were inoculated (one explant per test tube). After four weeks, the percentage of successful *in vitro* initiation was calculated.The regenerated shoots were subcultured every four weeks for six passages on Driver and Kuniyuki Walnut (DKW) medium (Driver & Kuniyuki, 1984) supplemented with 2 mg/L mT and solidified with 5 g/L (w/v) plant agar. All components were procured from Duchefa Biochemie BV (Haarlem, The Netherlands). The pH of the media was adjusted to 5.8 before adding agar, and all culture media was autoclaved at 120 °C for 20 minutes. In this stage, 720 mL culture jars (13.5 × 9 cm ø) with screw polypropylene caps were used as culture vessels. Each jar contained 100 mL of sterile medium, and five stem explants with 2-3 nodes were inoculated

The *in vitro* cultures were maintained at 23 ± 3 °C, under a light intensity of 32.4 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Philips CorePro LEDtube 1200 mm 16W865 CG, 1600 lm Cool Daylight) with a 16 h/8 h day/night photoperiod.After six subcultures, growth parameters were evaluated, including the number of shoots per explant, shoot length, roots per explant, and root length.

The rooted plants, obtained after six subcultures on DKW culture medium supplemented with 2 mg/L mT, were acclimatized in perlite using mini-greenhouses (Versay, T1, dimensions 39 × 25 × 7.5 cm, PVC). For each species, 30 *in vitro* rooted plantlets were acclimatized (ten plantlets per replicate), and the percentage of acclimatization was determined after four weeks.

Genetic fidelity assessment of *in vitro* raised plants using ISSR and SCoT markers

To assess the genetic fidelity of micropropagated plants of *S. rebaudiana*, *M. piperita,*

and *H. perforatum* to their mother plants, DNA was extracted from both the mother plant and one set for each molecular marker system used, which consisted of four *in vitro* plants that were randomly selected after four weeks of acclimatization in a mini-greenhouse. Biological material consisting of freshly harvested leaves was used for the genetic analyses.

DNA extraction

The extraction of total genomic DNA was performed using a DNeasy Plant Pro kit (Qiagen, Germany) following the protocol described by the supplier company.

PCR amplification

SCoT analysis

For the SCoT analysis, the PCR amplifications were carried out using the protocol described by Collard & Mackill (2009), with some modifications to the number of PCR amplification cycles and annealing temperature, depending on the primer selected (Hârţa et al., 2022; Samiee et al., 2023).

ISSR analysis

For the ISSR analysis, five ISSR primers were selected and PCR reactions were performed according to the protocol described by Morshedloo et al. 2014.

The SCoT and ISSR primers used in this study are shown in Table 1.

Table 1. The list of SCoT and ISSR primers selected for genetic stability analysis

Primer name	The 5'-3'nucleotide sequence of the Primer
SCoT 14	ACGACATGGCGACCACGC
SCoT 15	ACGACATGGCGACCGCGA
SCoT 24	CACCATGGCTACCACCAT
SCoT 26	ACCATGGCTACCACCGGG
ISSR 2	CACACACACACACACARC
ISSR 9	ACACACACACACACACYG
ISSR 12	AGAGAGAGAGAGAGAGT
ISSR 14	AGAGAGAGAGAGAGA GT
ISSR 32	CCCGTGTGTGTGTGTGT

The PCR-amplified products were separated by electrophoresis in agarose gels, under the conditions described in a previous study by Hârţa et al., 2024. To ensure the reproducibility of the results, PCR amplifications were repeated twice for each SCoT and ISSR primer.

Data analysis

One-way ANOVA followed by Tukey's HSD posthoc test ($P \leq 0.05$) was performed to determine the statistically significant differences between the mean values of the analysed morphological characteristics of vitro-plants. Values shown are means ± SE. For molecular markers analysis, gel images were evaluated using TL120 software (Nonlinear Dynamics, Newcastle upon Tyne, UK) to determine the molecular weight (bp) range of PCR amplified products. The number of amplified SCoT and ISSR monomorphic bands was also evaluated in this study.

RESULTS AND DISCUSSIONS

Micropropagation

In vitro culture initiation rates for *H. perforatum*, *M. piperita,* and *S. rebaudiana* on MS culture medium without PGRs were 64.44%, 68.89%, and 84.44%, respectively. The shoots obtained in the initiation stage were then maintained on the DKW culture medium supplemented with 2 mg/L mT for six subcultures of one month each (Figure 4). mT is an aromatic cytokinin known for its efficiency in shoot proliferation and root induction in economically important species. It is metabolized faster than conventional cytokinins and promotes plant growth and development, contributing to the alleviation of *in vitro* - induced morpho-anatomical and physiological disorders (Aremu et al., 2012; Ahmad & Anis, 2019; Souza et al., 2019; Elayaraja et al., 2019; Nowakowska & Pacholczak, 2020; Shekhawat et al., 2021; Manokari et al., 2021).

The presence of mT in the culture medium generated the highest number of shoots in *S. rebaudiana* (4.2 ± 0.12), while the longest shoots were observed in *M. piperita* (6.2 ± 0.46 cm) (Figures 1 and 2). It is noteworthy that, in all three species, the proliferated shoots did not exhibit morphophysiological abnormalities such as hyperhydric shoots, fragile leaves, or deformed structures. The beneficial effect of mT on the micropropagation of *S. rebaudiana* has also been reported when *in vitro* culture was conducted in RITA bioreactors (Ptak et al., 2023).

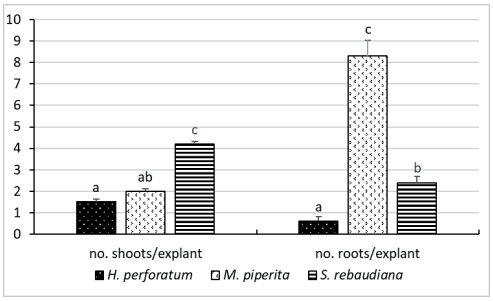


Figure 1. Number of shoots and number of roots per initial inoculum of *H. perforatum, M. piperita*, and *S. rebaudiana* after four weeks

In addition, DKW culture medium supplemented with 2 mg/L mT generated root formation in all three species. The highest rooting percentage was observed in *M. piperita* (80%), followed by *S. rebaudiana* (60%) and *H. perforatum* (40%). The highest number of roots per explant was 8.3 in *M. piperita*, followed by *S. rebaudiana* with 2.4 roots per explant (Figure 1). The lowest number of roots per explant was recorded in *H. perforatum* (0.6 ± 0.23), which also had the shortest roots, with an average length of 0.9 ± 0.32 cm.

Healthy explants, a good multiplication rate, and spontaneous rooting were also reported for *Aloe polyphylla* at a concentration of 5.0 mM mT (Bairu et al., 2007), at *Pelargonium* × *hortorum* (Mutui et al., 2012) and *Syzygium cumini* (Naaz et al., 2019).

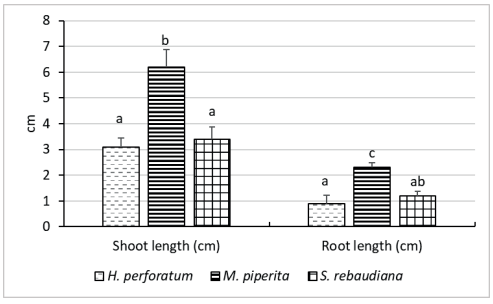


Figure 2. The length of shoots and roots of *H. perforatum, M. piperita*, and *S. rebaudiana*

The *in vitro* shoots rooted and then acclimatized *ex vitro* in perlite exhibited a 91.11% survival rate in *S. rebaudiana*, 90% in *M. piperita*, and 66.66% in *H. perforatum* (Figures 3 and 5). The present results are in accordance with those obtained in other studies conducted on *M. piperita* micropropagation (Sharma et al., 2019; Zayova et al., 2021), *S. rebaudiana* (Yücesan et al., 2016; Rodriguéz-Páez et al., 2024), and *H. perforatum* (Fascella et al., 2015).

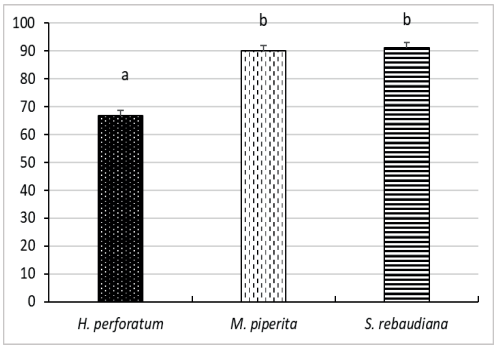


Figure 3. Perlite acclimation percentages

Genetic fidelity analysis

SCoT analysis. In total, 12 SCoT primers were used for the initial screening between the mother plants and the four randomly selected plants resulting from *in vitro* culture of each analysed medicinal and aromatic species. Four SCoT primers generated reproducible bands and were then used for genetic stability. The scorable bands for each SCoT primer varied from 7 (SCoT 24; SCoT 26) to 15 (SCoT 14). As can be seen in Table 2, the number of weight range of amplified bands (bp) varied between 300-2000 bp in *S. rebaudiana*, 300-2200 bp in *M. piperita*, and 300-2500 bp in *H. perforatum*. The highest total number of monomorphic SCoT bands was recorded in *H. perforatum*, namely 42, while the four selected SCoT primers generated 36 distinct and reproducible bands in *S. rebaudiana* (Table 2).

In this study, SCoT primers generated monomorphic PCR amplification products in all selected *in vitro* cultured plants, and no polymorphism was detected during SCoT analysis (Figure 6). Our results were consistent with those reported in previous studies (Tikendra et al., 2021; Biswas & Kumar, 2023; Bisht et al., 2024) which support that, when *in vitro* subcultured plants are used as commercial end products, assessing the genetic stability of acclimatized plants is essential.

ISSR analysis. According to Bisht et al. (2024) ISSR investigations use non-coding regions of DNA to assess genetic variability, diversity, and stability. Thus, the results of this study showed that ISSR markers are suitable for evaluating the

genetic fidelity of the analysed plant material grown under *in vitro* conditions.

A total of 11 ISSR primers were screened for genetic fidelity analysis of stevia, mint, and St. John's wort plants derived from *in vitro* shoot culture of which five ISSR primers produced reproducible monomorphic bands ranging from 300 to 3000 bp (Table 2). Maximum monomorphic bands (12) were produced by ISSR 12 primer in *H. perforatum*, whereas, minimum number of monomorphic bands (6) were produced by primer ISSR 32 in *M. piperita* (Table 2). The vitroplants and mother plants had a high level of genetic uniformity (100 %) and no genetic variation was identified (Figure 7). Previous studies have also documented the observed genetic homogeneity between the *in vitro* raised plants and the mother plants (Monalisa et al., 2024; Saritha et al., 2024; Awere et al., 2024).

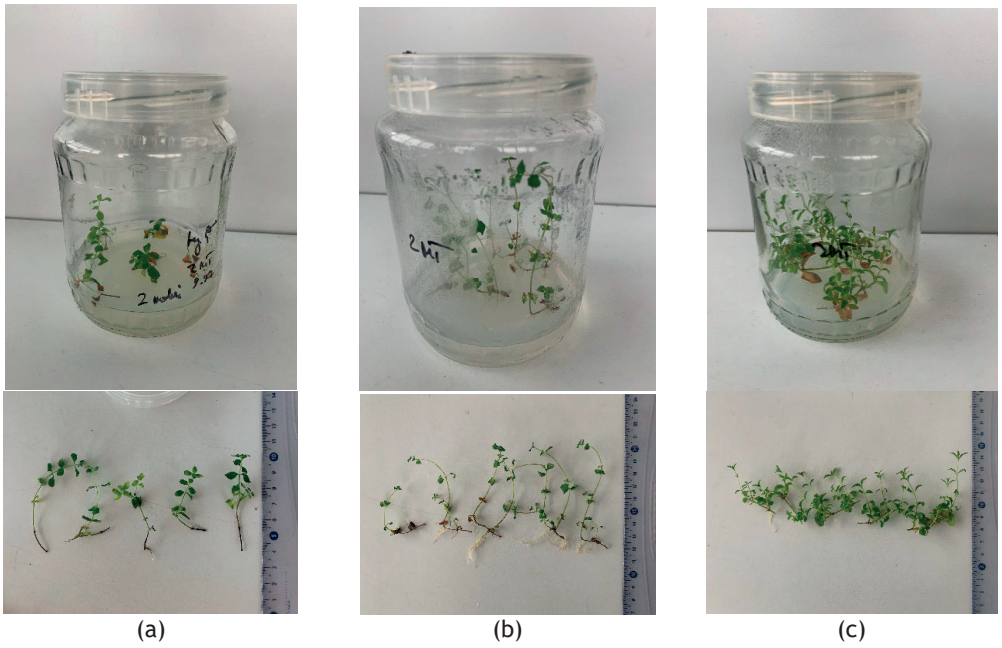


Figure 4. *In vitro* culture on DKW culture medium supplemented with 2 mg/L mT
(a) *H. perforatum;* (b) *M. piperita;* (c) *S. rebaudiana*



Figure 5. Acclimated plants: (a) *H. perforatum;* (b) *M. piperita;* (c) *S. rebaudiana*

Table 2. The number and size range (bp) of amplified SCoT and ISSR monomorphic bands

Primer name	Size range of monomorphic bands (bp)			Number of monomorphic bands		
	S. rebaudiana	*M. piperita*	*H. perforatum*	*S. rebaudiana*	*M. piperita*	*H. perforatum*
SCoT 14	400-2000	300-2200	450-1800	10	15	13
SCoT 15	300-1500	300-1700	300-1800	9	11	14
SCoT 24	350-1700	500-1500	500-1700	9	8	7
SCoT 26	400-2000	600-2000	550-2500	8	7	8
Total	-	-	-	36	41	42
ISSR 2	300-1500	400-2000	400-2000	7	8	10
ISSR 9	700-2200	500-1800	500-3000	8	10	11
ISSR 12	600-2000	800-3000	500-2200	10	8	12
ISSR 14	300-2500	400-1800	300-2200	7	8	10
Total	-	-	-	41	40	50

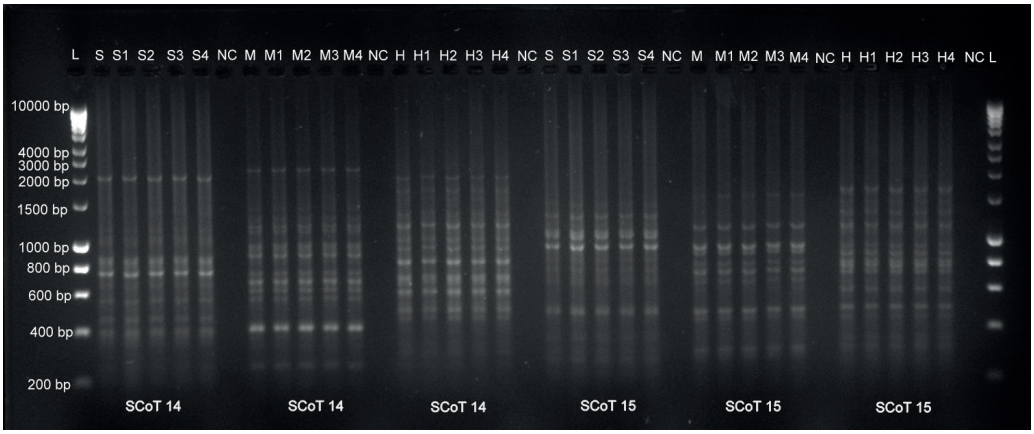


Figure 6. The SCoT profiles of mother plants and selected acclimated vitroplants of S. *rebaudiana* (S1-S4), *M. piperita* (M1-M4), and *H. perforatum* (H1-H4) generated by primers SCoT 14 and SCoT 15. Lanes: S, M, H – SCoT bands from the mother plants; L – molecular marker (1 Kb HyperLadder, Bioline, UK); NC – sample controls without DNA.

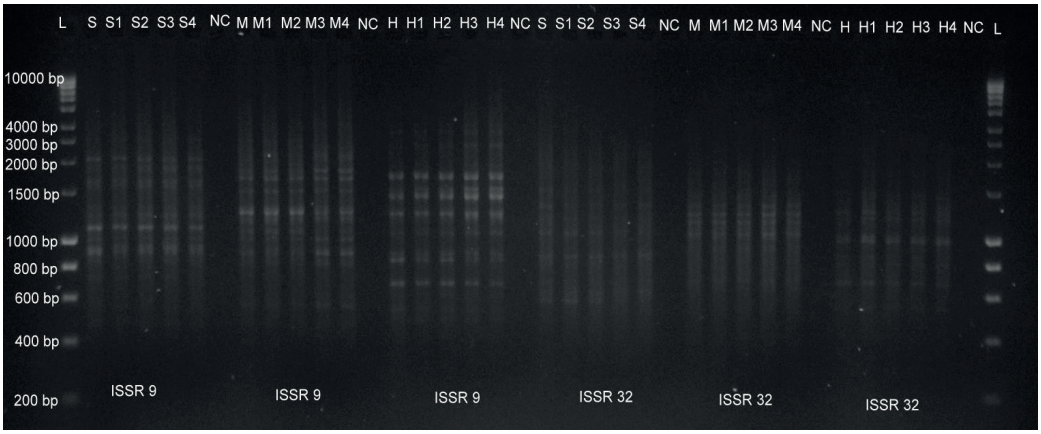


Figure 7. The ISSR profiles of mother plants and selected acclimated vitroplants of *S. rebaudiana* (S1-S4), *M. piperita* (M1-M4), and *H. perforatum* (H1-H4) generated by primers ISSR 9 and ISSR 32. Lanes: S, M, H – ISSR bands from the mother plants; L – molecular marker (1 Kb HyperLadder, Bioline, UK); NC – sample controls without DNA.

CONCLUSIONS

Applying mT during the *in vitro* multiplication stage of *H. perforatum*, *M. piperita*, and *S. rebaudiana* resulted in shoots free of morpho-physiological alterations and promoted root development, particularly in mint. Furthermore, genetic stability analysis using SCoT and ISSR markers revealed that the *in vitro*-raised plants were genetically true-to-type with the mother plants, confirming the stability of *in vitro* cultures when using mT as a cytokinin source.

ACKNOWLEDGEMENTS

This research was funded by the Romanian Ministry of Agriculture and Rural Development, contract ADER 5.2.3. / 2023.

REFERENCES

Afkar, S. (2024). Assessment of chemical compositions and antibacterial activity of the essential oil of *Mentha piperita* in response to salicylic acid. *Natural Product Research,* 38(20), 3562-3573.

Ahmad, A., & Anis, M. (2019). Meta-topolin improves *in vitro* morphogenesis, rhizogenesis and biochemical analysis in *Pterocarpus marsupium* Roxb.: a potential drug-yielding tree. *Journal of Plant Growth Regulation*, 38, 1007-1016.

Akın, B. (2020). Tissue culture techniques of medicinal and aromatic plants: History, cultivation and micropropagation. *Journal of Scientific Reports-A*, (045), 253-266.

Aremu, A. O., Bairu, M. W., Doležal, K., Finnie, J. F., & Van Staden, J. (2012). Topolins: a panacea to plant tissue culture challenges? *Plant Cell, Tissue and Organ Culture* (PCTOC), 108, 1-16.

Awere, C. O., Rakkammal, K., Ram, P. J., Kumar, K. P., Ragavan, K., Kumari, R. A., ... & Ramesh, M. (2024). Micropropagation, genetic fidelity and chromatographic analysis in Evolvulus alsinoides (L.): A potent multipurpose medicinal plant. *Industrial Crops and Products*, 213, 118444.

Bairu, M. W., Stirk, W. A., Dolezal, K., & Van Staden, J. (2007). Optimizing the micropropagation protocol for the endangered *Aloe polyphylla*: can meta-topolin and its derivatives serve as replacement for benzyladenine and zeatin?. *Plant Cell, Tissue and Organ Culture,* 90, 15-23.

Bisht, V., Rawat, J. M., Gaira, K. S., Purohit, S., Anand, J., Sinha, S., ... & Rawat, B. (2024). Assessment of genetic homogeneity of *in vitro* propagated apple root stock MM 104 using ISSR and SCoT primers. *BMC Plant Biology*, 24(1), 240.

Biswas, P., & Kumar, N. (2023). Application of molecular markers for the assessment of genetic fidelity of *in vitro* raised plants: current status and future prospects. *Molecular Marker Techniques: A Potential Approach of Crop Improvement*, 233-256.

Chakma, A., Afrin, F., Rasul, M. G., Maeda, H., Yuan, C., & Shah, A. K. M. A. (2023). Effects of extraction techniques on antioxidant and antibacterial activity of stevia (*Stevia rebaudiana* Bertoni) leaf extracts. *Food Chemistry Advances*, 3, 100494.

Chirumamilla, P., Gopu, C., Jogam, P., & Taduri, S. (2021). Highly efficient rapid micropropagation and assessment of genetic fidelity of regenerants by ISSR and SCoT markers of *Solanum khasianum* Clarke. *Plant Cell, Tissue and Organ Culture (PCTOC*), 144, 397-407.

Collard, B. C., & Mackill, D. J. (2009). Start codon targeted (SCoT) polymorphism: a simple, novel DNA marker technique for generating gene-targeted markers in plants. *Plant molecular biology reporter*, 27, 86-93.

Driver, J. A., & Kuniyuki, A. H. (1984). *In vitro* propagation of Paradox walnut rootstock. *HortScience*, 19(4), 507-509.

Elayaraja, D., Subramanyam, K., Vasudevan, V., Sathish, S., Kasthurirengan, S., Ganapathi, A., & Manickavasagam, M. (2019). Meta-Topolin (mT) enhances the *in vitro* regeneration frequency of *Sesamum indicum* (L.). *Biocatalysis and agricultural biotechnology*, 21, 101320.

Fascella, G., Airò, M., Mammano, M. M., Giardina, G., Carrubba, A., & Lazzara, S. (2015, April). Rooting and acclimatization of micropropagated *Hypericum perforatum* L. native to Sicily. *In VI International Symposium on Production and Establishment of Micropropagated Plants* 1155 (pp. 543-548).

Fierascu, R. C., Fierascu, I., Baroi, A. M., & Ortan, A. (2021). Selected aspects related to medicinal and aromatic plants as alternative sources of bioactive compounds. *International Journal of Molecular Sciences,* 22(4), 1521.

Gantait, S., & Mitra, M. (2021). Role of meta-topolin on *in vitro* shoot regeneration: an insight. *In Meta-topolin: a growth regulator for plant biotechnology and agriculture* (pp. 143-168). Singapore: Springer Singapore.

Haddou, M., Taibi, M., Elbouzidi, A., Loukili, E. H., Yahyaoui, M. I., Ou-Yahia, D., ... & El Guerrouj, B. (2023). Investigating the impact of irrigation water quality on secondary metabolites and chemical profile of *Mentha piperita* essential oil: analytical profiling, characterization, and potential pharmacological applications. *International Journal of Plant Biology*, 14(3), 638-657.

Hârţa, M., & Clapa, D. (2022). Micropropagation of ornamental Gesneriaceae species and genetic uniformity assessment of *in vitro* plants using scot markers. *Scientific Papers. Series B. Horticulture*, 66(1).

Hârţa, M., Clapa, D., Pop, R., Cordea, M. I., & Pamfil, D. (2024). *In vitro* propagation of *Saintpaulia ionantha* Wendl. genotypes and assessment of genetic stability of regenerated plants using CDDP markers. *Scientific Papers. Series B. Horticulture*, 68(1).

Hudz, N., Kobylinska, L., Pokajewicz, K., Horčinová Sedláčková, V., Fedin, R., Voloshyn, M., ... & Lipok,

J. (2023). *Mentha piperita*: essential oil and extracts, their biological activities, and perspectives on the development of new medicinal and cosmetic products. *Molecules*, 28(21), 7444.

Jayaprakash, K., Manokari, M., Badhepuri, M. K., Raj, M. C., Dey, A., & Shekhawat, M. S. (2021). Influence of meta-topolin on *in vitro* propagation and foliar micro-morpho-anatomical developments of *Oxystelma esculentum* (Lf) Sm. *Plant Cell, Tissue and Organ Culture* (PCTOC), 147, 325-337.

Kapoor, S., Chandel, R., Kaur, R., Kumar, S., Kumar, R., Janghu, S., ... & Kumar, V. (2023). The flower of *Hypericum perforatum* L.: A traditional source of bioactives for new food and pharmaceutical applications. *Biochemical Systematics and Ecology*, 110, 104702.

Lodhi, M. A., Ye, G. N., Weeden, N. F., & Reisch, B. I. (1994). A simple and efficient method for DNA extraction from grapevine cultivars and *Vitis* species. *Plant molecular biology Reporter*, 12, 6-13.

Manokari, M., Mehta, S. R., Priyadharshini, S., Badhepuri, M. K., Dulam, S., Jayaprakash, K., ... & Shekhawat, M. S. (2021). Meta-Topolin mediated improved micropropagation, foliar micro-morphological traits, biochemical profiling, and assessment of genetic fidelity in *Santalum album* L. *Industrial Crops and Products*, 171, 113931.

Mohagheghzadeh, A., Badr, P., Mohagheghzadeh, A., & Hemmati, S. (2023). *Hypericum perforatum* L. and the underlying molecular mechanisms for its choleretic, cholagogue, and regenerative properties. *Pharmaceutical*s, 16(6), 887.

Monalisa, K., Behera, S., Pidika, S. P., Nial, P. S., & Naik, S. K. (2024). *In vitro* propagation and assessment of genetic fidelity of *Blepharispermum subsessile* DC.: an endangered medicinal plant of India. *Vegetos*, 1-10.

Morshedloo, M. R., Moghadam, M. R. F., Ebadi, A., & Yazdani, D. (2015). Genetic relationships of Iranian *Hypericum perforatum* L. wild populations as evaluated by ISSR markers. *Plant systematics and evolution,* 301, 657-665.

Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia plantarum*, 15(3).

Mutui, T. M., Mibus, H., & Serek, M. (2012). Effect of meta-topolin on leaf senescence and rooting in *Pelargonium× hortorum* cuttings. *Postharvest biology and technology*, 63(1), 107-110.

Naaz, A., Hussain, S. A., Anis, M., & Alatar, A. A. (2019). Meta-topolin improved micropropagation in *Syzygium cumini* and acclimatization to *ex vitro* conditions. *Biol. Plant*, 63, 174-182.

Nowakowska, K., & Pacholczak, A. (2020). Comparison of the effect of meta-Topolin and benzyladenine during *Daphne mezereum* L. micropropagation. *Agronomy*, 10(12), 1994.

Papaefthimiou, M., Kontou, P. I., Bagos, P. G., & Braliou, G. G. (2023). Antioxidant activity of leaf extracts from *Stevia rebaudiana* Bertoni exerts attenuating effect on diseased experimental rats: A systematic review and meta-analysis. *Nutrients,* 15(15), 3325.

Ptak, A., Szewczyk, A., Simlat, M., Błażejczak, A., & Warchoł, M. (2023). Meta-Topolin-induced mass shoot multiplication and biosynthesis of valuable secondary metabolites in *Stevia rebaudiana* Bertoni bioreactor culture. *Scientific Reports*, 13(1), 15520.

Radomir, A. M., Stan, R., Peticilă, A. G., Clapa, D., Muşat, B., Vînătoru, C., & Sumedrea, D. I. (2024). Overview of bioactive compounds, biological properties and therapeutic effects of *Plectranthus amboinicus*. *Scientific Papers. Series B. Horticulture*, 68(1).

Rodriguéz-Páez, L. A., Pineda-Rodriguez, Y. Y., Pompelli, M. F., Jimenez-Ramirez, A. M., Genes-Avilez, O. J., Jaraba-Navas, J. d. D., Jarma-Orozco, A., Combatt-Caballero, E., Oviedo Zumaqué, L. E., Suarez-Padron, I. E., Oloriz-Ortega, M. I., & Rodríguez, N. V. (2024). Micropropagation Protocols for Three Elite Genotypes of *Stevia rebaudiana* Bertoni. *Horticulturae,* 10(4), 404.

Samiee, M., Hârța, M., Clapa, D., & Botu, M. (2023). Morphometric analysis and assessment of genetic diversity of willow (Salix sp.) genotypes using SCoT molecular markers. *Scientific Papers. Series B. Horticulture*, 67(1).

Saritha, K., Sandhya, D., Thirupathi, K., & Mohammed, M. (2024). Direct regeneration from leaf explants and genetic fidelity analysis of regenerates through ISSR markers in *Kedrostis foetidissima*: a medicinal climber. *Vegetos*, 37(4), 1669-1676.

Shasmita, Behera, S., Mishra, P., Samal, M., Mohapatra, D., Monalisa, K., & Naik, S. K. (2023). Recent advances in tissue culture and secondary metabolite production in *Hypericum perforatum* L. *Plant Cell, Tissue and Organ Culture (PCTOC)*, 154(1), 13-28.

Sharma, M., Sharma, M., Salgotra, R. K., Gupta, M., Singh, A. K., & Gupta, L. M. (2019). Development of an effective protocol for *in vitro* multiplication of peppermint (*Mentha piperita*). *The Indian Journal of Agricultural Science*s, 89(11), 1975-1978.

Shekhawat, M. S., Priyadharshini, S., Jogam, P., Kumar, V., & Manokari, M. (2021). Meta-topolin and liquid medium enhanced *in vitro* regeneration in *Scaevola taccada* (Gaertn.) Roxb. *In Vitro Cellular & Developmental Biology-Plan*t, 57, 296-306.

Souza, L. M. D., Silva, M. M. D. A., Herculano, L., Ulisses, C., & Camara, T. R. (2019). Meta-topolin: an alternative for the prevention of oxidative stress in sugarcane micropropagation. *Hoehnea*, 46, e1072018.

Śniegowska, J., Biesiada, A., & Gasiński, A. (2024). Influence of the Nitrogen Fertilization on the Yield, Biometric Characteristics and Chemical Composition of *Stevia rebaudiana* Bertoni Grown in Poland. *Molecules*, 29(8), 1865.

Thakur, M., Sharma, V., & Chauhan, A. (2021). Genetic fidelity assessment of long term *in vitro* shoot cultures and regenerated plants in *Japanese plum* cvs Santa Rosa and Frontier through RAPD, ISSR and SCoT markers. *South African Journal of Botany*, 140, 428-433

Tikendra, L., Potshangbam, A. M., Dey, A., Devi, T. R., Sahoo, M. R., & Nongdam, P. (2021). RAPD, ISSR, and SCoT markers based genetic stability assessment of micropropagated *Dendrobium fimbriatum* Lindl. var. oculatum Hk. f.-an important endangered orchid.

Physiology and Molecular Biology of Plants, 27, 341-357.

Yücesan, B., Mohammed, A., Büyükgöçmen, R., Altuğ, C., Kavas, Ö., Gürel, S., & Gürel, E. (2016). *In vitro* and *ex vitro* propagation of *Stevia rebaudiana* Bertoni with high Rebaudioside-A content—A commercial scale application. *Scientia Horticulturae*, 203, 20-28.

Zayova, E., Kirova, E., & Geneva, M. (2021). Optimized cultural conditions for rapid *in vitro* propagation and conservation of *Mentha piperita* L. *Comptes Rendus Acad. Bulg. Sci,* 74, 945-954.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

STRUCTURAL CHARACTERISTICS OF TREES OBTAINED THROUGH THE TRANSFORMATION OF SILVOPASTORAL SYSTEMS

Ghiţă Cristian CRAINIC[1], Florin Lucian COVACI[2]

[1]PhD Lecturer Eng., University of Oradea, Faculty of Environmental Protection, Department of Forestry and Forestry Engineering, Oradea, Romania
[2]PhD Student, University of Oradea, Doctoral School of Engineering Sciences, Agriculture Program, Oradea, Romania

Corresponding author email: gccrainic@yahoo.com

Abstract

The stands resulting from the transformation of silvopastoral systems present a series of specific structural, qualitative and synthesis characteristics. The structure of these stands is influenced by the species, their regeneration method and the time in which the massif state was achieved. The case study was carried out within the Codrii Cămării Forest District in Dobreşti, Bihor County, and has as its objectives the silvotechnical analysis and diagnosis of the stand formed by the transformation of a silvopastoral system. From the analysis of the horizontal, vertical and three-dimensional profiles made on the experimental samples in the studied stand, a series of differences are found between the base diameter, the base area, the total height, the pruned height and the crown diameter, for the analyzed trees. In some portions of the stand, the consistency index k varies in the range of 0.6 - 0.8, an aspect that negatively influences its growth and development process. Consequently, for the sustainable management of these stands with a specific structure, a series of works related to both mixed regenerations and young stands are necessary.

Key words: *silvopastoral system, transformation, stand, structural characteristics, silvotechnical interventions, sustainable management.*

INTRODUCTION

Within forest ecosystems, the forest phytocenosis, through the tree canopy, respectively the stand, builds their specificity. Currently, the forest can be defined in several ways, but the structural and functional characteristics are those that determine its specificity.

As a result, individual (isolated) trees and respectively tree biogroups are the defining elements of the forest phytocenosis and implicitly of the forest. Its interrelations with the forest zoocenosis and the elements of the forest site determine the establishment of a complex and unitary community of life, characterized by its own, specific environment. In this context, the forest offers forest products, services, and is characterized by a high mediogenic capacity (Crainic & Tăut, 2008; Cubbage, 2012; Nicolescu, 2009).

The expansion of forest areas, regardless of the owner and administrator, represents one of the strategies of national and international forestry policies (Abrudan et al., 2003).

The transformation of agrosilvopastoral systems into forest ecosystems represents a possibility for increasing (expanding) the national forest fund (Crainic et al., 2023). In this context, the areas occupied by individual trees and respectively by tree biogroups are included in the future stand, which will have a characteristic structure.

The woody vegetation on areas proposed for inclusion in the forest fund must be composed of tree species characteristic of the region's fundamental natural forest type and must exhibit a density index (k) greater than 0.4. Furthermore, the landowner is required to formally initiate the reclassification of the plot's land use to forestry, based on a technical-economic project specifically developed for this purpose.Individual trees exhibit a specific form, generally referred to as habitus, while trees within a closed stand are characterized by a forest (stand) form. (Crainic, 2024; Florescu & Nicolescu, 1996). Trees that grow in biogroups on the surface of agrosilvopastoral systems present an intermediate form, between the two forms presented previously. As a result, the structural elements of the future stand and implicitly its structure will have specific characteristics in this case.The structural charac-

teristics of stands can be highlighted in the horizontal plane and in the vertical plane. Those in the horizontal plane are represented by: composition, consistency, number of trees, average diameter, base area. In the vertical plane, the characteristics of stands are represented by: stratification, profile, closure and average height.
The qualitative characteristics are represented by: the origin and provenance of the stands, the production class, the quality, the age, the state of vegetation and the phytosanitary or health status.
A series of stand characteristics can be synthetically expressed through a series of indices, as follows: density and stocking indices, slenderness coefficient $Z_{\%}$ and spacing index $S_{\%}$ (Florescu & Nicolescu, 1996; Crainic et al., 2024) and provide important indications in the silvotechnical analysis and diagnosis process (Crainic et al., 2024; Crainic & Tăut, 2008; Nicolescu, 1995). The mathematical expressions related to these are presented in formulae 1-10.

$$I_N = \frac{N_{teren}}{N_{tabel}} \quad (1)$$

$$I_G = \frac{G_{teren}}{G_{tabel}} \quad (2)$$

$$I_v = \frac{V_{teren}}{V_{tabel}} \quad (3)$$

$$Z_{(\%)} = \frac{h}{d} \quad (4)$$

$$S_{(\%)} = \frac{a}{h_{dom}} \times 100 \quad (5)$$

$$S_{4(\%)} = \frac{a_4}{h_{dom}} \times 100 \quad (6)$$

$$S_{6(\%)} = \frac{a_6}{h_{dom}} \times 100 \quad (7)$$

$$h_{dom} = h + 0.15h = 1.15h \quad (8)$$

$$a_4 = \sqrt{\frac{10000}{N}} \quad (9)$$

$$a_6 = \sqrt{\frac{10000}{N\sqrt{\frac{3}{2}}}} \quad (10)$$

The meaning of the terms in formulae 1-10 is as follows: IN-density index determined based on the number of trees; I_G-density index determined based on the base area; I_V-density index determined based on the volume; N_{land}-number of trees per hectare, determined experimentally; G_{land}-base area per hectare, determined experimentally; V_{land}-volume per hectare, determined experimentally; N_{table}, G_{table}, V_{land}, theoretical values of the number of trees, area and volume, from the general production tables, for the same species, production class and age; $Z_{(\%)}$-slenderness coefficient of the stand; h-average height of the stand; d-average diameter of the stand; $S_{(\%)}$-Hart-Beking spacing index; hdom-dominant height of the stand; a-theoretical distance between trees;
S_4-spacing index related to the square disposal, a_4-theoretical distance between the trees when they are arranged in a square disposal; S_4-spacing index related to the quincunx disposal; a_6-theoretical distance between the trees when they are arranged in a quincunx device.
Also, the composition of the stands can be determined based on: the number of trees per hectare C_{pzN}; the base area per hectare C_{pzG} and the volume per hectare C_{pzV}.
Consequently, the structural and qualitative indicators related to a stand obtained through the transformation of an agrosilvopastoral system, provide a series of information regarding its structure and stability, as well as the complex of forest management practices that are necessary for sustainable management in the future (Crainic & Stamate 2009; Nair, 1993).

MATERIALS AND METHODS

The case study was carried out in Forest Compartment 60 of the forest fund of Dobreşti Commune, Bihor County. The respective forest fund is managed by the Codrii Cămării Forest District, within the Production Unit (P.U.) I Dobreşti - Figure 1.
The stand under study originated from the transformation and incorporation of an agrosilvopastoral system previously managed by the Dobreşti municipality into the communal forest fund. The research conducted in the case study is related to an ongoing doctoral study, which is being carried out in this location.
The objectives of the case study are represented by:
- analysis of the characteristics of the stand in Forest Compartment 60, which was formed by the transformation and introduction of an agrosilvopastoral system in the forest fund, during the period 2013-2024;
- establishment of the forest management practices necessary for the sustainable management of this stand.
The research methods used are represented by (Crainic et al., 2023): bibliographic documenttation, experiment, observation on the itinerary and stationary, statistical-mathematical inventtory, simulation, analysis, comparison and recording on digital photographic support.

Through bibliographic documentation, complex information was obtained regarding:
- structural and functional particularities related to agrosilvopastoral systems;

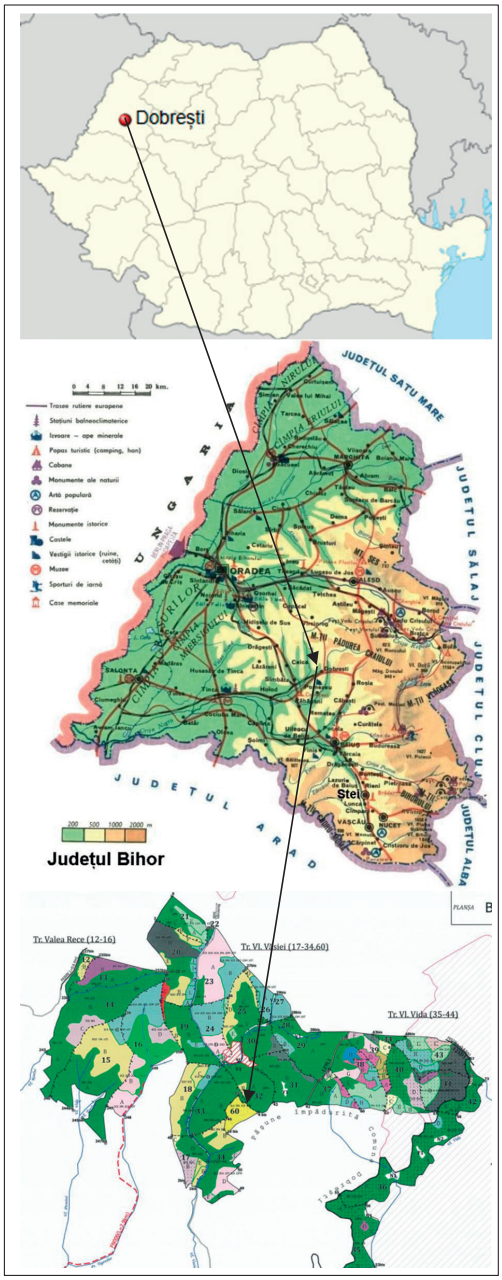


Figure 1. Research location (https://ro.wikipedia.org/wiki/Dobre%C8%99ti,_Bihor, https://pe-harta.ro/bihor/, Harta amenjistică a U.P. I Dobrești, 2018)

- specificities of the transformation and introduction of agrosilvopastoral systems into the national forest fund;
-structural characteristics related to the stands in the national forest fund;
- the complex of forest management practices necessary for the management of stands obtained through the transformation of agrosilvopastoral systems.

The experiment involved making observations, conducting a statistical and mathematical inventory, simulating the stand structure, and performing silvotechnical analysis and diagnosis.

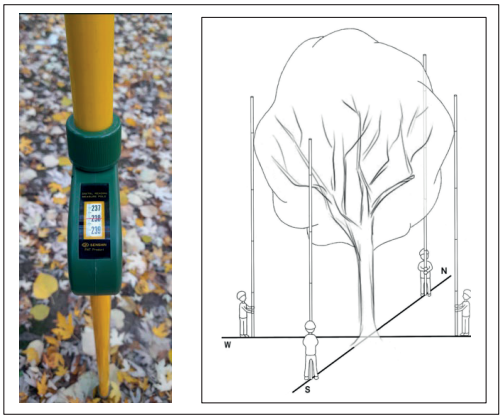
Through observations, a current and pertinent analysis of the objective reality in the field, in the study area, was carried out. As a result, observations were made on the itinerary and respectively stationary, at certain fixed points, representative for the research carried out. On these occasions, the dynamics of the growth and development process of the established forest vegetation, as well as its health condition, were analyzed.

The statistical and mathematical inventories were carried out on experimental plots, randomly placed on the surface of compartment 60.

The specific elements of the experimental plots were established in accordance with the Biometrics of Trees and Stands in Romania (Giurgiu et al., 1972) and the Forest management plan in force, and are represented by:
- area of the statistical population - of the plot, S = 7.6 ha;
- class of homogeneity of the stand;
- variation coefficient of the volume;
- age of the stand T = 15-20 years;
- shape of the sample areas - circle;
- size of the experimental areas S_i = 300 m^2;
- tolerance t = 10%;
- coverage probability p = 90%;
- number of experimental plots n = 14;
- distance between the centers of the experimental plots d = 54 m.

The 14 experimental surfaces, circular in shape, of established surface and radius, were placed with their centers in the corners of a square-shaped geometric grid (which was superimposed over the surface of plot 60), with the side equal to the distance d = 54 m, which was determined according to the methodology presented previously.

As a result, the following stages were completed in the statistical-mathematical inventory process:
- location of experimental areas on the field and materialization of their center;
- identification of the species,
- measurement of the basic diameter ($d_{1.30}$) at a height of 1.30 m from the plot;
- establishment of the quality class;
- establishment of the vegetation state;
- establishing the phytosanitary status.
These data were recorded in field sheets, for each experimental area separately.
To simulate the spatial structure of the stand, inventories were carried out on rectangular experimental areas, 20 m long and 15 m wide, in its representative areas. As a result, two experimental plots of 300 m^2 were located, one area located in the area where the biogroups of naturally regenerated trees from seed were promoted, and the other in the area where forest vegetation was artificially installed through plantations. The inventory of each tree in the experimental sample area involved the following operations:
- determination of the planimetric position in a local rectangular coordinate system;
- the diameter of the crowns was measured in two perpendicular directions;
- measurement of the total height and the height to the base of the crown, respectively;
- identification of the species.
These data were recorded in a specific field sheet, in which the total number of trees, length L and width l of the experimental plot, and respectively the average slope angle of the land, were mentioned in the corresponding columns. Subsequently, the data entered in this form were implemented in an Excel spreadsheet file, and their processing was carried out with the PROARB 2.0 program (Popa, 1999; Crainic et. al., 2024). The height of the trees was measured using a telescopic rod, graduated in centimeters, which ensured high accuracy (Figure 2 a).
The crown diameter of the trees was determined along two perpendicular directions, after marking on the ground the extreme points of the crown's circumference in those directions (Figure 2 b).
The following materials were used to carry out the research:
- forest management of the production unit I Dobreşti and the related management map;
- tree caliper for measuring the basic diameter $d_{1.30}$ of the inventoried trees;
- telescopic rod for determining the heights of the inventoried trees, up to 8 meters;
- dendrometer for measuring heights greater than 8 meters;
- PROARB 2.0 program for simulating the projections of the stand in the analyzed samples;
- Excel program for data processing and results explanation;
- MapSys11 digital cartography program, for locating and placing the experimental plots on the forest map.



a. Telescopic rod measurement b. Crown diameter

Figure 2. Aspects related to measuring the diameter of tree crowns, in two directions, with the telescopic rod (Crainic et al., 2024)

The experimental results obtained for the species in the inventoried stand were analyzed and compared with the theoretical values in the production tables, for the same species, production classes and ages.
Research, studies and observations at the aforementioned location began in the spring of 2013, and have been ongoing to this day.

RESULTS AND DISCUSSIONS

From the analysis and study of the description of the forest site and the vegetation related to compartment 60, presented in the forestry management related to P.U. I Dobreşti, and the observations made in the field, a series of important data were obtained, which are presented below.

As a result, the characteristics of the vegetation conditions and the stands in compartment 60 are represented by:
- area of the stand: 7.6 ha;
- typical luvic brown soil (2401);
- type of station: Phytoclimatic level of oak forests in the hilly area, with medium site quality, on luvisols with medium edaphic volume, and indicator flora consisting of grasses (5132);
- type of forest: Coastal sessile oak forest with *Gramineae* and *Luzula luzuloides*, of medium productivity (5131);
- land form: undulating slope;
- exposition: South-West;
- slope of the land: 22 g;
- altitude: 350-440 m;
- litter: continuous and thin layer;
- indicator flora: *Glecoma hirsuta - Geum urbanum;*
- forest type: natural fundamental of medium productivity, relatively even;
- current composition: the composition identified during the preparation of the forest management plan: 3Ce3Fa3La1Dt;
- target composition: 7Ce3Ca;
- exploitation age: 60 years;
- production class: III;
- vitality: normal;
- consistency: k = 0.7;
- growth: i_v = 1.2 m^3/ha/year.

After processing the recorded data related to the statistical-mathematical inventories, the total number of trees by species and diameter categories was obtained, for the cumulative area of the 14 experimental plots, respectively 0.42 ha. Subsequently, these results were extrapolated to the hectare (tables 1-8), in order to be able to compare them with the corresponding theoretical values from the production tables (Giurgiu et al., 1972).

Table 1. Number of trees registered on the experimental plots, and per hectare, for the *Quercus cerris* species

Crt. No.	D (cm)	Number of trees	
		pieces/0.42 ha	pieces/1.00 ha
1	4	64	152
2	6	113	269
3	8	117	279
4	10	77	184
5	12	54	128
6	14	43	102
7	16	17	41
8	18	13	32
9	20	9	21
10	22	5	13
11	**Total**	**513**	**1221**

Table 2. Number of trees registered on the experimental plots, for the *Larix decidua* species (La)

Crt. No.	D (cm)	Number of trees	
		pieces/0.42 ha	pieces/1.00 ha
1	4	8	18
2	6	16	39
3	8	12	29
4	10	6	15
5	**Total**	**42**	**101**

Table 3. Number of trees registered on the experimental plots, and per hectare, for the *Carpinus betulus* (Ca) species

Crt. No.	D(cm)	Number of trees	
		pieces/0.42 ha	pieces/1.00 ha
1	4	16	39
2	6	30	71
3	8	33	79
4	10	25	59
5	12	8	20
6	14	7	17
7	16	7	16
8	18	6	15
9	**Total**	**133**	**316**

Table 4. Number of trees registered on experimental areas, and per hectare, for the species *Fagus sylvatica*

Crt. No.	D (cm)	Number of trees	
		pieces/0.42 ha	pieces/1.00 ha
1	4	3	7
2	6	9	22
3	8	5	11
4	**Total**	**17**	**40**

Table 5. Number of trees registered on the experimental plots, for the *Quercus petraea* (Go) species

Crt. No.	D (cm)	Number of trees	
		pieces/0.42 ha	pieces/1.00 ha
1	4	13	31
2	6	17	41
3	8	22	52
4	10	10	24
5	12	8	19
6	**Total**	**70**	**167**

Table 6. Number of trees infested on the experimental plots, for the *Betula pendula* (Me) species

Crt. No.	D (cm)	Number of trees	
		pieces/0.42 ha	pieces/0.42 ha
1	4	5	11
2	6	17	40
3	8	20	47
4	10	15	35
5	12	9	21
6	14	7	17
7	**Total**	**72**	**171**

Table 7. Number of trees registered on the experimental plots, for the *Populus tremula* (Plt) species

Crt. No.	D(cm)	Number of trees	
		pieces/0.42 ha	pieces/0.42 ha
1	4	6	15
2	6	9	22
3	8	8	18
4	10	7	16
5	**Total**	**30**	**71**

The biogroups with turkey oak trees, which have regenerated naturally, are characterized by growth dynamics and active development, displaying lateral branches on the trunk at heights of 1.3-1.5 m (Figure 3).



Figure 3. Biogroup of trees of the Turkey oak species in the stand in compartment 60

From the analysis of the image in Figure 4, it can be seen that the biogroup formed by trees of the beech, hornbeam and larch species is relatively spaced, with shrub and subshrub vegetation also present between them.
From the field analysis conducted during the observations, it was found that the biogroup formed by trees of beech, sessile oak, and larch is spaced at a distance of approximately 4 meters, with shrub and subshrub vegetation present in the space between them, as shown in the image in Figure 4.



Figure 4. Biogroup of trees of larch, beech and hornbeam species in the stand in compartment 60

Specimens of hornbeam have naturally regenerated from seed and have established themselves in areas where the seedlings that were planted have dried out or were destroyed (damaged) by hunting species (Figure 5).



Figure 5. Biogroup of hornbeam trees in the stand in compartment 60



Figure 6. Specimens of the *Fagus sylvatica* species, which were planted in compartment 60 in 2013

From the analysis of the images in Figures 3 and 7, it can be seen that in biogroups with trees of the turkey oak and sessile oak species, artificial pruning must be carried out to remove branches from the base of the crown, to form a superior quality trunk (Florescu & Nicolescu, 1998).



Figure 7. Specimens of the *Quercus petraea* species, which were planted in compartment 60 in 2013



Figure 8. Specimens of the species Aspen - *Populus tremula* (Plt), naturally regenerated, in the stand in compartment 60

Also, the distribution of the number of trees by species and diameter categories per hectare was carried out for the stand in compartment 60 (Figure 9). Next, with the values of the number of trees by species and diameter categories, which were determined by extrapolation to the surface unit (ha), the unitary base area -g_i, multiple by diameter categories - $n_i \cdot g_i$, and cumulated per hectare was determined - $G = \Sigma n_i \cdot g_i$, for each species, and cumulated per hectare, for all species, with formulae 11, 12 and 13.

$$g_i = \frac{\pi \times d_i^2}{4} = 0{,}7854 \times d_i^2 \quad (11)$$

$n_i \cdot g_i$ (m^2) (12)

$\Sigma n_i \cdot g_i$ (m^2/ha) (13)

To determine the volume per species and respectively per stand, per hectare, the central diameter corresponding to the base area dgM was determined for each species, with formula 14.

$$d_{gM} = d_M + \frac{C \times \left(\frac{\sum gn}{2} - S_M \right)}{(gn)_M} \quad (14)$$

dg_M - central diameter of the base area;

d_M - lower limit of the median interval;

C - size of the interval between diameter categories;

$\Sigma n_i \cdot g_i$ - total (cumulative) base area;

S_M - cumulated base area up to the median interval;

$(gn)_M$ - the multiple base area of the median interval.

In the next step, the average height corresponding to the central diameter of the base surface was calculated - h_{dgM}, as the arithmetic mean of a number of 10-15 heights corresponding to trees with a base diameter $d_{1.30}$ equal to d_{gM} ± a diameter category, the case of the present study ± 2 cm.

The volume was determined by the volume series method, in accordance with the Biometrics of trees and stands in Romania (Giurgiu, Decei & Armăşescu, 1972). The experimental values, related to the base area and volume per species, reported per hectare, are presented synthetically in Tables 9 and 10.

Table 8. Record of the number of trees by diameter and species categories, per hectare, in the compartment 60

Crt. No.	D (cm)	Species (pieces/ha)							**N_{Stand}
		*N_{Ce}	N_{Ca}	N_{Fa}	N_{Go}	N_{La}	N_{Plt}	N_{Me}	(pieces/ha)
1	4	152	39	7	31	18	15	11	**273**
2	6	269	71	22	41	39	22	40	**504**
3	8	279	79	11	52	29	18	47	**515**
4	10	184	59	0	24	15	16	35	**333**
5	12	128	20	0	19	0	0	21	**188**
6	14	102	17	0	0	0	0	17	**136**
7	16	41	16	0	0	0	0	0	**57**
8	18	32	15	0	0	0	0	0	**47**
9	20	21	0	0	0	0	0	0	**21**
10	22	13	0	0	0	0	0	0	**13**
11	**Total**	**1221**	**316**	**40**	**167**	**101**	**71**	**171**	**2087**

*N_{Ce} - Total number of Turkey oak trees (*Quercus ceris*), per hectare.

**N_{Stand} - Total number of trees per hectare.

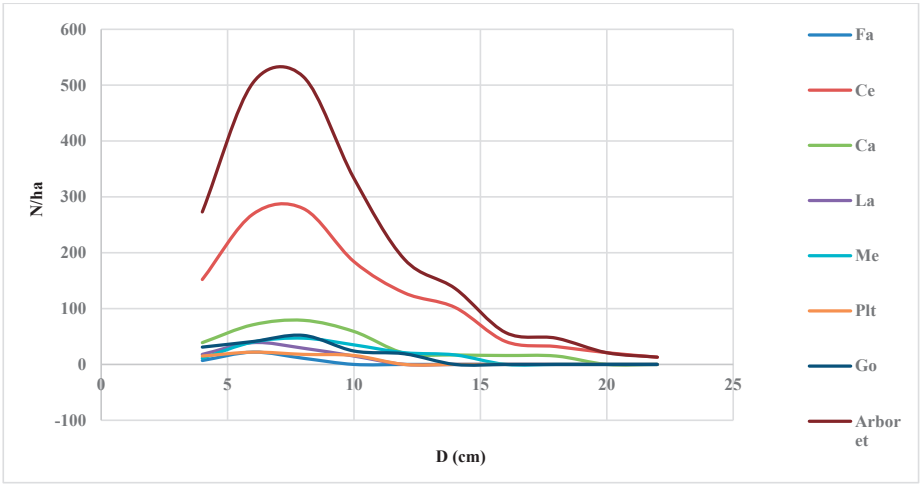


Figure 9. Distribution of the number of trees by diameter categories, per hectare, in the stand in compartment 60

Table 9. Evidence of multiple and cumulative base area, by species and by stand, per hectare, in the stand in compartment 60

Crt. No.	D (cm)	G (m^2./ha)							G_{Stand} (m^2./ha)
		$n_i \times g_{iCe}$	$n_i \times g_{iCa}$	$n_i \times g_{iFa}$	$n_i \times g_{iGo}$	$n_i \times g_{iLa}$	$n_i \times g_{iPlt}$	$n_i \times g_{iMe}$	
1	4	0,191	0,049	0,009	0,039	0,023	0,019	0,014	0,344
2	6	0,761	0,201	0,062	0,116	0,110	0,062	0,113	1,425
3	8	1,402	0,397	0,055	0,261	0,146	0,090	0,236	2,587
4	10	1,445	0,463	0,000	0,188	0,118	0,126	**0,275**	2,615
5	12	1,448	0,226	0,000	0,215	0,000	0,000	0,238	2,127
6	14	1,570	0,262	0,000	0,000	0,000	0,000	0,262	2,094
7	16	0,824	0,322	0,000	0,000	0,000	0,000	0,000	1,146
8	18	0,814	0,382	0,000	0,000	0,000	0,000	0,000	1,196
9	20	0,660	0,000	0,000	0,000	0,000	0,000	0,000	0,66
10	22	0,494	0,000	0,000	0,000	0,000	0,000	0,000	0,494
11	**Total**	***G_{Ce} = 9,610**	**G_{Ca} = 2,302**	**G_{Fa} = 0,126**	**G_{Go} = 0,820**	**G_{La} = 0,396**	**G_{Plt} = 0,297**	**G_{Me} = 1,137**	**G_{Stand} =14,688**

*G_{Ce} - the cumulative base area for the Turkey oak species (*Quercus cerris*).
**G_{Stand} - Cumulative base area of the stand.

Table 10. Volume records by species and by stand, per hectare, in the stand in compartment 60

Crt. No.	D(cm)	V (m^3./ha)							***V_{Stand} (m^3/ha)
		*$n_i \times Vu_{Ce}$	$n_i \times Vu_{Ca}$	$n_i \times Vu_{Fa}$	$n_i \times Vu_{Go}$	$n_i \times Vu_{La}$	$n_i \times Vu_{Plt}$	$n_i \times Vu_{Me}$	
1	6	2.421	0.639	0.154	0.41	0.273	0.198	0.28	4.375
2	8	5.301	1.422	0.165	1.04	0.406	0.324	0.705	9.363
3	10	6.072	1.829	-	0.816	0.36	0.496	0.875	10.448
4	12	6.400	0.96	-	1.007	-	-	0.84	9.207
5	14	7.446	1,173	-	-	-	-	0.969	9.588
6	16	4.059	1.488	-	-	-	-	-	5.547
7	18	4.128	1.800	-	-	-	-	-	5.928
8	20	3.423	-	-	-	-	-	-	3.423
9	22	2.600	-	-	-	-	-	-	2.600
10	**Total**	**V_{Ce}= 41,850**	**V_{Ca} = 9,311**	**V_{Fa} = 0,319**	**V_{Go} =3,273**	**V_{La} =1,039**	**V_{Plt} =1,018**	**V_{Me}=3,669**	**V_{Stand} = 60,479**

*$n_i \times Vu_{Ce}$- Multiple volume per diameter category for the Turkey oak species;
**V_{Ce}- Total volume for the Turkey oak species;
***V_{Stand} - total volume of the stand.

From the analysis of the data presented in Table 8, it is found that the total number of trees per hectare is N_{Crop} = 2087 pieces. Total base area of the stand per hectare G_{Crop} = 14,688 m^2/ha (table 9), and the total volume per hectare V_{Crop} = 60,479 m^3/ha (Table 10).

The composition of the stands was determined based on: the number of trees per hectare Cpz_N; the base area per hectare Cpz_G and the volume per hectare Cpz_V (table 11, figures 10-12). From the analysis of the elements presented in table 11 and in figures 10-12, it is found that it differs significantly, being influenced by the method of determination and respectively by the values of the dendrometric elements used.

Table 11. Evidence of structural characteristics related to the stand in compartment 60

Crt. No.	The values of the structural characteristics indices determined for the stand in compartment 60								
	*Cpz (N)	**Cpz (G)	***Cpz (V)	I_N	I_G	I_V	Z (%)	S_4 (%)	S_6 (%)
1	6Ce2Ca1Go1Me dis Fa La Plt	6Ce2Ca1Go1Me dis Fa La Plt	7Ce1Ca1Me1Go, dis La Plt Fa	0.7	1.2	1.1	67	26	28

*Cpz(N) - Composition depending on the number of trees;
**Cpz(G) - Composition depending on the base surface;
Cpz(V) - Composition by volume.

The Turkey oak, although not artificially regenerated, has the highest share, respectively 70%, the pioneer and secondary mixed species, represented by birch, aspen and hornbeam have a share of 20%, and the species sessile oak, beech, larch, bird cherry and ash, which were artificially introduced through plantations, account for 10% of the current composition of the stand.

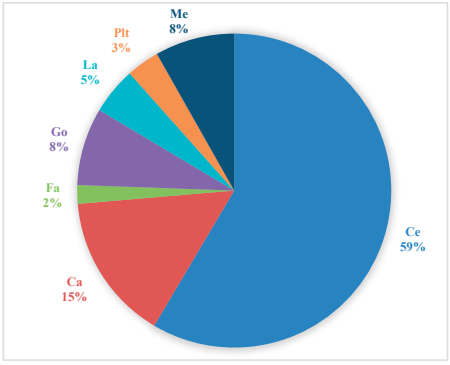


Figure 10. Composition of the stand in compartment 60, depending on the number of trees per hectare

The presence of oak species in a high proportion confirms the current trend of expanding their area in western Romania, an aspect also confirmed by a series of recent research (Pană et al., 2020)

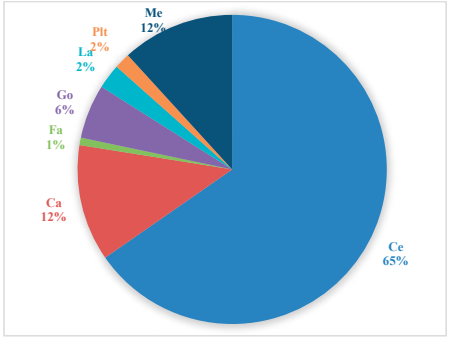


Figure 11. Composition of the stand in compartment 60, depending on the base area per hectare

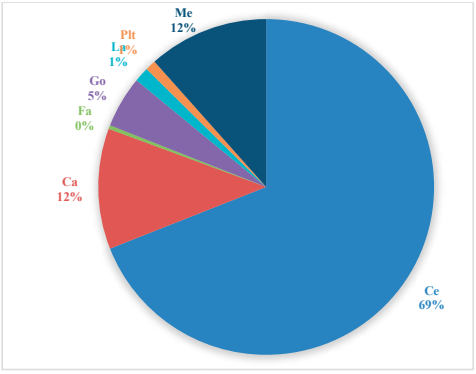


Figure 12. Composition of the stand in compartment 60, depending on the volume per hectare

The Hart-Beking spacing indices determined in the two variants have the values $s_{4\%}$ = 26% and $s_{6\%}$ = 28% (Table 11). Consequently, the stand is optimally spaced, and presents consolidated ecosystem stability.

The stand density index is sub-unit, and the density indices have super-unit values, in the range 1.1-1.2 (Table 11).

The value of the slenderness coefficient $Z_{\%}$ = 67% (Table 11) indicates that the stand is relatively stable to a possible destabilizing action of the prevailing winds, which blow in the studied area.

The complete destruction of biogroups of specimens of the species hornbeam, beech, larch, bird cherry and ash, on a cumulative area of approximately 1.8 ha, by deer and wild boar herds, determined a specific dynamics of the composition of the stand. As a result, grassy and shrubby vegetation has settled on this surface. The identified shrub species are represented by the mountain blackberry - *Rubus fruticosus* L., rosehip - *Rosa canina*, blackthorn - *Prunus spinosa*, hawthorn - *Crataegus monogyna Jacq.*, wild privet - *Ligustrum vulgare* L., dogwood - *Cornus sanguinea*, common spindle - *Eonimus europaeus*, warted spindle - *Euonymus verrucosus Scop.*

Also, a relatively small number of bird cherry, wild apple and wild pear specimens were identified, especially in areas with a lower stand stocking. Consequently, it is necessary to complete it with specific works, in preparation for the artificial installation of regeneration.

The data recorded on the field necessary for simulating the spatial structure of the stand Table 12 were processed with the PROARB 2.0 program, and the models for the stand structure in horizontal and vertical planes (Figures 13 and 16) and 3D perspective (Figures 14 and 15) were obtained for the two experimental plots.

In the parts where the stand has regenerated naturally, its spatial structure presents characteristics specific to the tree biogroups that have been integrated into the stand. As a result, some of the trees of the Turkey oak species present crowns with a large diameter, relatively low height and branches on a large portion of the trunk (Crainic, 2017, Fazal, Geertman & Toppen, 2012). Some of these branches dried up due to the fact that the massif state was achieved, and implicitly the natural pruning process was triggered.

The presence of biogroups of pioneering tree species, common poplar and birch, was found in the areas where the basic species did not naturally settle, which present a very active vegetation state (Dorog & Crainic, 2015).

In the areas where vegetation has been established artificially, by planting bare-rooted seedlings (Crainic et al., 2023) the tree stand has a relatively regular structure. In the areas where the seedlings planted in 2013 were affected, their completion was achieved in the following years, also through plantations. As a result, some differences were noticed between their main dendrometric characteristics, during the inventory and processing of the recorded data. A series of specimens of the larch species were identified, which were torn apart by specimens of the cervid species.

Table 12. Inventory of elements related to the simulation of the spatial structure of the stand in compartment 60, in the area with artificial regeneration

Crt. No.	X (m)	Y (m)	Dc1 (m)	Dc2 (m)	H (m)	He (m)	Species	No. of trees
1	0,5	0,5	3,124	3,552	5,625	2,438	La	25
2	4,5	0,5	3,142	3,811	5,353	2,951	Go	Slope
3	8,5	0,5	3,521	3,716	4,659	2,391	Fa	15
4	12,5	0,5	3,153	3,088	5,381	2,455	Go	Profile length
5	15,5	0,5	3,181	3,322	5,526	2,449	La	20
6	19,4	0,5	3,838	3,771	6,005	2,659	Go	Profile width
7	0,5	4,5	3,853	3,992	6,383	2,338	Ce	15
8	4,5	4,5	3,589	3,229	6,21	3,185	Ce	
9	8,5	4,5	3,151	3,229	6,181	2,485	Go	
10	12,5	4,5	3,165	3,552	5,708	2,779	Go	
11	16,5	4,5	3,117	3,772	5,364	3,441	Go	
12	19,8	4,5	3,658	3,732	5,449	2,665	Go	
13	0,5	8,5	3,151	3,822	5,852	2,554	La	
14	4,5	8,5	3,138	3,348	5,287	2,616	La	
15	8,5	8,5	3,023	3,322	6,31	4,144	Ca	
16	13,5	8,5	3,721	3,844	5,82	3,651	La	
17	16,5	8,5	3,156	3,003	5,15	2,564	Fa	
18	10,4	8,5	2,858	2,766	5,859	2,576	La	
19	0,5	12,5	3,921	2,891	5,294	3,332	La	
20	4,5	12,5	2,754	2,438	5,196	3,516	La	
21	8,5	12,5	3,821	2,885	5,664	3,85	Ca	
22	15,562	12,5	2,933	2,722	5,509	3,43	Fa	
23	18,559	12,5	2,858	2,971	5,845	2,609	Fa	
24	12,329	12,5	3,033	3,338	5,123	2,443	La	
25	19,551	10,007	2,571	2,682	6,122	3,881	Ci	

In the areas where the stand has regenerated naturally, its spatial structure presents characteristics specific to the tree biogroups that have been integrated into the stand (Figure 13).

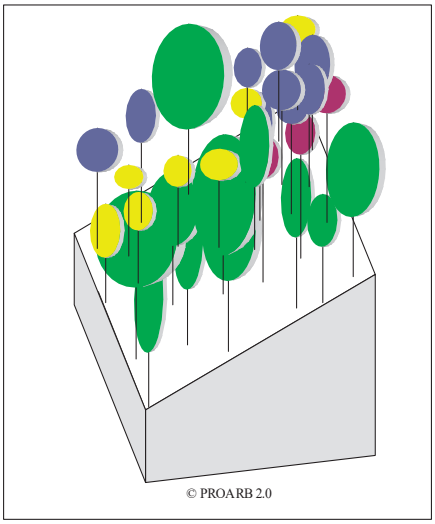


Figure 13. Horizontal and vertical structure of the stand in compartment 60, created with the PROARB 2.0 program, in the area with natural regeneration

In areas where the stand has been artificially regenerated, the stand has a relatively homogeneous spatial structure (Figure 14).

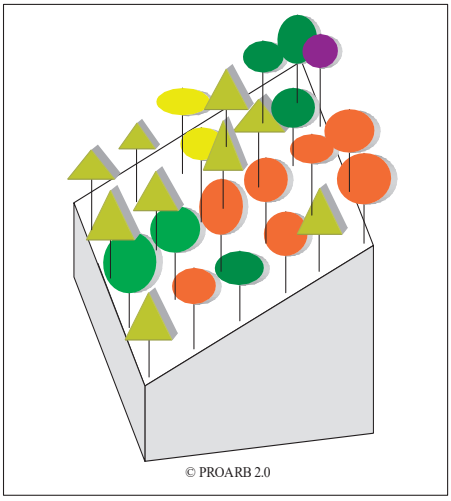


Figure 14. Horizontal and vertical structure of the stand in compartment 60, created with the PROARB 2.0 program, in the area with natural regeneration

The analysis and comparison of the obtained results substantiated (based on experimental and theoretical data) the conclusions regarding the structure and stability of the studied stand, as well as the specifics of the forest management practices necessary for the current phase of development.

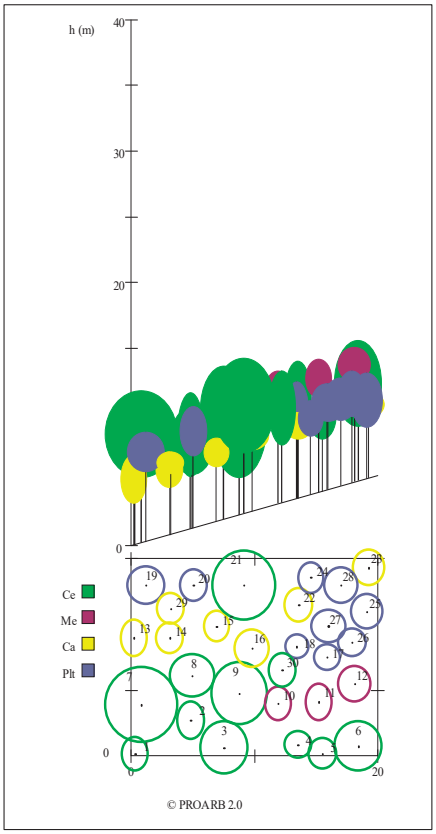


Figure 15. Perspective structure of the arboretum in compartment 60, created with the PROARB 2.0 program, in the area with natural regeneration

CONCLUSIONS

From the analysis of the results obtained by processing experimental data and field observations, it is observed that the stand in compartment 60, which was obtained by transforming an agrosilvopastoral system, presents a series of specific structural features, which are influenced
by the regeneration method and the works carried out over the 12 years.
The age difference between the biogroups of trees that were integrated into the stand and the seedlings that were planted, respectively, determined a series of differences in the homogeneity

of the dendrometric elements, an aspect that favored the outline of a relatively even structure.

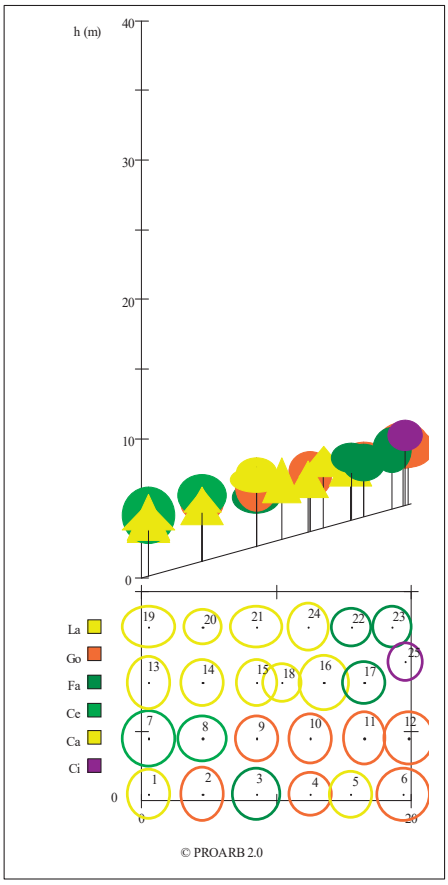


Figure 16. Perspective structure of the stand in compartment 60, created with the PROARB 2.0 program, in the area with artificial regeneration

For the optimal management of the stand in compartment 60, a series of works specific to the development stage and its structural particularities are necessary.

Consequently, on the areas where regeneration was destroyed by game animals, it is necessary to install forest vegetation through direct sowing, with acorns from native oak species, and with ash from neighboring beech stands.

The necessary forestry works are represented by clearings, artificial pruning and special hygiene works, to maintain an appropriate phytosanitary condition.

Adopting an exploitable age of 60 years ensures the conditions for promoting a future stable stand, which will build the fundamental natural type of forest through composition and structure.

The fencing of the perimeter of compartment 60 for a certain period of time is necessary to ensure its protection against fauna of hunting interest.

ACKNOWLEDGEMENTS

The case study was carried out with the support of the management and field staff of the Codrii Cămării Forest District R. A. Dobreşti, Bihor County. We also collaborated with the operator S.C. ECOPROD FOREST S.R.L., which carried out the works of installing forest vegetation and the respective transformation and introduction of the agrosilvopastoral system in the forest fund.

REFERENCES

Abrudan, I.V., Blujdea,V., Brown, S., Kostyushin, V., Pahonţu, C., Phillips, H., & Voicu, M. (2003). Prototype carbon fund: Afforestation of degraded agricultural land in Romania. *Forestry Journal*, No.1, Yar 118., 5-17.

Crainic, G. C., Curilă, M., Curilă S., Oneţ, C., Supuran, A., Bica, A. M., Şerban, E., Cioflan, F. M., Irimie, F. (2024). Establishing the intensity of interventions in young beech (*Fagus sylvatica* L.) stands based on a spline regression mathematical model. *Not Bot Horti Agrobo*, 52(4):14021. Retrieved January 25, 2025DOI: 10.15835/nbha/52414021.

Crainic, G. C., Covaci, F., Sabău, N.C., Sicoe, S. I,, Irimie (Cioflan), F., Bodog, M. F. & Iovan, C.I. (2023). Works specific to the introduction of silvopastoral systems in the forest fund. *Scientific Papers. Series E. Land Reclamation, Earth Observation & Surveying, Environmental Engineering*. Vol. XII, 2023 Print ISSN 2285-6064, CD-ROM ISSN 2285-6072, Online ISSN 2393-5138, ISSN-L 2285-6064.

Crainic, G.C. (2017). Specific Works For The Maintenance Of Agroforestry Systems. *Annals of the University of Oradea, Environmental Protection Fascicle*, Vol. XXIX, 2017, ISSN - 1224-6255, 153-164.

Crainic, G.C., & Stamate, G. (2009). Agro-Forestry Systems - a Possible Valorization Alternative of the Forestry Potential in Some Extreme Sites. *Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Horticulture.* Vol. 66, No 1, ISSN - 1843-5394, 572-577.

Crainic, G.C., & Tăut, F.C. (2008). Aspects referring to the thickness and spacing of some beech and oak forests at the Production Unit III In Varciorog, Forest District Dobresti, Oradea Forest Office. *Annals of the University of Oradea, Fascicle: Environmental Protection*, 13(13):253-260. ISSN 1224-6255. Retrieved January 25, 2025, from

http://protmed.uoradea.ro/english/faculty/annals/environmental_protection/2008/2008.html.

Cubbage, F., Balmelli, G., Bussoni, A., Noellemeyer, E., Pachas, A.N., Fassola, H., Colcombet, L., Rossner, B., Frey, G., Dube, F., Lopes de Silva, M., Stevenson, H., Hamilton, J., & Hubbard, W. (2012). Comparing silvopastoral systems and prospects in eight regions of the world. *Springer Science+Business Media B.V., Agroforest Syst*, 2012, DOI 10.1007/s10457-012-9482-z, 86:303 - 314.

Dorog, L.S., & Crainic, G.C. (2015). Shrubs and trees regeneration in silvopastorale systems in the counties of bihor and arad. *Jurnal of Agriculture Science*. Vol. 47 Issue 4 University of Agricultural Sciences and Veterinary Medicine of the Banat, Timişoara. Retrieved January 25, 2025, from https://www.cabdirect.org/cabdirect/abstract/20163022043, 64-69.

Fazal, S., Geertman, S., & Toppen, F. (2012). Interpretation of Trends in Land Transformations - A Case of Green Heart Region (The Netherlands). *Natural Resources*, Vol. 3 No. 3, 107-117. doi: 10.4236/nr.2012.33015.

Florescu, I.I., Nicolescu, N.V. (1998). *Silviculture, Silvotechnics, Vol. II*. Braşov, RO: Transilvania University of Braşov Publishing House.

Florescu, I.I., & Nicolescu, N.V. (1996). *Forestry, Vol. I, Forest Study*. Braşov, RO: Lux-Librix Publishing House.

Giurgiu, V, Decei, I., & Armăşescu, S. (1972). *Biometrics of trees and stands in Romania, Tables for the statistical inventory of trees*. Bucharest, RO CERES Publishing House.

Nicolescu, N.V. (2009). *Forestry*. Braşov, RO: Aldus Publishing House.

Nair Ramachandran, P.K. (1993). *An Introduction to Agroforestry*. Dordrecht/Boston/London in Cooperation with International Centre for Research in Agroforestry, Academic Publishers.

Nicolescu, N. (1995). *Silviculture, Guide to practical works*. Braşov, RO: Transilvania University of Braşov Publishing House.

Popa, I. (1999). Useful computer applications in forestry research. The Carota Program and the Proarb Program. *Forestry Journal*, 2:41-42.

Stănescu, V., Şofletea, N., Popescu O. (1997). *The woody forest flora of Romania*. Bucharest, RO: Ceres Publishing House.

****Forest management of Production Unit I Dobreşti, Bihor County*. (2018).

****Forestry map of Production Unit I Dobreşti*, Bihor County. (2018).

(https://ro.wikipedia.org/wiki/Dobre%C8%99ti,_Bihor, https://pe-harta.ro/bihor/.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

OXALIS ACETOSSELA IN FORESTS. A SYSTEMATIC BIBLIOMETRIC STUDY OVER THE LAST 47 YEARS

Lucian DINCĂ[1], Vlad CRIȘAN[1], Gabriel MURARIU[2], Eliza ȚUPU[3]

[1]National Institute for Research and Development in Forestry
"Marin Drăcea", 128 Eroilor Street, 077190, Voluntari, Ilfov, Romania
[2]"Dunărea de Jos" University of Galați, Faculty of Sciences and Environmental,
Department of Chemistry, Physics and Environment,
47 Domnească Street, 800008, Galați, Romania
[3]"Răsvan Angheluță" Natural Sciences Museum Complex Galați,
11 Regimentul Street, 800340, Galați, Romania

Corresponding author email: vlad_crsn@yahoo.com

Abstract

Oxalis acetosella (wood sorrel) is an herbaceous plant commonly found in beech and spruce forests, indicating fertile areas. By analyzing articles in Web of Science and using VOS viewer, Excel, and Geochart, 91 publications from 1976 to 2023 were reviewed. These articles span 19 research areas, with the highest numbers in Environmental Sciences-Ecology, Plant Sciences, Forestry, and Biodiversity Conservation. Around 2-3 articles have been published annually, with a peak of seven articles in 1998. The studies feature authors from 27 countries, primarily from Sweden, Germany, and the USA, with affiliations mostly at Lund University, Swedish University of Agricultural Sciences, and the University of Tartu. Articles were published in 61 journals, with Forest Ecology and Management, Ecography, and Vegetatio being the most representative. Frequently used key words include "vegetation", "Oxalis acetosella", "growth", "dynamics" and "plants". The evolution of key words shows a shift from "beech forest", "nitrogen deposition", and "Oxalis acetosella" to terms that reflect the effects of various factors on wood sorrel, such as "impact", "disturbance", "dynamics", and "diversity".

Key words*: topic, articles, wood sorrel, key words, journals.*

INTRODUCTION

Oxalis acetosella L. (wood sorrel) is an herbaceous species commonly found in the understory of northern hardwood forests (Tessier et al., 2001). It begins producing seeds earlier in its life cycle compared to many other herbaceous plants (Bierzychudek, 1982). This long-lived herb exhibits extensive rhizomatous growth and is primarily found in relatively undisturbed forest understories, where it can reach very high densities (Shorina, 1985; Grime et al., 1988; Berg, 2002; Murariu et al., 2021).
Oxalis acetosella thrives in soils with high nutrient levels but relies heavily on the shelter provided by the tree canopy (Spârchez et al., 2017; Crișan et al., 2024). It is a typical forb in mesic and fertile upland forest sites from southern Finland (Kuusipalo, 1987). Its presence is often considered a reliable indicator of forest stand fertility (Kuusipalo, 1985). The plant requires substantial nutrients from the soil substrate (Dincă et al., 2014) and serves as a dependable predictor of site fertility (Dincă et al., 2020; Crișan et al., 2023). Additionally, it is highly shade-tolerant, surviving under dense canopy shading that many other forest species cannot withstand (Packham & Willis, 1977).
Review articles have been written about different plants from the forest floor (Wright, 2002; Hermy & Verheyen, 2007; Schulze et al., 2016), but there is only one article about *Oxalis acetosella*. This synthesis article refers to changes in the occurrence and abundance of plant species in a Norwegian boreal coniferous forest, 1988-1993, where several plant species with preference for richer sites have been observed. Most notably, an *Oxalis acetosella* decrease during this period.
There are also numerous bibliometric studies in domains such ash Environment (Ho, 2007; Chen et al., 2009; Borrett et al., 2018: Ruiz-Real et al., 2018) and Forestry (Polinko & Coupland, 2021; Sullivan, 2022; Fernandeset al., 2022; Van

Winkle et al., 2022; Santillán-Fernández et al., 2023).

MATERIALS AND METHODS

A bibliometric analysis was conducted to examine the global scientific research on indicator plants in mountain forests between 1980 and 2023. This study utilised the Science Citation Index Expanded (SCI-Expanded) from the Web of Science database, identifying 716 publications. Data were obtained from the online SCI-Expanded platform, a multidiscciplinary resource managed by the Institute for Scientific Information (ISI) in Philadelphia, PA, USA.

For more than 40 years, ISI - now part of Thomson Reuters - has provided exclusive bibliographic resources, enabling large-scale development of bibliometric indicators. These citation indexes, now integrated into the Web of Science, remain primary sources for bibliometric data.

The database was searched using the term "*Oxalis acetosella* in forests" to compile relevant publications from 1996 onward.

The analysis focused on ten areas: (1) publiccation types, (2) Web of Science categories, (3) publication years, (4) countries, (5) institutions, (6) languages, (7) journals, (8) publishers, (9) authors, and (10) keywords.

Data processing employed Web of Science Core Collection tools [128], Excel [129], Geochart [130], and VOSviewer version 1.6.20 [51], which facilitates cluster analysis and visualisation. Only research articles and reviews were included in the study.

RESULTS AND DISCUSSIONS

A total of 98 publications were identified and analysed. Of these, 91 are articles (representing 93% of total publications), 5 are proceeding papers (5%) and 2 are review articles (2%), (Figure 1).

The published articles can be divided in 19 research areas, with the most ones being: Environmental Sciences Ecology (46 articles), Plant sciences (35 articles), Forestry (27 articles) and Biodiversity conservation (9 articles) (Figure 2).

The first article on this topic was published in a renowned scientific journal in 1976. In general, two-three articles were published each year, with the largest number of articles (7 articles) published in 1998 (Figure 3).

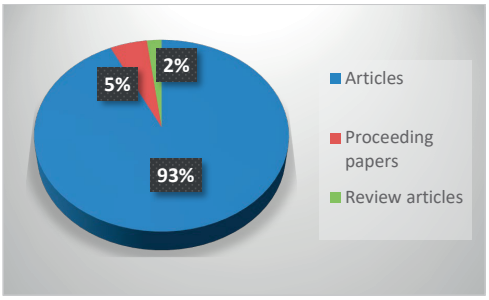


Figure 1. Distribution of the main types of publications used in the bibliometric analysis.

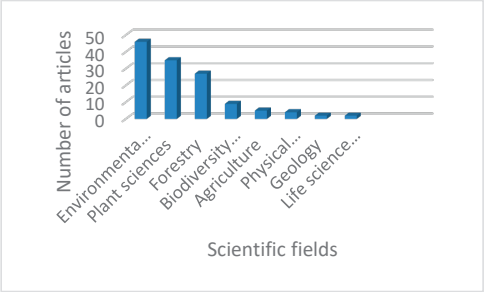


Figure 2. Distribution of the main 10 scientific fields of publications used in the bibliometric analysis.

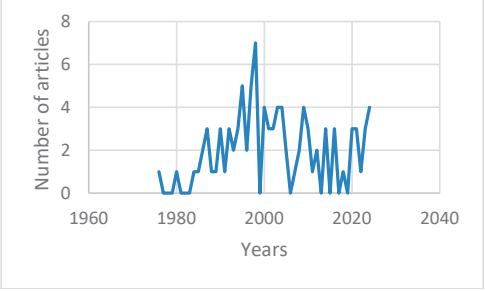


Figure 3. Distribution of articles published per year.

Over 200 authors have published articles on this topic, with the most articles written by Ursula Falkengren-Grerup (9 articles), Jörg Brunet and G. Tyler (each with 8 articles) Hermann Rodenkirchen (7 articles) and Kris Verheyen (6 articles).

The published articles have authors from 27 countries, from three continents, with the following hierarchy based on the largest number of articles: Sweden (23 articles), Germany (18

articles), USA (13 articles), Belgium and Estonia (each with 8 articles), Czech Republic and France (each with 6 articles) (Figure 4).

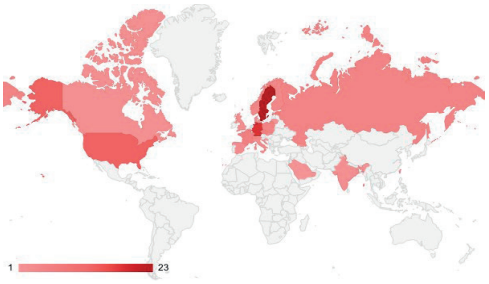


Figure 4. Geography of the use of *Oxalis acetosella* in forests in published articles

The links between the countries with authors that have published about this subject show that there are 3 clusters that contain at least 5 countries. These are: cluster 1 (Belgium, Estonia, France, Germany, Sweden, Taiwan); cluster 2 (Czech Republic, Georgia, India, Italy, Saudi Arabia); cluster 3 (Finland, Norway, Poland, Spain, Switzerland) (Figure 5).



Figure 5. Countries with authors of articles on *Oxalis acetosella* in forests. The node size and thickness of the connecting lines are proportional to the number of documents assigned to each country. The connections represent the collaboration network among countries

The institutions where the authors that have published articles on this subject work include a top tier represented by institutions from Sweden: (Lund University - with 12 published articles and Swedish University of Agricultural Sciences - with 9 articles) and Estonia (University of Tartu - with 8 articles and Tartu University Institute of Ecology Earth Science - with 8 articles). They are followed by the State University of New York Suny System and University of Munich (each with 7 articles). This aspect is in accordance with the hierarchy of countries from where authors originated, where Sweden occupies the first place, Estonia the fourth place, USA the third place and Germany the second place.

Articles on this topic are published in 61 journals. Based on the number of published articles, the most representative journals are Forest Ecology and Management (11 articles), Journal of the Torrey Botanical Society (4 articles) and Ecography and Vegetation (each with 3 articles). Based on total link strength, the most important journals are Forest Ecology and Management, Ecography and Vegetation (Table 1).

Table 1. The most representative journals where articles about *Oxalis acetosella* in forests have been published.

Crt. No.	Journal	Documents	Citations	Total link strength
1	Forest Ecology and Management	11	348	20
2	Ecography	3	118	15
3	Vegetatio	3	86	15
4	American Journal of Botany	1	7	14
5	Journal of the Torrey Botanical Society	4	117	12
6	Oecologia	3	311	12
7	Nordic Journal of Botany	2	51	11
8	Acta Oecologica-International Journal	3	43	10
9	Flora	3	40	9
10	Plant and Soil	3	36	7
11	Environmental and Experimental Botany	2	35	5
12	Journal of Ecology	2	126	5
13	Landscape Ecology	2	12	5

We have identified 7 clusters that contain at least 3 journals. The first cluster, containing 6 journals, includes: Biological conservation; Environmental and Experimental Botany;

Journal of Applied Ecology; Journal of Ecology; Nordic Journal of Botany; Water air and soil pollution. The second cluster, with 5 journals contains: American Journal of Botany; Botany-Botanique; Environmental pollution; Forest Ecology and Management; Journal of vegetation science (Figure 6).

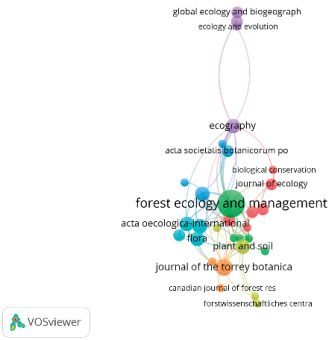


Figure 6. Main journals where articles on *Oxalis acetosella* in forests have been published. The node size and thickness of the connecting lines are proportional to the number of documents assigned to each country. The connections represent the collaboration network among journals

There are 32 publishers, with the most prominent being Elsevier (26 articles), Wiley (16 articles) and Springer Nature (15 articles).
The most frequently used key words are vegetation, *Oxalis-acetosella* (if we consider the two forms in which the term is used: *Oxalis-acetosella* and *Oxalis-acetosella*), growth, dynamics and plants (Table 2).
Amongst the key words with a high frequency, we can also note the ones regarding the forest types in which the plant appears (deciduous forest, beech forest, *Picea abies*). As other authors have also noted, wood sorrel is characteristic for beech forests (Kutnar et al., 2002; Godefroid et al., 2005; Ujházy et al., 2007) as well as for spruce forests (Dinca et al., 2022; Kikkeva et al., 2024; Matuszkiewicz et al., 2024).

Table 2. The most commonly used keywords in articles about the *Oxalis acetosella* in forests

Crt. No.	Key word	Occurrences	Total link strength
1	vegetation	28	88
2	Growth	13	45
3	Dynamics	13	42
4	deciduous forest	10	40
5	disturbance	9	38
6	Soil	9	35
7	plants	11	33
8	forest	10	32
9	*Oxalis acetosella*	10	29
10	diversity	7	24
11	vascular plants	7	24
12	communities	7	23
13	*Oxalis-acetosella*	6	23
14	beech forest	5	21
15	nitrogen	4	20
16	nitrogen deposition	7	20
17	*Picea abies*	4	20
18	understorey vegetation	3	17

We have identified 7 clusters for grouping key words (with more than 5 key words). Among them, two have more than 10 key words. The first contains the following key words: Catskill mountains, climate, deciduous forest, disturbance, field layer, herb layer, Northern hardwood forest, *Oxalis* acetosella, succession, understory and woodland; while the second one includes: abundance, bryophytes, deposition, dynamics, ground vegetation, light, nitrogen, *Picea abies*, understorey vegetation and vascular plants (Figure 7).

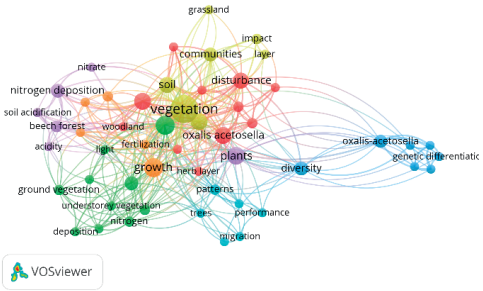


Figure 7. Authors' key words regarding *Oxalis acetosella* in forests. The node size and thickness of the connecting lines are proportional to the number of documents assigned to each country. The connections represent the collaboration network among key words

If we analyse the usage of key words over years, we can see that in the beginning (1994-2000), key words such as beech forest, nitrogen deposition and pH were mainly used. Then, during 2000-2004, the main key words were *Oxalis acetosella*, soil, deciduous forest, growth, vegetation; followed by communities,

dynamics, disturbance, nitrogen during 2005-2008, while impact and diversity were mainly used during the last period (2006-2012). From this aspect, we can see that the last two periods have used key words that pertain to the results of different factors on wood sorrel: impact, disturbance, dynamics, diversity.

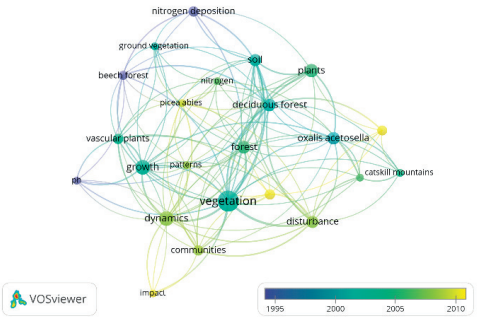


Figure 8. Distribution of key words concerning *Oxalis acetosella* in forests over the years. The node size and thickness of the connecting lines are proportional to the number of documents assigned to each country. The connections represent the collaboration network among key words

CONCLUSIONS

A total of 98 publications were identified and analyzed. Of these, 91 are articles, 5 are proceeding papers and 2 are review articles. The published articles can be included in 19 research areas, with the most representative being Environmental Sciences Ecology, Plant sciences, Forestry and Biodiversity conservation. After the first article was published in 1976, an average of 2-3 articles followed each year. Most articles belong to Ursula Falkengren-Grerup, Jörg Brunet and G. Tyler. The published articles have authors from 27 countries, from three continents, with the first countries being Sweden, Germany and USA. The institutions where these authors belong are especially from Sweden: (Lund University and Swedish University of Agricultural Sciences) and Estonia (University of Tartu), while the most important journals are Forest Ecology and Management, Ecography and Vegetation. The most frequently used key words are vegetation, *Oxalis-acetosella*, growth, dynamics and plants. In total, we have identified 3 clusters for countries and 7 clusters for key words.

REFERENCES

Berg, H. (2002). Population dynamics in Oxalis acetosella: the significance of sexual reproduction in a clonal, cleistogamous forest herb. *Ecography*, *25*(2), 233-243.

Bierzychudek, P. (1982). Life histories and demography of shade-tolerant temperate forest herbs: a review. *New phytologist*, 90(4), 757-776.

Borrett, S. R., Sheble, L., Moody, J., & Anway, E. C. (2018). Bibliometric review of ecological network analysis: 2010-2016. *Ecological Modelling*, *382*, 63-82.

Chen, J., Zhang, B., Ma, K., & Jiang, Z. (2009). Bibliometric analysis of status quo of conservation biology in China. *Biodiversity Science*, *17*(4), 423.

Crişan, V. E., Dincă, L., Bragă, C., Murariu, G., Tupu, E., Mocanu, G. D., & Drasovean, R. (2023). The Configuration of Romanian Carpathians Landscape Controls the Volume Diversity of *Picea abies* (L.) Stands. *Land*, *12*(2), 406.

Crişan, V., Dincă, L., Târziu, D., Oneţ, A., Oneţ, C., & Cântar, I. C. (2024). A Comparison between Uneven-Aged Forest Stands from the Southern Carpathians and Those from the Banat Mountains. *Sustainability*, *16*(3), 1109.

Dincă, L., Sparchez, G., & Dincă, M. (2014). Romanian's forest soil GIS map and database and their ecological implications. *Carpathian Journal of Earth and Environmental Sciences*, *9*(2), 133-142.

Dincă, L., Murariu, G., Enescu, C. M., Achim, F., Georgescu, L., Murariu, A., ... & Holonec, L. (2020). Productivity differences between southern and northern slopes of Southern Carpathians (Romania) for Norway spruce, silver fir, birch and black alder. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, *48*(2), 1070-1084.

Dinca, L., Marin, M., Radu, V., Murariu, G., Drasovean, R., Cretu, R., ... & Timiș-Gânsac, V. (2022). Which are the best site and stand conditions for silver fir (*Abies alba* Mill.) located in the Carpathian Mountains? *Diversity*, *14*(7), 547.

Fernandes, A. A., Adams, C., de Araujo, L. G., Romanelli, J. P., Santos, J. P. B., & Rodrigues, R. R. (2022). Forest landscape restoration and local stakeholders: A global bibliometric mapping analysis. *Sustainability*, *14*(23), 16165.

Godefroid, S., Rucquoij, S., & Koedam, N. (2005). To what extent do forest herbs recover after clearcutting in beech forest? *Forest Ecology and Management*, *210*(1-3), 39-53.

Grime, J. P., Hodgson, J. G. & Hunt, R. (1988). *Comparative plant ecology*. – Unwin Hyman.

Hermy, M., & Verheyen, K. (2007). Legacies of the past in the present-day forest biodiversity: a review of past land-use effects on forest plant species composition and diversity. *Sustainability and diversity of forest ecosystems: An interdisciplinary approach*, 361-371.

Ho, Y. S. (2007). Bibliometric analysis of adsorption technology in environmental science. *Journal of environmental protection science*, *1*(1), 1-11.

Kikeeva, A. V., Romashkin, I. V., Nukolova, A. Y., Fomina, E. V., & Kryshen, A. M. (2024). Influence of Picea Abies Logs on the Distribution of Vascular Plants in Old-Growth Spruce Forests. *Forests*, *15*(5), 884.

Kutnar, L., Ódor, P., & Dort, K. V. (2002). Vascular plants on beech dead wood in two Slovenian forest reserves. *Zbornik gozdarstva in lesarstva*, (69), 135-153.

Kuusipalo, J. (1985). An ecological study of upland forest site classification in southern Finland. *Acta For. Fenn*., 192: 1-77.

Kuusipalo, J. (1987). Relative importance of factors controlling the success of Oxalis acetosella: an example of linear modelling in ecological research. *Vegetatio*, *70*, 171-179.

Matuszkiewicz, J. M., Affek, A. N., Zaniewski, P., & Kołaczkowska, E. (2024). Early response of understory vegetation to the mass dieback of Norway spruce in the European lowland temperate forest. *Forest Ecosystems*, *11*, 100177.

Murariu, G., Dinca, L., Tudose, N., Crisan, V., Georgescu, L., Munteanu, D., ... & Mocanu, G. D. (2021). Structural characteristics of the main resinous stands from Southern Carpathians, Romania. *Forests*, *12*(8), 1029.

Økland, R. H. (1995). Changes in the occurrence and abundance of plant species in a Norwegian boreal coniferous forest, 1988–1993. *Nordic Journal of Botany*, *15*(4), 415-438.

Packham, J. R. & Willis, A. J. (1977). The effects of shading on *Oxalis acetosella. J. Ecol*., 65: 619-642.

Polinko, A. D., & Coupland, K. (2021). Paradigm shifts in forestry and forest research: a bibliometric analysis. *Canadian Journal of Forest Research*, *51*(2), 154-162.

Ruiz-Real, J. L., Uribe-Toril, J., De Pablo Valenciano, J., & Gázquez-Abad, J. C. (2018). Worldwide research on circular economy and environment: A bibliometric analysis. *International journal of environmental research and public health*, *15*(12), 2699.

Santillán-Fernández, A., Vásquez-Bautista, N., Pelcastre-Ruiz, L. M., Ortigoza-García, C. A., Padilla-Herrera, E., Tadeo-Noble, A. E., ... & Bautista-Ortega, J. (2023). Bibliometric Analysis of Forestry Research in Mexico Published by Mexican Journals. *Forests*, *14*(3), 648.

Schulze, E. D., Aas, G., Grimm, G. W., Gossner, M. M., Walentowski, H., Ammer, C., ... & von Gadow, K. (2016). A review on plant diversity and forest management of European beech forests. *European Journal of Forest Research*, *135*, 51-67.

Shorina, I. I. (1985). Seasonal dynamics of coenopopulations of *Oxalis acetosella* in relation to its biology. – In: I. I. Serebrjakova (ed.), *Dynamics of plant coenopopulations*. Nauka, pp. 36–45, in Russian.

Spârchez, G., Dincă, L. C., Marin, G., Dincă, M., & Enescu, R. E. (2017). Variation of eutric cambisols' chemical properties based on altitudinal and geomorphologic zoning. *Environmental Engineering & Management Journal (EEMJ)*, *16*(12).

Sullivan, A. (2022). Bridging the divide between rural and urban community-based forestry: A bibliometric review. *Forest Policy and Economics*, *144*, 102826.

Tessier, J. T., McNaughton, S. J., & Raynal, D. J. (2001). Influence of nutrient availability and tree wildling density on nutrient uptake by Oxalis acetosella and Acer saccharum. *Environmental and experimental botany*, *45*(1), 11-20.

Ujházy, K., Krizová, E., Ondruš, M., Vigoda, M., & Abrudan, V. I. (2007). Short-term vegetation change in the fir-beech primeval forest (Badínsky prales, Central Slovakia). In *Forest and sustainable development. Proceedings of the biennial international symposium (2007) Editura Universităţii Transilvania (Braşov)* (pp. 213-218).

Van Winkle, T., Kotval-K, Z., Machemer, P., & Kotval, Z. (2022). Health and the Urban Environment: A Bibliometric Mapping of Knowledge Structure and Trends. *Sustainability*, *14*(19), 12320.

Wright, J. S. (2002). Plant diversity in tropical forests: a review of mechanisms of species coexistence. *Oecologia*, *130*, 1-14.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

EFFECT OF ESSENTIAL OILS ON QUALITY AND SHELF LIFE OF STRAWBERRIES DURING COLD STORAGE

Sorina DINU[1], Lavinia-Diana-Nicoleta BUTURUGĂ-BARBU[2], Maria-Cristina LUMÎNARE[1, 2], Daniel-Nicolae COJANU[1, 2], Viorel FĂTU[1], Oana-Alina BOIU-SICUIA[1, 2]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăști Blvd, District 1, Bucharest, Romania
[2]Research Development Institute for Plant Protection, 8 Ion Ionescu de la Brad Blvd, District 1, 013813, Bucharest, Romania

Corresponding author email: oana.sicuia@bth.usamv.ro

Abstract

Strawberries hold significant economic value in many countries. However, due to their delicate texture and thin skin, they are prone to rapid spoilage and susceptible to various pathogens. Currently, chemicals are the primary method used to prevent pathogen infections and degradation of fresh fruits. Therefore, this research focuses on identifying organic, eco-friendly treatments that can extend the shelf life of these fruits. Essential oils (EOs) are potential candidates for prolonging strawberry shelf life and protecting against post-harvest fungal pathogens. Current study evaluates the in vitro activity of several EOs against Botrytis cinerea and the in vivo effects of EO treatments on the quality and physicochemical properties of strawberries (Fragaria × ananassa cv. Alba) stored at 4 ± 1°C for 15 days. Parameters such as weight loss, decay percentage, fruit firmness, soluble solid content, and total polyphenols were measured at three intervals over 15 days of cold storage. Cinnamon EO was particularly effective in reducing rot by inhibiting fungal growth, aligning with in vitro results. Notably, EO treatments extended the shelf life and delayed decay of strawberries up to 15 days under cold storage.

Key words*: Botrytis cinerea*, *essential oils, fruit quality, shelf life, strawberries.*

INTRODUCTION

The use of essential oils (EOs) as antimicrobial additives within food industry is garnering heightened attention, attributed to their abundance of biologically active compounds that possess well-documented antimicrobial and antioxidant properties (Manso et al., 2014; Calo et al., 2015; Llana-Ruiz-Cabello et al., 2015; Wrona et al., 2015; Atarés and Chiralt, 2016). EOs are complex volatile substances, comprising mainly terpenes and other aromatic compounds, found in various pant parts (Bakkali et al., 2008).
They play on important role in plant protection against pest and diseases (Tzortzakis & Economakis, 2007).
Microbial spoilage and enzymatic activity are two major factors affecting products' shelf-life and inducing organoleptic qualities loss. Therefore, using efficient storage methods, including biologic solutions to prevent microbial spoilage and modulate enzymatic inactivation, can improve the products quality during storage and prolong food shelf-life.
Studies carried out in this pathway have shown that EOs exhibit strong antimicrobial activity against post-harvest fungal pathogens, mainly on *Botrytis* and *Colletotrichum*.
Thus, EOs can be used as preservatives to extend the shelf-life of small fruits, such as strawberries (*Fragaria* × *ananassa* Duch.), raspberries (*Rubus* spp.), blueberries (*Vaccinium* spp.) and grapes (*Vitis vinifera* L.), as well as to prevent the development of microorganisms that degrade other fresh products (Vital et al., 2016; Nazari et al., 2019; Simionato et al., 2019).
There are several ways of using EOs to protect perishable fruits from spoilage, such as volatiles, fruit coating or packaging coating (Figure 1).
The aim of this study was to evaluate *in vitro* activity of essential oils against fungal phytopathogen *Botrytis cinerea*, the causal agent of grey rot. Also, *in vivo* EOs activity on the physical qualities of strawberries (*Fragaria* × *ananassa* cv. Alba), in storage conditions at 4 ± 1°C, for 15 days were assessed.

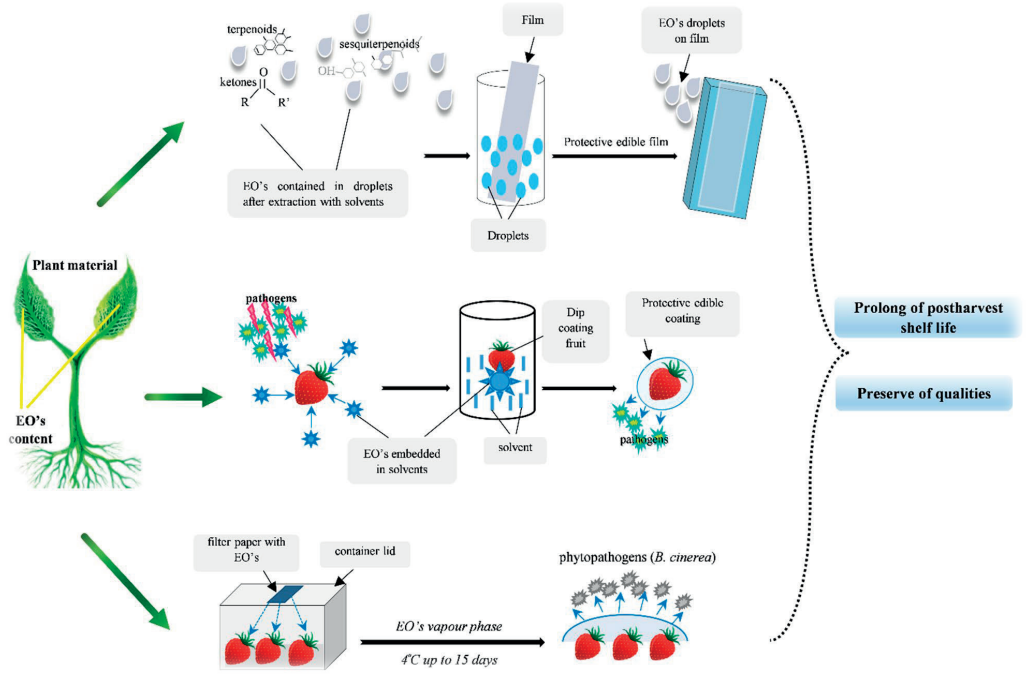


Figure 1. Potential usage of EOs in fruits protection against pathogens during storage (after Herman et al., 2019, amended by Dinu)

MATERIALS AND METODS

Experimental conditions

All experiments were conducted at the Research-Development Institute for Plant Protection, within the Laboratory of Plant Protection Bioproducts. The *in vitro* studies were performed during spring, for March to April 2024, while the *in vivo* trials and related analysis were performed during Mai to June 2024.

Commercial EOs

Commercial EOs purchased from a Romanian brand, providing natural biologic products, were used in this study. These EOs were obtained by steam distillation from different plant parts, such as bark, leaves, flowers, and flower buds of cinnamon (*Cinnamomum verum*), peppermint (*Menta piperita*) and thyme (*Thymus vulgaris*), respectively.

Fungal pathogen

A wild strain of *Botrytis cinerea* was used in both *in vitro* and *in vivo* trials. This strain was isolated from naturally contaminated strawberry fruits expressing grey mould infection.

In vitro assessment of EOs antimicrobial activity

The antifungal activity of EO's against *B. cinerea* was determined by disk diffusion technique. The phytopathogenic fungus was refreshed on solid Potato Dextrose Agar medium (PDA medium; Carl Roth GmbH+Co.KG, Karlsruhe, Germany) to obtain an abundant growth. For the *in vitro* tests, calibrated mycelium plugs (7 mm Ø), collected from fresh fungal cultures, were inoculated on PDA, in the centre of 90 mm Ø plates. At 30 mm form the fungal inoculum, sterile paper discs, 5 mm Ø filter, were placed on medium' surface, 1 disc per plate, and impregnated with 20 µL of EO. Three replicates were prepared for each EO. Control plates only inoculated with the fungal phytopathogen were also prepared. All plates were sealed with 2 layers of parafilm M foil and alimentary foil, then incubated at 25°C, for five days. Antimicrobial activity of the EOs was assessed by measuring the fungal growth in the

test and control plates, for calculating the inhibition rate, according to the equation:

$$\% \text{ inhibition} = R_M - R_{UE} / R_M \times 100$$

where R_M is the radius of fungal mycelium growth in control plate, and R_{UE} is the radius of fungal mycelium growth into the direction of EO's impregnated disc.

***In vivo* testing of EOs on stored strawberries**

Strawberries (*Fragaria* × *ananassa*) of the Alba variety were harvested at the commercial ripening stage (at least 75% of the fruit surface red) from a private farm located in Giurgiu County (latitude 44.1779084º N, longitude 26.3579245º E). For the *in vivo* trials, fruits with uniform appearance (colour and shape), and no signs of fungal contamination or physical damage were selected. The fruits were then placed transparent polyethylene containers (L x l x h = 120 × 100 × 50 mm), 5 strawberries / box. Adhesive sponges of 5 mm squares were taped on the containers' lids and impregnated with 20 µL EOs of peppermint (UEM), thyme (UEC) or cinnamon (UES). The containers were sealed with parafilm to avoid vapor leaks. A control variant was also prepared by adding 20 µL of distilled water to the square sponge (Figure 2).



Figure 2. Strawberry fruits prepared for the *in vivo* trial with EOs treatments

To determine the physicochemical parameters of the stored fruits, strawberries from each experimental variant, including control, were analysed after 5, 10 and 15 days of storage at 4±0.5°C. The performed analyses included weight loss, decay percentage, fruit firmness, soluble solid content and total phenolic content.

Fruits weight loss quantification

Each strawberry fruit was separately weighed and labelled at the beginning of the experiment and at each sampling date of 5, 10, and 15 days of storage. To determine weight loss (WL), the following equation was used:

$$WL\% = (IW-FW)/IW \times 100$$

where IW is initial weight of the fruits, and FW is their final weight.

Decay percentage evaluation

The percentage of fruits degradation was determined according to Rokaya et al. (2016) method, with some modifications. The decay percentage (D %) was calculated with the following formula:

$$D\% = WIF/ WAF \times 100$$

where WAF is total weight of fruits before storage and WIF is the weight of decayed strawberries after 5, 10 and 15 days of storage. Decayed fruits were considered those exhibiting brown spots, pulp softening, lesions, spoilage or mycelial coverage.

Fruit firmness analysis

The strawberries firmness was measured using a digital penetrometer equipped with a 6 mm Ø cylindrical probe (Velickova et al., 2013). Firmness was expressed as kg/cm^2 strength and was determined after 5, 10 and 15 days of storage at 4°C.

Measurement of the soluble solid content

A portable refractometer with automatic temperature compensation was used to measure the total solid content (SSC). Fruit samples were crushed, and 2-3 drops were distributed on the prismatic element of the measuring equipment, ensuring that the juice covered the entire reading surface. The SSC values were expressed as degree Brix and converted into g of sugars/L, considering 1°Bx is 10.04 g/L at 20°C.

Determination of total phenolic content

The total phenolic content (TPC) in strawberries was evaluated using Folin-Ciocalteu reagent, according to Singleton et al. (1999) method. Extracts are diluted with distilled water in a 1:4 (*v/v*) ratio, and 20 µL of the diluted extract were added to the reaction mixture. To this, 1.7 mL of distilled water and 100 µL of Folin-Ciocalteu

reagent are added. The mixture was incubated for 5 minutes at room temperature to allow the reaction to develop. Following incubation, 0.3 mL of a 20% sodium carbonate solution was added to stabilize the reaction. A control sample was prepared by replacing the extract with distilled water. The optical density of the samples was measured at 765 nm, and the results expressed as equivalent gallic acid per mL (EAG/mL) using a standard curve.

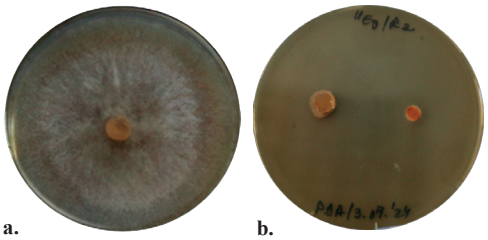
Statistical analysis

The data were statistically analysed using a one-way ANOVA, performed with GraphPad Prism software. Prior to analysis, the data were examined to verify compliance with the assumptions of normality and homogeneity. Normality was assessed using the Shapiro-Wilk test, while homogeneity of variances was evaluated through Levene's test. Post-hoc comparisons of all possible treatment pairs within each of the three storage periods (5, 10 and 15 days) were conducted using Tukey's test. Additionally, the interaction between the two variable factors, storage duration and applied treatments, was analysed using a two-way ANOVA test.

RESULTS AND DISCUSSIONS

***In vitro* EOs antimicrobial activity**

According to the *in vitro* analysis, all three tested EOs, of peppermint (UEM), thyme (UEC) and cinnamon (UES), have completely inhibited *B. cinerea* growth during the 5 days of incubation at 25°C. Mine wile, within the control plates, the pathogen has completely colonised the agar surface (Figure 3).



a. **b.**

Figure 3. EOs antifungal activity against *B. cinerea* (after 5 days of incubation at 25°C):
a. Fungal control plate, b. Complete fungal growth inhibition using cinnamon EO

Currently, there are many research findings on the effectiveness of plant volatiles to control *in vitro* microbial growth and extend the shelf life of fresh products (Rojas-Graü et al., 2007; Rokaya et al., 2016). Therefore, EOs with bioactive potential can be used as alternative method to protect food and vegetable from important pathogens, due to their antibacterial, antifungal and antioxidant properties. Moreover, plant volatile bioproducts tend to have a low mammalian toxicity and broad-spectrum antimicrobial activity, being less dangerous than synthetic compounds and generally more acceptable to the consumer (Kizil et al, 2010; Hyldgaard et al., 2012; Calo et al., 2015; Aloui & Khwaldia, 2016; Rahmawati et al., 2017; Torres-Alvarez et al, 2017).

***In vivo* effect of EOs on stored strawberries**

Fresh healthy strawberries preserved at 4±0.5°C in the presence, and absence of EOs were analysed during 15 days of storage. The physicochemical parameters of the fruits were examined after 5, 10 and 15 days of storage.

Fruits weight loss was the first analysed parameter, as rapid water loss of fruits is considered one of the important factors that contributing to their perishability (Bakkali et al, 2008).

As expected, strawberries weight loss increased during storage (Figure 4). However, the EO treatments did not statistically influenced the measured results ($F_{(3, 24)} = 2.157$, $P = 0.1194$). Fruits respiration and moisture evaporation through the fruit skin most probably contributed to this aspect.

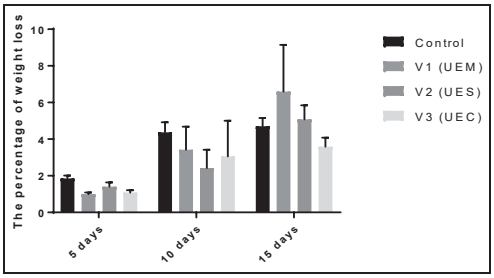


Figure 4. The diagram of the weight loss percentage of strawberries during storage at 4°C

After the first 5 days of storage at 4°C, the weight loss was relatively low across all treatments, while the control group showed significantly higher weight loss compared to the EOs treated variants (p-values < 0.05).

The reduction in weight loss, due to the EO treatments, during the first five days of fruits storage, is attributed to the antioxidant activity of EOs that acted as a protective barrier (Bakkali et al, 2008; Aloui & Khwaldia, 2016; Martínez et al., 2018).

The EOs most probably decreased the gas and water exchange between the fruits and the environment.

On the 10th days, a significant increase in weight loss was observed across all treatments, although UES treatments remain more effective in preserving fruit weight compared to the control. Despite these differences in weight loss among treatments, the variation was not statistically significant (p-values > 0.05). A similar trend was recorded on the 15th days of storage, the fruits continued to lose weight, with no statistically significant differences among the experimental variants, control and the EOs treatments.

Beside weight loss, fruit depreciation was also analysed during storage.

Signs of fungal degradation were observed in the control group (untreated fruits) from the 5th day of storage.

Fruit degradation was accentuated while extending the storage period.

In the EOs treated variants, first signs of fungal degradation were observed in fruit treated with UEM, on the 10th day of storage, however considerably lower than in the untreated control. In the 15th day of storage, the strawberry fruits treated with thyme and cinnamon EOs also started to show decaying signs, while the untreated fruits, followed by those treated with UEM revealed high percentage of decomposition. The decay percentage of cold stored strawberries is graphical presented below (Figure 5).

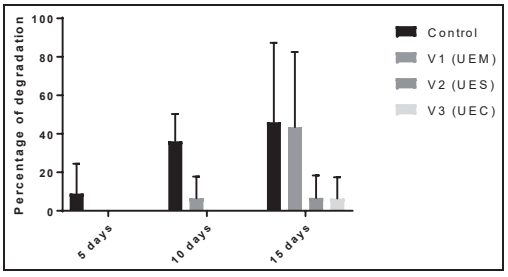


Figure 5. Strawberries decaying percentage during 15 days of storage at 4°C

Strawberries cold storage, in the presence of thyme and cinnamon EOs significantly reduced fruits degradation and inhibited fungal growth on the fruit surface. Correlating these results with those from the in vitro tests, it could be concluded that the antimicrobial effects of the EOs contributed to fruit preservation. Similar aspects being also presented in other similar studies (Kizil et al, 2010; Aloui & Khwaldia, 2016; Torres-Alvarez et al, 2017).

Firmness is considered an important quality attribute for strawberries, indicating their quality and predicting their shelf life throughout the market chain. Thus, a higher strawberry firmness is correlated with a lower fruit decay score (https://edepot.wur.nl/503212).

Within the EOs tests, fruits firmness was evaluated in the 5th, 10th and 15th day of storage. Results showed that the pulp firmness decreased during storage in all experimental variants (Figure 6).

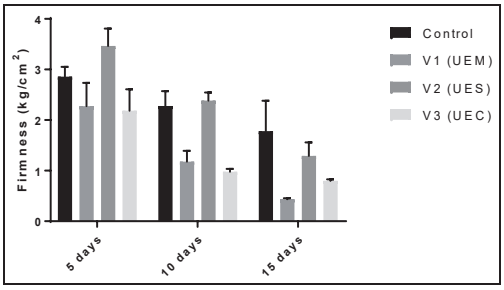


Figure 6. The effect of EOs on strawberries firmness during storage at 4°C

After five days of storage, strawberries from the UES experimental variant revealed the highest firmness parameter, significantly different from the other treatments (UEM $p = 0.0183$; UEC $p = 0.0123$). However, by the end of the 15 days of cold storage, strawberries' firmness decreased considerably, especially when peppermint and thyme EOs were used. Severino et al. (2015) reported fruit firmness maintenance for 15 days in cold storage conditions, as well as other quality attributes of fruits treated with peppermint, mandarin and lemongrass EOs. This was probably due to the selective gas and water permeability of the coating material, thus reducing respiration rate, enzymatic activities and most metabolic changes, thus delaying ripening and over-softening of strawberries (Janjarasskul & Krochta, 2010). Several other

authors also reported that strawberry fruit tissue becomes soft due to the loss of cell wall structure by increasing enzymatic activity, which leads to decreased fruit resistance during the shelf life (Aloui & Khwaldia, 2016).
Regarding the solid soluble content (SSC) the registered values remained at similar levels in samples treated with cinnamon and thyme EOs, throughout the cold storage period of 15 days (Figure 7).

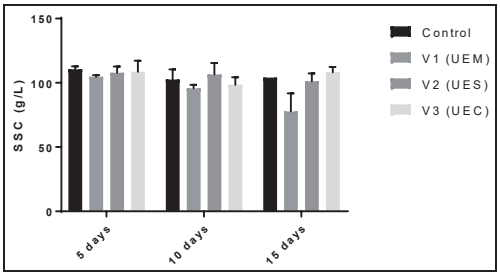


Figure 7. The effect of EO's on solid soluble content (SSC) of strawberries during storage at 4°C

Regarding the UEM treatment, SSC values were significantly different from both the control (p = 0.0156) and the other treatment variants (V2: p = 0.0278; V3: p = 0.0068), revealing a lower SSC concentration.
The reduction of SSC values in fruits treated with EOs could be associated with the reduced water loss from the fruit surface due to EO's ability to decrease the migration of water from fruit surface to the environment (Aloui & Khwaldia, 2016).
Evaluating the total phenolic content (TPC) in EO's treated and untreated strawberry fruits a wide values fluctuation was retrieved among treatments and storage time (Figure 8).

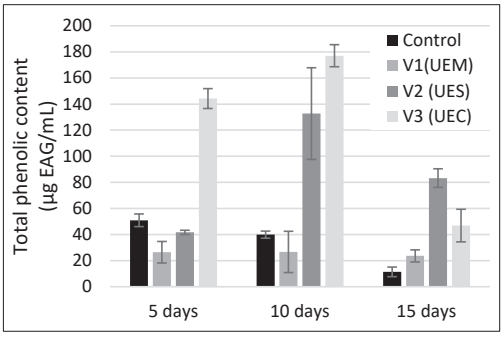


Figure 8. The effect of EO's on total phenolic content (TPC) of strawberries during storage at 4°C

TPC was significantly higher in UEC treated fruits compared the untreaded ones, during the cold storage period of 15 days. After the first 5 days of storage at 4°C, the TPC in UEC treated strawberries was 144.22±7.63 µg EAG/mL, meaning 2.8 times higher in thyme EO treated fruits compared to the control. After 10 days of storage, the TPC increased up to 177.08±8.46 µg EAG/mL, being 4.4 times higher in UEC treated fruits compared to the untreated ones. At this storage time (10 days at 4°C), the UES treatment also revealed an increased TPC in the fruits (132.71±35.15 µg EAG/mL), 3.3 times higher than in the untreated control (39.93±2.71 µg EAG/mL). After the 15 days of cold storage, the TPC values decreased. However, both UES and UEC treatments induced a significantly higher TPC in the stored fruits, 7.3 and 4.1 times higher, respectively, than in the untreated control (11.39±3.68 µg EAG/mL).
Thyme EO is known to significantly enhance strawberry preservation, especially due to its antimicrobial and antioxidant properties. Studies have shown that thyme EO encapsulated in zein nanofiber films to protect strawberry quality (Ansarifar & Moradinezhad, 2021). Moreover, thyme oil is recommended also for soil application in strawberry fields to improve yield and reduce disease abundance (Cato et al., 2024).

CONCLUSIONS

These *in vitro* and *in vivo* studies showed the perspective of using certain plant EO's for strawberry fruits preservation. The antimicrobial activity of peppermint, thyme and cinnamon EO's against *Botrytis cinerea* phytopathogen revealed a complete inhibition of the grey mould mycelium growth. Moreover, the cinnamon and thyme EO's treatments applied to the strawberry fruits, stored at 4°C, were effective in reducing fruits rot, by inhibiting the fungal growth on strawberries surface. Several quality parameters, such as fruit firmness, SSC and TPC were also increased in cinnamon and thyme EO's treated fruits. Therefore, the application of such plant extracts based on EO's may serve as alternative to conventional treatments, offering potential advantages in limiting damage caused by grey mould in organic farming systems.

REFERENCES

Aloui, H., & Khwaldia, K. (2016). Natural Antimicrobial Edible Coatings for Microbial Safety and Food Quality Enhancement. *Comprehensive Reviews in Food Science and Food Safety*, *15* (6), 1080–1103.

Ansarifar, E., & Moradinezhad, F. (2021). Preservation of strawberry fruit quality via the use of active packaging with encapsulated thyme essential oil in zein nanofiber film. *International Journal of Food Science & Technology, 56* (9),4239–4247.

Atarés, L., & Chiralt, A. (2016). Essential oils as additives in biodegradable films and coatings for active food packaging. *Trends in Food Science & Technology, 48,* 51–62.

Bakkali, F., Averbeck, S., Averbeck, D., & Idaomar, M. (2008). Biological effects of essential oils – A review. *Food and Chemical Toxicology, 46* (2)*,* 446–475.

Calo, J. R., Crandall, P. G., O'Bryan, C. A., & Ricke, S. C. (2015). Essential oils as antimicrobials in food systems – A review. *Food Control, 54*, 111–119.

Cato, A. J., McWhirt, A. L., & Rojas, A. (2024). Impact of soil-applied thyme oil on strawberry yield and disease abundance. *International Journal of Fruit Science, 24*(1), 130–141.

Hyldgaard, M., Mygind, T., & Meyer, R. L. (2012). Essential oils in food preservation: mode of action, synergies, and interactions with food matrix components. *Frontiers in Microbiology*, *3*, 12.

Janjarasskul, T., & Krochta, J. M. (2010). Edible packaging materials. *Annual Review of Food Science and Technology, 1*, 415–448.

Kizil, S., Hasimi, N., Tolan, V., Kilinc, E., & Yuksel, U. (2010). Mineral content, essential oil components and biological activity of two mentha species (*M. piperita* L., *M. spicata* L.). *Turkish Journal of Field Crops, 15* (2), 148–153.

Llana-Ruiz-Cabello, M., Pichardo, S., Maisanaba, S., Puerto, M., Prieto, A. I., Gutiérrez-Praena, D., Jos, A., & Cameán, A. M. (2015). *In vitro* toxicological evaluation of essential oils and their main compounds used in active food packaging: A review. *Food and chemical toxicology : an international journal published for the British Industrial Biological Research Association*, *81*, 9–27.

Manso, S., Pezo, D., Gómez-Lus, R., & Nerín, C. (2014). Diminution of aflatoxin B1 production caused by an active packaging containing cinnamon essential oil. *Food Control*, *45,* 101–108.

Martínez, K., Ortiz, M., Albis, A., Gilma Gutiérrez Castañeda, C., Valencia, M. E., & Grande Tovar, C. D. (2018). The effect of edible chitosan coatings incorporated with *Thymus capitatus* essential oil on the shelf-life of strawberry (*Fragaria x ananassa*) during cold storage. *Biomolecules*, *8*(4), 155. https://doi.org/10.3390/biom8040155

Nazari, M., Ghanbarzadeh, B., Kafil, H. S., Zeinali, M., & Hamishehkar, H. (2019). Garlic essential oil nanophytosomes as a natural food preservative: Its application in yogurt as food model. *Colloid and Interface Science Communications, 30*, 100176.

Rahmawati, D., Chandra, M., Santoso, S., & Puteri, M. G. (2017). Application of lemon peel essential oil with edible coating agent to prolong shelf life of tofu and strawberry. *AIP Conference Proceedings, 1803*, 020037.

Herman, R. A., Ayepa, E., Shittu, S., Fometu S. S., & Wang, J. (2019). Essential Oils and Their Applications -A Mini Review. *Advances in Nutrition & Food Science,* 4(4), 1–13.

Rojas-Graü, M. A., Raybaudi-Massilia, R. M., Soliva-Fortuny, R., Avena-Bustillos, R. J., McHugh, T. H., & Martín-Belloso, O. (2007). Apple puree-alginate edible coating as carrier of antimicrobial agents to prolong shelf-life of fresh-cut apples. *Postharvest Biology and Technology, 45*(2), 254–264.

Rokaya, P.R., Baral, D.R., Gautam, D.M., Shrestha, A.K., & Paudyal, K.P. (2016). Effect of postharvest treatments on quality and shelf life of mandarin (*Citrus reticulata* Blanco). *American Journal of Plant Sciences, 7*, 1098–1105.

Severino, R., Ferrari, G., Vu, K.D., Donsì, F., Salmieri, S., & Lacroix, M. (2015). Antimicrobial effects of modified chitosan based coating containing nanoemulsion of essential oils, modified atmosphere packaging and gamma irradiation against *Escherichia coli* O157:H7 and *Salmonella typhimurium* on green beans. *Food Control, 50*, 215–222.

Simionato, I., Domingues, F.C., Nerín, C., & Silva, F. (2019). Encapsulation of cinnamon oil in cyclodextrin nanosponges and their potential use for antimicrobial food packaging. *Food and Chemical Toxicology, 132*, 110647.

Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology, 299*, 152–178.

Torres-Alvarez, C., González, A.N., Rodríguez, J., Castillo, S.L., Leos-Rivas, C., & Báez-González, J. (2017). Chemical composition, antimicrobial, and antioxidant activities of orange essential oil and its concentrated oils. *CyTA Journal of Food, 15*(1), 129–135.

Tzortzakis, N. G., & Economakis, C. D., (2007). Antifungal activity of lemongrass (*Cympopogon citratus* L.) essential oil against key postharvest pathogens. *Innovative Food Science & Emerging Technologies, 8*(2)*,* 253–258.

Velickova, E., Winkelhausen, E., Kuzmanova, S., Alves, V.D., & Moldão-Martins, M. (2013). Impact of chitosan-beeswax edible coatings on the quality of fresh strawberries (*Fragaria ananassa* cv Camarosa) under commercial storage conditions. *LWT Food Science and Technology, 52*, 80–92.

Vital, A. C., Guerrero, A., Monteschio, J.de O., Valero, M. V., Carvalho, C. B., de Abreu Filho, B. A., Madrona, G. S., & do Prado, I. N. (2016). Effect of edible and active coating (with rosemary and oregano essential oils) on beef characteristics and consumer acceptability. *PloS one*, *11*(8), e0160535.

Wrona, M., Bentayeb, K., & Nerín, C. (2015). A novel active packaging for extending the shelf-life of fresh mushrooms (*Agaricus bisporus*). *Food Control, 54*, 200–207.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

CRAFTED BARREL - AGED BEER: TRADITION AND INNOVATION IN FLAVOR EVOLUTION - REVIEW

Mihai FRÎNCU[1], Corina DUMITRACHE[5], Diana-Iuliana BĂRBULESCU[2], Mihaela BEGEA[3, 4], Carmen-Rodica VRÎNCEANU[5], Răzvan-Ionuț TEODORESCU[5]

[1]Research Center for Studies of Food Quality and Agricultural Products, University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăști Blvd, District 1, 011464, Bucharest, Romania
[2]Faculty of Biotechnologies, University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăști Blvd, District 1, 011464, Bucharest, Romania
[3]National University of Science and Technology Politehnica Bucharest, 313 Splaiul Independentei, District 6, Bucharest, Romania
[4]Faculty of Biotechnical Systems Engineering, National University of Sciences and Technology Politehnica Bucharest, 060042, Bucharest, Romania
[5]Faculty of Land Reclamation and Environmental Engineering, University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăști Blvd, District 1, 011464, Bucharest, Romania

Corresponding author email: corina_dumitrache17@yahoo.com

Abstract

Barrel-aging beer is a practice that dates back to the early days of brewing, when wooden barrels served as essential tools for storage and transportation. Over time, brewers observed that barrels could enhance the flavor of beer, transforming a practical necessity into a valued tradition. This process has been revitalized in the modern brewing industry to craft beverages with unique and complex sensory profiles. Barrel-aging involves storing beer in barrels made from various types of wood, often previously used to age spirits such as whiskey or rum. The interaction between beer and wood releases aromatic compounds like vanillin, γ-nonalactone, and volatile phenols, which contribute to the beer's enhanced flavor and character. This paper investigates the key factors influencing the sensory characteristics of barrel-aged beer, focusing on oxidation, wood compound extraction, and biochemical transformations during aging. It also highlights the beer styles best suited for barrel-aging, such as stouts, porters, and strong ales, which benefit most from the process. Finally, this paper underscores how barrel-aging merges tradition and innovation, offering brewers new ways to diversify and refine the sensory profile of modern beers.

Key words*: barrel-aged beer, aromatic compounds, yeast, flavor, craft beer.*

INTRODUCTION

Beer has significantly influenced cultural, economic, and technological advancements in human societies, with its origins tracing back to ancient civilizations such as Mesopotamia, where evidence suggests beer production dates back over 13,000 years (Paulette, 2024; Pilcher, 2025). Initially, brewing techniques were rudimentary, but they evolved through technological advancements, as seen in the ancient Hymn to Ninkasi, which outlines the brewing process (Paulette, 2024). The introduction of hops in the thirteenth century revolutionized beer preservation and trade, leading to the commercialization of beer and the emergence of distinct styles, reflecting social distinctions among consumers (Pilcher, 2025; Withers, 2017). Recent research highlights the importance of sustainability in beer production, emphasizing the environmental impacts of brewing processes and advocating for improved technologies and practices, such as Life Cycle Assessment (LCA) to evaluate ecological footprints (D'Ascanio et al., 2024). Nowadays, barrel aging exemplifies the fusion of traditional brewing practices with modern scientific insights, enhancing the sensory complexity of beer through complex interactions between the

beverage and wood (Noviello et al., 2024; Paulette, 2024; Seo, 2024; Winstel et al., 2024; Paulette, 2024).

The transition from necessity to craft beer technique marks a profound shift in brewing practices and cultural significance over millennia. Originally, beer production served as a means of grain preservation and nutrition, playing a key role in the shift from nomadic to agrarian societies around 14,000 BP (Cabras et al., 2023). Over time, brewing evolved beyond its practical origins, becoming a dynamic cultural expression shaped by tradition, innovation, and advancements in technology (Mastanjević et al., 2019; Valentoni et al., 2022; Villacreces et al., 2022). This ongoing evolution underscores beer's role as a dynamic cultural artifact, shaped by both historical context and contemporary innovation (Billingsley, 2024; Kimberley, 2018).

Advances in chemometrics provide insights into aging compounds, aiding brewers in quality control (Coelho et al., 2019; Mutz et al., 2020). Additionally, craft beer's role in tourism underscores its cultural and economic significance, reinforcing the need for strategic approaches to enhance consumer experience (Tham et al., 2024; Withers, 2017). These aspects highlight aging as a key factor in sustaining the integrity and market value of craft beer.

MATERIALS AND METHODS

This review was based on an extensive selection of scientific publications, web pages data and relevant academic books covering the chemistry, microbiology, and sensory aspects of barrel-aged beer. Literature was retrieved from databases such as ScienceDirect, Google Scholar, Web of Science, and SpringerLink, focusing on works published between 2015 and 2025 (approximately 80% of all references), with the remaining citations representing historical or foundational sources that consolidate the theoretical background and contextualize traditional practices. Search terms included: barrel-aged beer, oak wood compounds, beer maturation in spirit barrels, volatile aroma compounds, yeast metabolism during aging, and flavor development in craft beer. Both recent experimental studies and historical references on wood aging and fermentation processes were included. Priority was given to sources discussing the influence of wood, the residual compounds from previously stored beverages, and the role of yeast in shaping the final sensory profile. Emphasis was placed on identifying how barrel-aging contributes to the transformation of beer aroma and flavor, with particular attention to beer styles most suitable for this maturation technique, such as stouts, porters, and strong ales.

Barrel composition and types of wood used

The barrel-aging process significantly shapes the chemical composition and aromatic complexity of beer through its interactions with wood, with factors such as the wood type and barrel charring playing a pivotal role. In addition to the compounds derived directly from the wood, the impact of the previously stored beverage must also be considered (in the case of using recycled barrels from the spirits or wine industry). Essentially, the barrel walls act as a reservoir of aromas: bourbon, whiskey, or wine barrels will release residual components into the beer, such as aldehydes, esters, and alcohols specific to those beverages (Bhowmik, 2023). Different oak species, such as American and French oak, introduce distinct phenolic compounds that enhance both flavor and aroma. For example, American oak can increase certain phenolic acid concentrations by up to tenfold compared to French oak (Carpena et al., 2020; Machado, 2019). The extraction of wood-derived compounds, including pyrazines and pyrroles, further contributes to the sensory profile of matured beers, influencing aroma, taste, and color (Picard et al., 2021; Silva et al., 2022; Tarko et al., 2023). Additionally, the aging process fosters the development of complex flavors, particularly in sour beers, where microbial activity adds an additional depth to the profile (Bossaert et al., 2021; Bossaert, Kocijan, et al., 2022). Ultimately, the interplay between wood characteristics and beer composition is fundamental to achieving the desired sensory attributes (Castro & Ross, 2018; Karathanos et al., 2024; Machado, 2019; Winstel et al., 2024).

In order to highlight the influence of barrel maturation on the aromatic profile of beer, it's

essential to examine the impact of different types of barrels used. The specific characteristics of each type of wood, as well as previous maturation processes, significantly contribute to the chemical composition and sensory complexity of the beer.

Maturation in whiskey barrel

The maturation of whiskey in oak barrels leads to a diverse and intricate aromatic profile, shaped by a range of chemical processes. The interaction between the spirit and the barrel releases a variety of volatile compounds that define its sensory attributes. For instance, key components found in American Bourbon, such as (E)-β-damascenone and vanillin, originate both from the oak barrel and the spirit absorbed in the wood, impart distinctive caramel and smoky notes (Sterckx, 2012; Tarko et al., 2023). The structural changes in oak during toasting and charring facilitate the formation of roasted aroma compounds, including pyrazines and pyrroles, which deepen the overall complexity of the whiskey (Picard et al., 2021; Goullet et al., 2023). Additionally, terpenoids extracted from the wood contribute subtle floral and minty undertones, further refining the whiskey's sensory character (Picard et al., 2023). The Maillard reaction, occurring as a result of barrel heating, generates maltol and furaneol - compounds essential for the development of a rich, toasted caramel aroma (Tamanna & Mahmood, 2015; Starowicz & Zieliński, 2019). As the beer matures, residual whiskey compounds previously absorbed by the oak, such as lactones, furfurals, and ethanol derivatives, gradually dissolve into the beer, contributing to a richer and more layered flavor profile.

Maturation in rum barrel

Oak barrels previously used for rum aging can significantly influence the sensory profile of barrel-aged beer, imparting rich notes of molasses, brown sugar, and tropical fruits. The interaction between beer and the residual compounds absorbed by the wood during rum maturation enhances both sweetness and complexity. Key aroma compounds such as vanillin and phenolic derivatives extracted from the oak contribute to the beer's overall depth and balance. Additionally, the presence of ethyl esters, which increase during both fermentation and aging (some originating from the beer itself and others extracted from the residual spirit) further intensifies fruity and caramel-like notes, complementing the malt backbone of the beer (Franitza et al., 2016). The porous structure of the barrel allows controlled oxygen exchange, promoting the slow oxidation of phenolic and aromatic compounds, which can enhance the perception of vanilla, caramel, and dried fruit notes. The residual impact of molasses-based fermentation, a defining element of rum production, also influences the final character of barrel-aged beer by introducing subtle yet distinct layers of dried fruit and spice. Ultimately, the combination of raw material origins, brewing techniques, and barrel-aging conditions creates a complex and evolving flavor profile unique to beer matured in rum barrels (Mangwanda et al., 2021; Maza Gomez, 2002; Yi, 2013).

Maturation in wine barrel

The sensory characteristics of wine, including its aroma, taste, and color, are primarily influenced by its composition, which is shaped by various factors throughout fruit cultivation and wine production (Barbulescu et al., 2022; Frincu et al., 2023; Frîncu et al., 2024). Key components such as sugars, organic acids, polyphenols (including anthocyanins), and yeasts activity play a critical role in determining the wine's quality and sensory attributes (Martínez-Gil et al., 2022; Recamales et al., 2006; Styger et al., 2011; Viviani et al., 2007). Beer aged in wine barrels gains complexity from the wood and residual wine compounds, enriching its aroma and flavor profile (Bossaert, Winne, et al., 2022). Oak barrels previously used for wine maturation contribute lactones, tannins, and phenolic compounds, enhancing sensory attributes like vanilla, coconut, dried fruits, and spices (Oliver & Colicchio, 2011). The porous structure of oak barrels facilitates micro-oxygenation, promoting controlled oxidation and stabilizing polyphenols. This reaction softens astringency, deepens color, and refines overall sensory perception (Gomez-Cordoves & Gonzalez-SanJose, 2002; Ribéreau-Gayon, 2006). The degree of wood toasting significantly influences aromatic profiles: lightly toasted

barrels release more ellagitannins, stabilizing color and enhancing subtle fruity notes, whereas medium to heavy toasting favors smoky and spicy phenolic compounds (Díaz-Maroto et al., 2008; Poisson & Schieberle, 2008). Red wine barrels particularly introduce complex, tannic, and fruity characteristics due to residual anthocyanins and tannins interacting with beer compounds (Del Fresno et al., 2020; Maga, 1989). White wine barrels, however, provide more subtle fruity, floral, and vanilla nuances (González-Centeno et al., 2020; Stegăruș et al., 2021). However, the maturation in wine barrels carries risks, such as excessive oxidation, potentially leading to undesired off-flavors or color deterioration, necessitating careful control of the aging process to maximize beneficial impacts while minimizing negative effects (Del Fresno et al., 2020; Ortega-Heras et al., 2007; Picard et al., 2021).

Cognac and brandy barrel maturation

Cognac and brandy barrels play a significant role in shaping the sensory complexity of barrel-aged beer, imparting layered notes of dried fruit, nuts, and caramel (Caldeira et al., 2010; Tarko et al., 2023). The toasting process applied to oak used for cognac and brandy production is particularly important, as it modifies the chemical composition of the wood, facilitating the extraction of key aromatic compounds during beer maturation. In this context, the interaction enhances the flavor complexity, contributes to a smoother mouthfeel, and intensifies the aromatic profile of the final product (Gadrat et al., 2022; Tsakiris et al., 2014). When beer is aged in barrels previously used for grape distillates, residual compounds from cognac or brandy, along with wood-derived elements, slowly infuse the beer, imparting nutty, and caramelized notes, enriching its complexity (Butrón-Benítez et al., 2024). Furthermore, volatile compounds released during aging amplify these sensory characteristics, reinforcing the perception of fruit-forward and oaky flavors (Uselmann & Schieberle, 2015). As with whiskey and rum barrels, the residual spirit absorbed into the wood also contributes to the beer's complexity, introducing ethanol derivatives and esterified compounds unique to each barrel's previous contents. Ultimately, the intricate exchange between beer and cognac or brandy barrels plays a crucial role in developing sophisticated flavor profiles unique to this aging method.

Table 1. Summary of key aroma compounds and sensory notes contributed by different barrel wood species and previous beverages

Barrel wood species	Previous beverage	Key aroma compounds	Dominant sensory notes
American oak	Bourbon whiskey	Vanillin, (E)-β-damascenone, pyrazines, pyrroles, furfurals, terpenoids	Caramel, vanilla, smoke, roasted nuts, minty, toffee
American/French oak	Molasses-based rum	Vanillin, phenolic derivatives, ethyl esters, lactones	Brown sugar, molasses, tropical fruits, dried fruits, spice, vanilla, caramel
French oak	Red wine	Tannins, anthocyanins, organic acids, phenolic compounds, lactones	Plum, fig, vanilla, oak, spice, dried fruits, coconut
French oak	White wine	Volatile esters, ellagitannins, phenolic aldehydes, lactones	Fruity, floral, vanilla, creamy, slightly oxidized
American/French oak	Grape distillates	Ethanol derivatives, esters, nutty aldehydes	Dried fruits, nuts, caramel, soft oak, sherry-like notes

Types of beer suitable for barrel aging

Maturation is a key stage in the beer production process, particularly when it comes to craft beers. During this phase, the beer naturally clarifies, and its flavors blend and develop harmoniously. Historically, beer maturation was carried out in wooden barrels, whose essences enriched the beer's aromatic profile, imparting distinctive taste nuances and a unique character. Nowadays, maturation takes place in stainless steel vessels, which allow for strict quality control by minimizing oxidation and the risk of microbiological contamination. However, this modern solution comes with a trade-off: a subtle loss of the flavor complexity once imparted by wooden aging. The suitability of a particular beer style for barrel aging is influenced by several factors, including the

style of the beer, the type of wood used, and the desired flavor profile. Certain beer styles are better suited to this process due to their robust aromatic character and their ability to withstand the complex interactions resulting from contact with wood.

Ale beers are particularly well-suited for barrel maturation due to their malt-forward profile, low bitterness, and inherent caramelized sweetness, which are enhanced through aging in oak barrels (Sterckx, 2012). The interaction between the beer and the wood during maturation leads to the development of complex flavor profiles, including notes of vanilla, toffee, and mild smokiness (Wyler et al., 2015). Stout and Porter beers are among the most popular styles for barrel aging, as their roasted, coffee-like flavors complement the vanilla, oak, and caramel notes imparted by the barrel (Schneider, 2023). Additionally, their high alcohol content and rich mouthfeel make them particularly well-suited for extended maturation periods, allowing the development of complex and harmonious flavor profiles (Nilsen, 2024).

Barley wine beers, both English and American styles, are well-suited for barrel aging due to their high alcohol content, residual sweetness, and robust malt character (Craft Beer & Brewing, 2010). These attributes provide a stable base that interacts favorably with the barrel, developing flavors of dried fruits, sherry, oak, and caramel over time (Bjergso & Pang, 2015). The aging process enhances their complexity, making them ideal candidates for long-term maturation in wood barrels previously used for wine, bourbon, or other spirits.

Belgian Dark Strong Ales and Quadrupels are particularly well-suited for barrel aging due to their complex malt profiles and phenolic yeast characteristics, which harmonize with the flavors imparted by wood. The process of aging these beers in barrels, especially those previously used for red wine or brandy, enhances their sensory attributes by intensifying notes of fig, plum, vanilla, and subtle spices, while also smoothing the perception of alcohol (Sanna & Pretti, 2015). Also, wood aging significantly influences the flavor profile of beverages, as seen in the increased woody, vanilla-like, and spicy notes in beers aged with oak chips (Sterckx, 2012).

Sour ales (Vrînceanu,et al, 2022), including Lambic, Gueuze, Flanders Red, and Oud Bruin, are characterized by their unique fermentation processes that occur in wooden barrels, which harbor diverse microbiota such as *Brettanomyces*, *Lactobacillus*, and *Pediococcus*. These microorganisms contribute significantly to the beers' acidity and complex flavor profiles, which often feature tart fruit notes, earthy undertones, and a vinous character (De Roos et al., 2019). The fermentation process is influenced by various factors, including the barrel's microbial community, which can vary based on cleaning procedures and barrel type, as well as environmental conditions during fermentation (Bongaerts et al., 2021). Traditional sour ales rely on spontaneous inoculation rather than pure cultures (Nasuti et al., 2023a), allowing for a rich interplay of microorganisms that evolve over time, leading to distinctive taste variations and enhancing the overall quality of the final product (De Roos And & De Vuyst, 2022; Spitaels et al., 2017).

Saison and other farmhouse ales can be successfully aged in barrels, particularly when aiming for rustic or mixed-fermentation profiles. Their dry body, spicy phenolics, and moderate ester content offer a clean base that allows wood and secondary fermentation to shine (McCarthy & Ewing-Mulligan, 2006). Barrel aging introduces layers of hay, leather, white wine, and soft oak tannins, especially when using barrels previously containing white wine or cider (Alexander & Valliere, 2019).

The Role of Yeasts in Barrel-Aging

Yeasts play a crucial role in the barrel-aging process of beer, contributing significantly to the flavor and aroma profiles of the final product. During barrel aging, various yeast strains, including *Saccharomyces cerevisiae*, *Brettanomyces*, and *Pichia*, dominate the microbiome, each imparting unique sensorial attributes to the beer (Iorizzo et al., 2021; Nasuti et al., 2023b; Shayevitz et al., 2020; Vrînceanu et al., 2025). The metabolic activity of yeasts varies considerably depending on the species and the environment from which they were isolated, influencing their contribution to the beer’s sensory characteristics (Bărbulescu et al., 2021; Dumitrache et al., 2020). The

presence of these yeasts in wooden barrels can significantly alter microbiome profiles, affecting beer consistency and quality, not only through their role in fermentation but also by reducing undesirable aged aroma compounds such as aldehydes, responsible for off-flavors like cardboard (Saison et al., 2010; Shayevitz et al., 2020). Yeasts play a critical role not only in fermentation but also in reducing undesirable aged aroma compounds, such as aldehydes, responsible for off-flavors like cardboard and ribes (blackcurrant-like notes), by metabolically converting these aldehydes into corresponding alcohols, thus enhancing beer flavor stability (Maicas, 2020; Saison, 2011). The chronological lifespan of yeast cells is crucial, as it affects their reductive metabolism capacity, essential for enhancing beer shelf life by removing staling compounds, while the selection of yeast strains, including non-*Saccharomyces* yeasts, has gained importance among brewers aiming to innovate and develop specialty beers with unique organoleptic profiles (Wauters et al., 2021). Moreover, optimization processes targeting yeast performance are equally important, as they can significantly enhance the aroma complexity and overall quality of fermented beverages (Barbulescu et al., 2024; Dumitrache et al., 2024). Furthermore, selecting yeast strains, particularly non-*Saccharomyces* species, is increasingly important for brewers aiming to innovate and create specialty beers with unique sensory profiles, as these yeasts possess specific enzymatic activities that distinctly influence beer taste and aroma, offering valuable biotechnological potential to the brewing industry (Iorizzo et al., 2021; Marongiu et al., 2015; Mascia et al., 2016)). Overall, yeasts have a multifaceted role in barrel-aged beer production, encompassing fermentation, flavor enhancement, and opportunities for innovation.

CONCLUSIONS

Barrel-aging is a traditional yet increasingly innovative practice in craft brewing, offering a unique avenue for flavor development through the complex interaction between beer, wood, and microorganisms. This review highlights the multifactorial nature of the maturation process, where variables such as wood species, toasting levels, the previous contents of the barrel (whiskey, rum, wine, or cognac), and the metabolic activity of yeasts play critical roles in shaping the final sensory profile.

American oak contributes intense flavors such as vanilla, coconut, and sweet spice, while European oaks offer more subtle, tannin-rich notes. The reuse of barrels previously containing spirits or wines allows for the transfer of residual compounds that enhance complexity and depth. Yeasts, particularly *Brettanomyces* and certain non-*Saccharomyces* strains, not only survive barrel conditions but also actively contribute to aroma stabilization by reducing oxidative off-flavors and producing distinctive esters and phenols.

The aging process is especially suited to high-alcohol, full-bodied styles such as imperial stouts, porters, and strong ales, which can better integrate and express the compounds extracted during maturation. While barrel-aging can significantly elevate a beer's aromatic profile, it also poses challenges related to oxidation, microbial variability, and process reproducibility.

Future directions in this field may include the exploration of novel wood types, greater control of micro-oxygenation, and tailored microbial inoculations to standardize and optimize flavor outcomes. Barrel-aging thus represents a compelling convergence of tradition, biochemistry, and craftsmanship within the evolving landscape of specialty beer production.

REFERENCES

Alexander, T. R., & Valliere, B. L. E. (2019). *The Professional Handbook of Cider Tasting*. CABI.

Barbulescu, I. D., Ciric, A. I., Begea, M., Matei, P. M., Moisac, A., Nita, A., Frincu, M., Banita, C.-D., Teodorescu, R. I., Tudor, V., & Dumitrache, C. (2022). Tamaioasa Romaneasca and Busuioaca De Bohotin Grapes- Valuable Sources for Wine Production. *SCIENTIFIC PAPERS-SERIES B-HORTICULTURE*, *66*(2), 171–176.

Bărbulescu, I. D., Ghica, M. V., Begea, M., Albu Kaya, M. G., Teodorescu, R. I., Popa, L., Mărculescu, S. I., Cîrîc, A. I., Dumitrache, C., Lupuliasa, D., Matei, F., & Dinu-Pîrvu, C.-E. (2021). Optimization of the Fermentation Conditions for Brewing Yeast Biomass Production Using the Response Surface Methodology and Taguchi Technique. *Agriculture*, *11*(12), 1237. https://doi.org/10.3390/agriculture11121237

Barbulescu, I. D., Radoi-Encea, R. S., Dumitrache, C., Ghica, M. V., Frincu, M., Begea, M., Tudor, V., Boroiu, E.-M., & Teodorescu, R. I. (2024). Optimisation of Culture Media Factors for Active Wine Yeast Biomass Production. *SCIENTIFIC PAPERS-SERIES E-LAND RECLAMATION EARTH OBSERVATION & SURVEYING ENVIRONMENTAL ENGINEERING*, *13*, 866–875.

Bjergso, M. B., & Pang, P. (2015). *Mikkeller's Book of Beer*. Jacqui Small.

Bongaerts, D., De Roos, J., & De Vuyst, L. (2021). Technological and Environmental Features Determine the Uniqueness of the Lambic Beer Microbiota and Production Process. *Applied and Environmental Microbiology*, *87*(18), e00612-21. https://doi.org/10.1128/AEM.00612-21

Bossaert, S., Kocijan, T., Winne, V., Van Opstaele, F., Schlich, J., Herrera-Malaver, B., Verstrepen, K. J., De Rouck, G., Lievens, B., & Crauwels, S. (2022). *Development of a tractable model system to mimic wood-ageing of beer on a lab scale*. https://doi.org/10.1101/2022.03.11.483928

Bossaert, S., Winne, V., Van Opstaele, F., Buyse, J., Verreth, C., Herrera-Malaver, B., Van Geel, M., Verstrepen, K. J., Crauwels, S., De Rouck, G., & Lievens, B. (2021). Description of the temporal dynamics in microbial community composition and beer chemistry in sour beer production via barrel ageing of finished beers. *International Journal of Food Microbiology*, *339*, 109030. https://doi.org/10.1016/j.ijfoodmicro.2020.109030

Bossaert, S., Winne, V., Van Opstaele, F., Buyse, J., Verreth, C., Herrera-Malaver, B., Verstrepen, K. J., De Rouck, G., Crauwels, S., & Lievens, B. (2022). Impact of wood species on microbial community composition, beer chemistry and sensory characteristics during barrel-ageing of beer. *International Journal of Food Science & Technology*, *57*(2), 1122–1136. https://doi.org/10.1111/ijfs.15479

Butrón-Benítez, D., Valcárcel-Muñoz, M. J., García-Moreno, M. V., Rodríguez-Dodero, M. C., & Guillén-Sánchez, D. A. (2024). Chemical and Sensory Profile of Grape Distillates Aged in Quercus alba Casks Previously Used for Sherry Wine or Brandy. *Molecules*, *29*(22), Article 22. https://doi.org/10.3390/molecules29225303

Cabras, I., Kogler, D. F., Davies, R. B., & Higgins, D. (2023). Beer, brewing, and regional studies. *Regional Studies*, *57*(10), 1905–1908. https://doi.org/10.1080/00343404.2023.2216066

Caldeira, I., Anjos, O., Portal, V., Belchior, A. P., & Canas, S. (2010). Sensory and chemical modifications of wine-brandy aged with chestnut and oak wood fragments in comparison to wooden barrel. *Analytica Chimica Acta*, *660*, 43–52. https://doi.org/10.1016/j.aca.2009.10.059

Carpena, M., Pereira, A. G., Prieto, M. A., & Simal-Gandara, J. (2020). Wine Aging Technology: Fundamental Role of Wood Barrels. *Foods*, *9*(9), Article 9. https://doi.org/10.3390/foods9091160

Castro, L., & Ross, C. (2018). Correlation Between Sensory Descriptive Analysis and Volatile Composition of Beer Using Multivariate Analysis: The Effect of the Nonvolatile Matrix on the Sensory Perception and Volatile Fraction Behavior. *Journal of the American Society of Brewing Chemists*, *76*, 86–95. https://doi.org/10.1080/03610470.2018.1433943

Coelho, E., Magalhães, J., Pereira, F. B., Macieira, F., Domingues, L., & Oliveira, J. M. (2019). Volatile fingerprinting differentiates diverse-aged craft beers. *LWT*, *108*, 129–136. https://doi.org/10.1016/j.lwt.2019.03.044

D'Ascanio, V., Greco, D., Abbasciano, M., & Avantaggiato, G. (2024). Optimization and in-house validation of the analytical procedure for official control of bentonites as aflatoxin inactivators. *Food Chemistry*, *432*, 137198. https://doi.org/10.1016/j.foodchem.2023.137198

De Roos And, J., & De Vuyst, L. (2022). Lambic Beer, A Unique Blend of Tradition and Good Microorganisms. In F. J. De Bruijn, H. Smidt, L. S. Cocolin, M. Sauer, D. Dowling, & L. Thomashow (Eds.), *Good Microbes in Medicine, Food Production, Biotechnology, Bioremediation, and Agriculture* (1st ed., pp. 225–235). Wiley. https://doi.org/10.1002/9781119762621.ch18

De Roos, J., Van Der Veken, D., & De Vuyst, L. (2019). The Interior Surfaces of Wooden Barrels Are an Additional Microbial Inoculation Source for Lambic Beer Production. *Applied and Environmental Microbiology*, *85*(1), e02226-18. https://doi.org/10.1128/AEM.02226-18

Del Fresno, J. M., Morata, A., Loira, I., Escott, C., & Suárez Lepe, J. A. (2020). Evolution of the Phenolic Fraction and Aromatic Profile of Red Wines Aged in Oak Barrels. *ACS Omega*, *5*(13), 7235–7243. https://doi.org/10.1021/acsomega.9b03854

Díaz-Maroto, M. C., Guchu, E., Castro-Vázquez, L., De Torres, C., & Pérez-Coello, M. S. (2008). Aroma-active compounds of American, French, Hungarian and Russian oak woods, studied by GC–MS and GC–O. *Flavour and Fragrance Journal*, *23*(2), 93–98. https://doi.org/10.1002/ffj.1859

Dumitrache, C., Frîncu, M., Alexandru, T., Diana, I., Tudor, V., & Ionuț, R. (2020). Identification by PCR ITS-RFLP technique of new yeast isolates from Pietroasa vineyard. *Scientific Papers. Series B, Horticulture*, *LXIV*(1), 287–293. https://horticulturejournal.usamv.ro/pdf/2020/issue_1/Art43.pdf

Dumitrache, C., Ghica, M. V., Frîncu, M., Bărbulescu, I. D., Begea, M., Diguță, C. F., Baniță, C., Cotea, V. V., Israel-Roming, F., & Teodorescu, R. I. (2024). Bioprocess Optimization by Taguchi Design and Response Surface Analysis for Obtaining Active Yeast Used in Vinification. *Fermentation*, *10*(8), 413. https://doi.org/10.3390/fermentation10080413

Franitza, L., Granvogl, M., & Schieberle, P. (2016). Influence of the Production Process on the Key Aroma Compounds of Rum: From Molasses to the Spirit. *Journal of Agricultural and Food Chemistry*, *64*(47), 9041–9053. https://doi.org/10.1021/acs.jafc.6b04046

Frîncu, M., Dumitrache, C., & Ionuț, R. (2024). *PHYSICO-CHEMICAL COMPOSITION OF*

DIFFERENT GRAPE VARIETIES FROM PIETROASA VINEYARD.

Frincu, M., Dumitrache, C., Petre, A.-C., Mot, A., Teodorescu, R. I., Barbulescu, D. I., Tudor, V., & Matei, F. (2023). Physico-Chemical Characterization of Some Sources of Grape Marc from Pietroasa Vineyard. *AGROLIFE SCIENTIFIC JOURNAL*, *12*(1), 81–86.

Gadrat, M., Lavergne, J., Emo, C., Teissedre, P. L., & Chira, K. (2022). Sensory characterisation of Cognac eaux-de-vie aged in barrels subjected to different toasting processes. *OENO One*, *56*(1), Article 1. https://doi.org/10.20870/oeno-one.2022.56.1.4853

Gomez-Cordoves, C., & Gonzalez-SanJose, M. L. (2002, May 1). *Interpretation of Color Variables during the Aging of Red Wines: Relationship with Families of Phenolic Compounds* (world). ACS Publications; American Chemical Society. https://doi.org/10.1021/jf00051a001

González-Centeno, M. R., Teissedre, P.-L., Thibon, C., & Chira, K. (2020). Does barrel size influence white wine aging quality? *IVES Technical Reviews, Vine and Wine*. https://doi.org/10.20870/IVES-TR.2020.3305

Goullet, A., Hanaei, F., Vallet, N., Nonier, M.-F., Vivas, N., Vivas, N., & Picard, M. (2023). A sensory characterization of some N-heterocycles in model spirit matrix: Influence of ethanol and several chemical features on aroma perception. *Flavour and Fragrance Journal*, *38*(2), 108–120. https://doi.org/10.1002/ffj.3729

Iorizzo, M., Coppola, F., Letizia, F., Testa, B., & Sorrentino, E. (2021). Role of Yeasts in the Brewing Process: Tradition and Innovation. *Processes*, *9*(5), 839. https://doi.org/10.3390/pr9050839

Karathanos, A., Soultani, G., Kontoudakis, N., & Kotseridis, Y. (2024). Impact of Different Wood Types on the Chemical Composition and Sensory Profile of Aged Tsipouro: A Comparative Study. *Beverages*, *10*(3), Article 3. https://doi.org/10.3390/beverages10030076

Machado, S. (2019). *Craft beer in Portugal: A study about consumers, perceptions, drivers and barriers of consumption*. repositorio.ucp.pt. https://repositorio.ucp.pt/handle/10400.14/29285

Maga, J. A. (1989). The contribution of wood to the flavor of alcoholic beverages. *Food Reviews International*, *5*(1), 39–99. https://doi.org/10.1080/87559128909540844

Maicas, S. (2020). The Role of Yeasts in Fermentation Processes. *Microorganisms*, *8*(8), 1142. https://doi.org/10.3390/microorganisms8081142

Mangwanda, T., Johnson, J., Mani, J., Jackson, S., Chandra, S., Mckeown, T., White, S., & Naiker, M. (2021). Processes, Challenges and Optimisation of Rum Production from Molasses-A Contemporary Review. *Fermentation*, *7*, 21. https://doi.org/10.3390/fermentation7010021

Marongiu, A., Zara, G., Legras, J. L., & ... (2015). Novel starters for old processes: Use of Saccharomyces cerevisiae strains isolated from artisanal sourdough for craft beer production at a brewery scale. *Journal of Industrial …*. https://doi.org/10.1007/s10295-014-1525-1

Martínez-Gil, A. M., Del Alamo-Sanza, M., Del Barrio-Galán, R., & Nevares, I. (2022). Alternative Woods in Oenology: Volatile Compounds Characterisation of Woods with Respect to Traditional Oak and Effect on Aroma in Wine, a Review. *Applied Sciences*, *12*(4), 2101. https://doi.org/10.3390/app12042101

Mascia, I., Fadda, C., Karabín, M., Dostálek, P., & Del Caro, A. (2016). Aging of craft durum wheat beer fermented with sourdough yeasts. *LWT*, *65*, 487–494. https://doi.org/10.1016/j.lwt.2015.08.026

Mastanjević, K., Krstanović, V., Lukinac, J., Jukić, M., Lučan, M., & Mastanjević, K. (2019). Craft brewing – is it really about the sensory revolution? *KVASNY PRUMYSL*, *65*(1), 13–16. https://doi.org/10.18832/kp2019.65.13

Maza Gomez, S. (2002). Rum aroma descriptive analysis. *LSU Master's Theses*. https://doi.org/10.31390/gradschool_theses.2770

McCarthy, E., & Ewing-Mulligan, M. (2006). *Wine for dummies* (4th ed). Wiley Pub.

Mutz, Y. S., Rosario, D. K. A., & Conte-Junior, C. A. (2020). Insights into chemical and sensorial aspects to understand and manage beer aging using chemometrics. *Comprehensive Reviews in Food Science and Food Safety*, *19*(6), 3774–3801. https://doi.org/10.1111/1541-4337.12642

Nasuti, C., Ruffini, J., Sola, L., Di Bacco, M., Raimondi, S., Candeliere, F., & Solieri, L. (2023a). *Sour Beer as Bioreservoir of Novel Craft Ale Yeast Cultures*. https://doi.org/10.20944/preprints202307.0964.v1

Nasuti, C., Ruffini, J., Sola, L., Di Bacco, M., Raimondi, S., Candeliere, F., & Solieri, L. (2023b). Sour Beer as Bioreservoir of Novel Craft Ale Yeast Cultures. *Microorganisms*, *11*(9), 2138. https://doi.org/10.3390/microorganisms11092138

Nilsen, D. (2024). *How To Pair Bourbon Barrel-Aged Beers with Craft Chocolate*. Www.Beantobarstool.Com. https://www.beantobarstool.com/blog/pairing-bba

Noviello, M., Paradiso, V. M., Natrella, G., Gambacorta, G., Faccia, M., & Caponio, F. (2024). Application of toasted vine-shoot chips and ultrasound treatment in the ageing of Primitivo wine. *Ultrasonics Sonochemistry*, *104*, 106826. https://doi.org/10.1016/j.ultsonch.2024.106826

Oliver, G., & Colicchio, T. (2011). *The Oxford Companion to Beer*. Oxford University Press. https://books.google.ro/books?id=oWQdjnVo2B0C

Ortega-Heras, M., González-Sanjosé, M. L., & González-Huerta, C. (2007). Consideration of the influence of aging process, type of wine and oenological classic parameters on the levels of wood volatile compounds present in red wines. *Food Chemistry*, *103*(4), 1434–1448. https://doi.org/10.1016/j.foodchem.2006.10.060

Paulette, T. (2024). Beer in world history. In T. Paulette, *In the Land of Ninkasi* (1st ed., pp. 1–16). Oxford University PressNew York. https://doi.org/10.1093/oso/9780197682449.003.0001

Picard, M., Nonier, M.-F., Vivas, N., & Vivas, N. (2021). The dynamic of roasted aroma compounds release from oak wood: Investigation of the heating barrel process and some spirit maturation parameters. *Wood Science and Technology*, *55*(6), 1821–1839. https://doi.org/10.1007/s00226-021-01330-5

Picard, M., Oulieu, C., Nonier, M.-F., Vivas, N., & Vivas, N. (2023). The role of oak wood in the mint and floral notes of whisky: Identification of common terpenoids by aromatic fractionation. *Journal of the Institute of Brewing*, *129*(1), 62–79. https://doi.org/10.58430/jib.v129i1.8

Pilcher, J. M. (2025). Before Hops. In J. M. Pilcher, *Hopped Up* (1st ed., pp. 17–41). Oxford University PressNew York. https://doi.org/10.1093/oso/9780197676042.003.0002

Poisson, L., & Schieberle, P. (2008). Characterization of the Most Odor-Active Compounds in an American Bourbon Whisky by Application of the Aroma Extract Dilution Analysis. *Journal of Agricultural and Food Chemistry*, *56*(14), 5813–5819. https://doi.org/10.1021/jf800382m

Recamales, Á. F., Sayago, A., González-Miret, M. L., & Hernanz, D. (2006). The effect of time and storage conditions on the phenolic composition and colour of white wine. *Food Research International*, *39*(2), 220–229. https://doi.org/10.1016/j.foodres.2005.07.009

Ribéreau-Gayon, P. (2006). *Handbook of enology: Volume 2: The chemistry of wine stabilization and treatments* (2nd ed). John Wiley.

Saison, D. (2011). Improved Flavor Stability by Aging Beer in the Presence of Yeast. *Journal of the American Society of Brewing Chemists*, *69*. https://doi.org/10.1094/ASBCJ-2011-0127-01

Saison, D., De Schutter, D. P., Vanbeneden, N., Daenen, L., Delvaux, F., & Delvaux, F. R. (2010). Decrease of Aged Beer Aroma by the Reducing Activity of Brewing Yeast. *Journal of Agricultural and Food Chemistry*, *58*(5), 3107–3115. https://doi.org/10.1021/jf9037387

Sanna, V., & Pretti, L. (2015). Effect of wine barrel ageing or sapa addition on total polyphenol content and antioxidant activities of some Italian craft beers. *International Journal of Food Science & Technology*, *50*(3), 700–707. https://doi.org/10.1111/ijfs.12666

Schneider, B. M. (2023). Flavor Compounds Found in Barrel Aged Beers. In *Chemistry of Alcoholic Beverages* (Vol. 1455, pp. 159–172). American Chemical Society. https://doi.org/10.1021/bk-2023-1455.ch008

Seo, M.-J. (2024). Fermented Foods and Food Microorganisms: Antioxidant Benefits and Biotechnological Advancements. *Antioxidants*, *13*(9), 1120. https://doi.org/10.3390/antiox13091120

Shayevitz, A., Harrison, K., & Curtin, C. D. (2020). *Barrel-Induced Variation in the Microbiome and Mycobiome of Aged Sour Ale and Imperial Porter Beer* (p. 299343 Bytes) [Dataset]. Taylor & Francis. https://doi.org/10.6084/M9.FIGSHARE.12848766

Silva, H. G. da, Silva, H. G. da, Souza, P. G. de, & Pinheiro, C. L. (2022). Avaliação físico-química e sensorial da cerveja artesanal estilo Wood e Barrel Aged adicionada de chip de jatobá (Hymenaea courbaril L.). *Brazilian Journal of Science*, *1*(3), Article 3. https://doi.org/10.14295/bjs.v1i3.39

Spitaels, F., Wieme, A. D., Snauwaert, I., Vuyst, L. D., & Vandamme, P. (2017). Microbial Ecology of Traditional Beer Fermentations. In *Brewing Microbiology: Current Research, Omics and Microbial Ecology*. Caister Academic Press. https://doi.org/10.21775/9781910190616.07

Starowicz, M., & Zieliński, H. (2019). How Maillard Reaction Influences Sensorial Properties (Color, Flavor and Texture) of Food Products? *Food Reviews International*, *35*, 1–19. https://doi.org/10.1080/87559129.2019.1600538

Stegăruș, D. I., Călugăr, A., Tanase, C., Muscă, A., Botoran, O. R., Manolache, M., Babeș, A. C., Bunea, C., Gál, E., Bunea, A., & Coldea, T. E. (2021). Influence of Oak Chips and Oak Barrel Ageing on Volatile Profile in Chardonnay Wine of Romania. *Applied Sciences*, *11*(8), Article 8. https://doi.org/10.3390/app11083691

Sterckx. (2012). Wood Aging of Beer. Part II: Influence of Wood Aging Parameters on Monophenol Concentrations. *Journal of the American Society of Brewing Chemists*, *70*. https://doi.org/10.1094/ASBCJ-2011-1201-02

Styger, G., Prior, B., & Bauer, F. F. (2011). Wine flavor and aroma. *Journal of Industrial Microbiology and Biotechnology*, *38*(9), 1145. https://doi.org/10.1007/s10295-011-1018-4

Tamanna, N., & Mahmood, N. (2015). Food Processing and Maillard Reaction Products: Effect on Human Health and Nutrition. *International Journal of Food Science*, *2015*, 526762. https://doi.org/10.1155/2015/526762

Tarko, T., Krankowski, F., & Duda-Chodak, A. (2023). The Impact of Compounds Extracted from Wood on the Quality of Alcoholic Beverages. *Molecules*, *28*(2), Article 2. https://doi.org/10.3390/molecules28020620

Tham, A., Campton, J., & Cooper-McKenzie, B. (2024). Tapping into flavourful journeys: A systematic review and application of craft beer tourism frameworks. *International Journal of Wine Business Research*, *36*(2), 184–206. https://doi.org/10.1108/IJWBR-03-2023-0013

Tsakiris, A., Kallithraka, S., & Kourkoutas, Y. (2014). Grape brandy production, composition and sensory evaluation: Grape brandy production, composition and evaluation. *Journal of the Science of Food and Agriculture*, *94*(3), 404–414. https://doi.org/10.1002/jsfa.6377

Uselmann, V., & Schieberle, P. (2015). Decoding the Combinatorial Aroma Code of a Commercial Cognac by Application of the Sensomics Concept and First Insights into Differences from a German Brandy. *Journal of Agricultural and Food Chemistry*, *63*(7), 1948–1956. https://doi.org/10.1021/jf506307x

Valentoni, A., Santoru, A., Sanna, M., Fanari, M., Porcu, M. C., Fadda, A., Sanna, D., & Pretti, L. (2022). Evolution of sensory analysis attributes and volatile aging markers in bottle fermented craft beers during

storage at different temperatures. *Food Chemistry Advances*, *1*, 100151. https://doi.org/10.1016/j.focha.2022.100151

Villacreces, S., Blanco, C. A., & Caballero, I. (2022). Developments and characteristics of craft beer production processes. In *Food bioscience*. Elsevier. https://www.sciencedirect.com/science/article/pii/S2212429221006209

Viviani, A., Moreno, J., & Peinado, R. A. (2007). Differentiation of young red wines obtained in a warm climate region. *International Journal of Food Science and Technology*, *42*(5), 523–527. https://doi.org/10.1111/j.1365-2621.2006.01240.x

Vrînceanu, C.-R., Diguță, F. C., Cudalbeanu, M. D., Ortan, A., Mihai, C., Bărbulescu, I. D., Frîncu, M., Begea, M., Matei, F., & Teodorescu, R. I. (2025). Exploring the Potential of Torulaspora delbrueckii, Starmerella bacillaris, and Saccharomyces cerevisiae as a Probiotic Starter for Craft Beer Production. *Foods*, *14*(9), Article 9. https://doi.org/10.3390/foods14091608

Vrînceanu, C.R.; Bărbulescu, I.D.; Matei, F.; Begea, M.; Tudor, V.; Frîncu, M.; Teodorescu R.I. Review: Actual approaches for the craft beer fermentations. Sci Pap-Ser B-Hortic, 2022, LXVI(1), 944-949

Wauters, R., Britton, S. J., & Verstrepen, K. J. (2021). Old yeasts, young beer—The industrial relevance of yeast chronological life span. *Yeast*, *38*(6), 339–351. https://doi.org/10.1002/yea.3650

Winstel, D., Gammacurta, M., Waffo-Téguo, P., & Marchal, A. (2024). Identification of Two New Taste-Active Compounds in Oak Wood: Structural Elucidation of Potential β-Methyl-γ-octalactone Precursors and Quantification in Spirits. *Journal of Agricultural and Food Chemistry*, *72*(37), 20592–20602. https://doi.org/10.1021/acs.jafc.4c04799

Withers, E. T. (2017). The impact and implications of craft beer research: An interdisciplinary literature review. *Craft Beverages and Tourism, Volume 1: The Rise of …*. https://doi.org/10.1007/978-3-319-49852-2_2

Yi, Z. (2013). *Method for producing compound rum from sugarcane juice and sugarcane molasses* (China Patent CN101760378B). https://patents.google.com/patent/CN101760378B/en

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

LEONTOPODIUM NIVALE SSP. *ALPINUM* (CASS.) BIOTECHNOLOGY APPLIED FOR ECONOMIC AND CONSERVATIVE PURPOSE

Mihaela Irina HOLOBIUC, Alexandra-Gabriela CIOCAN, Rodica CATANĂ

Institute of Biology Bucharest of Romanian Academy, 296 Splaiul Independentei, 060031, District 6, P.O. Box 56-53, Bucharest, Romania

Corresponding author email: alexandra.ciocan@ibiol.ro

Abstract

Plant biotechnology ensures the production of a wide range of compounds for industry, pharmacy, cosmetics and medicine. Tissue cultures can provide useful metabolites all-round the year under controlled conditions with reduced expenses. L. nivale subsp. alpinum (Cass.) is a protected taxon, of ornamental value and economic importance.
Our aim was to produce highly regenerative cultures for mass production and synthetic seeds development, as mean for multiplication and preservation.
The effect of different growth factors was studied concerning regeneration through direct morphogenesis and somatic embryogenesis. The presence of 2.4-D and Dicamba alone or combined with low levels of kinetin favoured somatic embryogenesis. Histological samples of embryogenic aggregates were analysed using optical and scanning electron microscopy. Somatic embryogenesis provides a large mass of plant material for basic studies and for economic purposes.
For synthetic seeds production, embryogenic aggregates of 2-3 mm diameter were incubated in 3% sodium alginate in a modified $CaCl_2$-free MS liquid medium. Subsequently, dehydrated seeds were cultured on MS agar solidified medium added with growth regulators to test the artificial seeds' growth.
We improved the regeneration rate through somatic embryogenesis and, subsequently, produced artificial seeds useful for multiplication and conservative purposes.

Key words*: Leontopodium nivale, somatic embryogenesis, synthetic seeds.*

INTRODUCTION

L. nivale ssp. *alpinum* (syn. *L. alpinum*) is a perennial alpine herbaceous, hemicryptophyte taxon known as Edelweiss, growing on rocky substrates as limestones in different European alpine area as in the Alps, Pirini, Rhodopes and Carpathian Mountains. This taxon is threatened in several European countries and populations in the past declined due to extensive collection; having an extent distribution and lacking information on population reduction, it is listed as Least Concern (Khela, 2013). *L. nivale* flowers are highly prized and have been collected from the wild (Pace et al., 2009), a practice that is now regulated or banned in many European countries (Kozuharova et al., 2018).

This alpine plant became of great interest in the latest years for pharmaceutics and cosmetics industry, owing to its valuable compounds with biological activities (Tauchen & Kokoska, 2016). Specifically, different classes of compounds such as terpenoids (analogues of sesquiterpenes, bisabolenes), phenylpropanoids (phenolic acids, flavonoids, coumarins, lignans), fatty acids and polyacetylenes, extracts and compounds have been characterized and found to have a broad spectrum of pharmacological activities on the cardiovascular and nervous system, but also anti-inflammatory, antimicrobial, antioxidant and chemo-protective effects.

The Edelweiss extract proved to be a valuable constituent of anti-aging skin treatments, being included in a wide number of dermo-cosmetical products already existing on the market, due to its antioxidant and anti-inflammatory effects (Campiche et al., 2022). Owing to the large economic and industrial demand for compounds and Edelweiss' protected status in many countries, alternative methods were developed, of which the biotechnological approach proved to be reliable and affordable (Pralea et al., 2021).

Small scale *ex situ* cultivation (Kozuharova et al., 2018) and *in vitro* biotechnological approaches are economically viable alternatives to the production via chemical synthesis of useful compounds (Ciocan et al., 2023).

Biotechnology has the potential for large-scale plant production in horticulture, forestry and medicinal plants.

The elaboration of highly reproducible, efficient micropropagation protocols provides continuous plant production (Anis & Ahmad, 2016) and sustains the germplasm preservation in a tissue bank, ensuring material for slow-growth culture and cryopreservation (Kulus, 2016). Plant tissue cultures can provide high-quality planting material throughout the year, irrespective of season and weather (Shahzad et al., 2017).

In vitro seed germination, vegetative propagation and acclimatization methods contribute to propagule production, both for preservation strategies and for translocation or reintroduction projects (Chauhan, 2016).

In the case of endangered species, the process might require modified, improved or combined strategies for *ex situ* preservation (Sarasan et al., 2006; Reed et al., 2011; Hurdu et al., 2022).

Micropropagation represents an alternative to overcome population decline in the natural habitat due to climatic changes and antropization and also the establishment of valuable metabolite-producing cell lines (Fasciani et al., 2011).

The biotechnological approach generally targets three main directions: (1) the production and optimization of callus and suspension cultures for useful secondary metabolites' production, including different ways of stimulation or elicitation; (2) the production of hairy roots after genetic transformation (Wawrosch et al., 2014) and (3) *in vitro* micropropagation and the production of secondary metabolites (Hook, 1994). In our work, we aimed to improve the *in vitro* production of *L. nivale* ssp. *alpinum* plant material useful for ornamental and economic purposes, starting from aseptically germinated seeds, induction and evaluation of the micropropagation through direct morphogenesis and somatic embryogenesis, along with the characterization of somatic embryogenesis, as an optimal mean to multiply and to preserve this taxon through synthetic seeds production.

MATERIALS AND METHODS

Seed sterilization

Commercial seeds (Germany) were sterilized following a three step protocol: (a) a two-hour wash in running tap water; (b) short immersion in 70^0 ethanol, followed by a treatment with mercuric chloride 0.1% (12 minutes) and (c) three washes with sterile distilled water. The sterilized seeds were cultivated on MS (Murashige & Skoog, 1962) agar solidified (8 g/l) medium, added with 20 g/l sucrose, cultured 6-10 seeds/ Petri dish.

In vitro culture initiation

Seedlings germinated after 7-10 days and were used as explants for establishing *in vitro* cultures. Different media variants were tested, based on the MS formula (Murashige, Skoog, 1962) and supplemented with Gamborg B5 vitamins (Gamborg et al., 1968), 30 g/l sucrose, 7 g/ l agar (Duchefa Biochemie), pH adjusted to 5.8 and additional compounds (Table 1).

The subcultures were performed every 4 weeks. Cultures were maintained in the growth chamber with a light intensity of 55 µECmu-$2s^{-1}$ at 25°C and a photoperiod of 16 light /8 h darkness.

Direct morphogenesis

To induce the morphogenetic response, 5 seedlings were inoculated in 6 cm/10 cm high glass jars in 3 replicates/variant, on media based on the MS formula added with 30 g/l sucrose, Gamborg vitamins and different plant regulators (Table 1). After regeneration, 9-10 shoots were further cultivated in autoclavable polypropylene dishes (9x10x10 cm) on Murashige Skoog medium, supplemented with 20 g/l sucrose, 0.5 g/l charcoal and solidified with 8 g/l Duschefa agar.

Callus and somatic embryogenesis induction

For the callus and somatic embryogenesis induction, fragments of *in vitro* regenerated seedlings (roots and leaves) were used as explants. Fragments of about 5 mm long were cultured on 6 cm diameter Petri dishes, culturing five explants x 3 repetitions/medium variant. Several media variants based on the MS formula (Murashige & Skoog, 1962) added with 30 g/l sucrose and B5 vitamins according Gamborg formula were tested (Table 2). After one month, embryogenic calli of 1 cm diameter were subcultured on MS 1/2 hormone free medium supplemented with 500 mg/l charcoal,

20 g/l sucrose, solidified with 8 g/l Duschefa agar, at pH 5.8, to ensure embryos' development.

Table 1. Media variants tested for direct morphogenesis

Media	Growth factors (µM)				Other compounds (mg/L)		
	BAP	Kin	Zea	NAA	Glut	$CaCO_3$	Ch
MSL 1	4.44	-	-	0.18	-	-	-
MSL 2	-	4.6	-	0.18	-	-	-
MSL 3	4.44	4.6	-	0.90	-	-	-
MSL 4	4.44	4.6	-	0.36	-	-	-
MSL 5	4.44	4.6	-	0.36	-	100	-
MSL 6	4.44	4.6	-	0.36	250	-	-
MSL 7	4.44	4.6	-	0.36	-	-	300
MSL 8	-	-	4.56	0.18	-	-	-

Legend: 6-BAP - 6-benzylaminopurine; Kin - kinetin; Zea - zeatin; NAA - 1-naphthaleneacetic acid; IAA - indole-3-acetic acid; Glut - glutamine; Ch- casein hydrolisate

Table 2. Media variants tested for callus and somatic embryogenesis induction

Variants	Growth factors (µM)				
	2,4 D	Dic	IBA	NAA	Kin
MSL9	-	2,26	-	-	-
MSL10	2.26	-	-	-	
MSL11	-	4.52	4.9	-	0.92
MSL12	4.52	-	4.9	-	0.92
MSL13	-	2.26	-	-	0.92
MSL14	2.26	-	-	-	0.92
MSL15	-	4.52	-	-	0.92
MSL16	4.52	-	-	-	0.92
MSL17	9.04	-	-	-	4.6
MSL18	4.52	-	-	-	9.2
MSL19	-	-	-	1.8	9.2
MSL20	-	-	-	3.6	4.6

Legend: Kin - kinetin; 2,4-D - 2,4-dichlorophenoxyacetic acid; Dic- Dicamba; IBA - indole butyric acid

In vitro culture characterization

The efficiency of plant production via direct morphogenesis was evaluated taking into account the mean number of regenerants/initial inoculum and the maximum shoots length (cm) after two months.

The effect of different combinations of growth factors was analysed regarding callus production and regeneration through morphogenesis and somatic embryogenesis. Somatic embryogenesis was evaluated taking into account the explant's source (leaves and roots). The rate of callusing of initial explants (the number of calli developed/ initial explant x100) was evaluated in the first month of culture.

The rate of regenerative calli (the number of regenerated calli/total number of cultured calli x100) and the mean number of regenerants/ explant were recorded for each experimental variant after two months. Ten initial explants were taken into consideration for each variant for somatic embryos evaluation.

Microscopical analysis

Optical microscope

For the histological observations, the aggregates of embryogenic cultures were prepared in several stages: (a) fixation in formaldehyde 3.7%; (b) serial dehydration in ethanol for 15 minutes (70, 80, 96°); (c) 6-hour immersion in butyl alcohol; (d) immersion of the samples in benzene 2 times x 15 minutes and (e) embedding in paraffin at 60° for 2 hours. Paraffin-embedded sections were sectioned using an LKB microtome at 7 µm. For removal of paraffin, the sections were immersed in xylene for 30 minutes, transferred in alcohol series 96-15° for 15 minutes, washed and stained in Hemalaun Mayer for 10 minutes, followed by washing in running tap water.

For stereomicroscopy analysis, embryogenic cultures were visualized under a binocular microscope Stemi-2000C Zeiss. For optical microscope analysis, fresh embryogenic cultures were stained with 0.1% methylene blue dissolved in a drop of distilled water. The samples were prepared by spreading the plant material on a slide and covered with glass.

For scanning electron microscopy (SEM), embryogenic aggregates were fixed in 3% glutaraldehyde adjusted to pH 6.8 in 0.1 M cacodylate phosphate buffer for 24 h at 4 °C. The tissue was washed in the same buffer, post fixated for 2 h in a similarly buffered 1% osmium tetroxide solution, dehydrated in ethanol series (50, 70, 90, 100°) for 10 minutes, air dehydrated for 130 minutes and finally mounted on slide and coated with gold nanoparticles. The prepared samples were examined and photographed using a scanning electron microscope Jeol JSM 6610LV and 20 kV acceleration.

Synthetic seeds production

Fragments of embryogenic aggregates of 2-3 mm diameter were incubated in 3% sodium alginate (10 min) in a modified $CaCl_2$-free MS liquid medium supplemented with 87 mM sucrose and sterilized by autoclaving. Using a cut pipette tip (5 mm diameter), they were gently dropped into liquid MS supplemented with 0.1 M $CaCl_2$ and left for 30 min. The obtained artificial seeds were washed with sterile distilled water and osmotically dehydrated in

100 ml liquid MS medium, with gradually increased sucrose concentrations: 0.25 M (24 h), 0.50 M (24 h), 0.75 (24 h), being kept in agitation using an orbital shaker at 130 rpm, in the dark. Sterile synthetic seeds were kept one week at 4°C. Subsequently, seeds were cultured on MSL9 or MSL10 agar solidified medium to allow further development.

Statistical analysis

The experimental data obtained were checked for normality, using Shapiro-Wilk's test and homogeneity of variance, using Levene's test. Afterward, they were subjected to a one-way analysis of variance (ANOVA), to assess the statistical significance, followed by a Tukey HSD test to determine the differences between means. All analyses were performed using the software R 4.4.1 (R Foundation for Statistical Computing, Austria) and Origin, Version 2024 (OriginLab Corporation, Northampton, MA, USA). Error bars of graphics represent the standard deviation of mean value (±SD) and *p*-values $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***) were considered statistically significant.

RESULTS AND DISCUSSIONS

Seed germination

The germination of seeds started after 7-10 days. The registered rate of sterilization was 80% and a maximum germination rate of 70 % was obtained after two weeks.

After the initiation of aseptic tissue cultures, plant regeneration was conducted through two main developmental processes: morphogenesis and somatic embryogenesis, based on plant cell totipotency.

In the morphogenetic process, developed roots and shoots establish vascular connections with the tissues of the origin explant, while in the case of somatic embryos (Se), no vascular connections with the explant tissues are present, being similar to zygotic embryos, with a bipolar structure, but characterized by the absence of developmental arrest and the endosperm presence (Faure et al., 1998).

Direct morphogenesis

In vitro culture evaluation

The mean number of shoots/inoculum varied between 5(on the MSL3 medium variant (Figure 3 a) and 32 (on MSL6), the values being significantly different ($p < 0.001$) among tested media (Figure 1).

Concerning the maximum length of regenerated shoots, the values varied between 1.5 cm for shoots regenerated on MSL1 and 2.6 cm for shoots regenerated on MSL3, with significant differences ($p < 0.001$) between media variants (Figure 2).

The addition of benzyl amino purine combined with kinetin and alfa naphthyl acetic acid, in presence of glutamine proved to be more beneficial and improved the regeneration rate through direct morphogenesis, with a maximum of 38 shoots/initial explants recorded (Figure 3 b).

Also, regeneration was stimulated by zeatin added in the MSL8 variant, however shoots growth was slower.

Neo shoots further developed on the primary shoots, increasing the number of regenerants after two months. The shoots rooted easily, but required to be detached and further cultivated on MS hormone-free medium for growth (Figure 3 c), the entire multiplication cycle taking more than three months.

The plants can be acclimated when necessary using the successful protocol described by Kotzuharova et al (2018).

In *L. nivale*, Hook (1994) first reported *in vitro* regeneration through shooting on apical buds and seedling explants cultured on MS medium supplemented with low levels of hormones, but the regeneration rate was not registered. Callus was also induced on MS medium added with 0.22 mg/l 2.4-D and 0.18 NAA, next used for suspension cultures establishment.

In the same taxon, Pace et al. (2009) succeeded in inducing callus from cotyledons from *in vitro* germinated seeds, but on Hoagland medium (Hoagland & Arnon, 1938) supplemented with 2,4-dichlorophenoxyacetic acid.

The regeneration of shoots occurred after the transfer of callus on a medium supplemented with 0.25 mg/L NAA and 0.50 mg/L6-BAP.

In this report, through indirect morphogenesis, over 30 shoots per callus were obtained and rooting was possible in the presence of auxin.

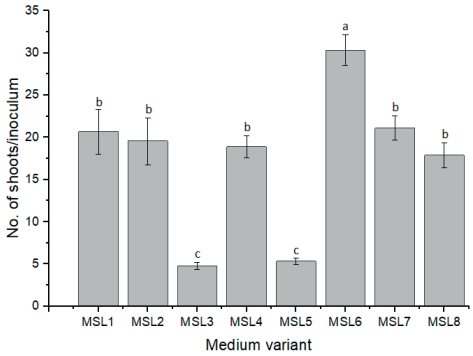


Figure 1. The effect of different medium variants on the number of shoots/inoculum. The data were recorded at the end of the experiment and are presented as mean ± SD. Values with different letters are significantly different ($p < 0.001$), using ANOVA and Multiple pairwise-comparison Tukey test (n= 9)

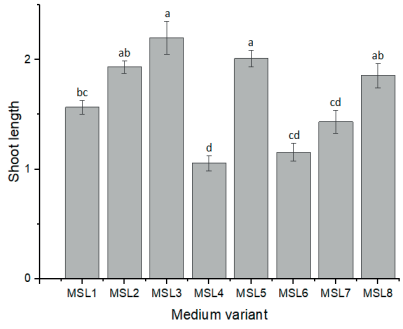


Figure 2. The effect of different medium variants on the maximum shoot length (cm). The data were recorded at the end of the experiment and are presented as mean ± SD. Values with different letters are significantly different ($p < 0.001$), using ANOVA and Multiple pairwise-comparison Tukey test (n = 9)

Using shoot tips, fragments of cotyledons and hypocotyls excised from 10-day-old seedlings, Trejgell et al. (2010) also reported the highest frequency of axillary shoot proliferation on shoot tip explants, the average of 15.1 shoots/ explant being obtained on medium supplemented with 4.4 µM BAP and 0.54 µM NAA.
Vasile et al. (2011) also reported a good regeneration in *L. alpinum* on the medium Adenin sulphate, with a high number of regenerants, but without specifying the duration of cultures and number of passages. In our experiment, the multiplication rate through direct morphogenesis, starting from aseptically germinated seeds, increased in subsequent stages, reaching over 30 regenerants/initial explant (maximum 38) after two months of culture on variant ML6, supplemented with BAP, NAA and glutamine (Figure 3b).
On the other hand, variants MSL1, MSL2, MSL7 and MSL8 ensured an acceptable regeneration.



Figure 3 a, b, c. Aspects of direct morphogenesis in *L. nivale* (scale bar = 1 cm)

The rooting occurred easily without additional treatments, but the transfer of mini-plantlets on large Phytatray vessels with MS medium added with charcoal, improved growth (Figure 3 c).

Somatic embryogenesis

Somatic embryogenesis is a multistep process, starting with proembryogenic masses that progress to the next stages maintaining the bipolar pattern of cell. Auxins and cytokinins are essential to maintain proembryogenic proliferation (Xu et al., 2013).
The process was induced as a result of auxin presence or dominance in the culture medium.
The dedifferentiation and redifferentiation occurred directly on the explant surface, callus cells also appeared (Figure 4 a, b), followed by embryogenic stages (Figure 5 b).

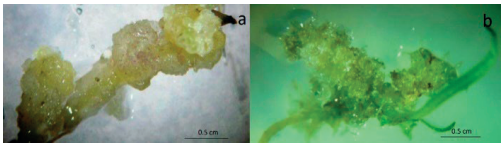


Figure 4. The induction of callus on MSL9 variant starting from root explant (a) and leaves (b)

Firstly, proembryogenic callus developed (Figure 5 b), followed by embryogenic stages (Figure 5 c).
Generally, the variants supplemented with 2.4-D and Dicamba ensured a good callusing rate, varying between 90 and 100% (Table 3). The low auxin level supplemented media variants (MSL9 and MSL10) induced a good response, while the variants containing low levels of auxins and kinetin also determined a positive embryogenetic response, somatic embryos evolving even on hormone added media.

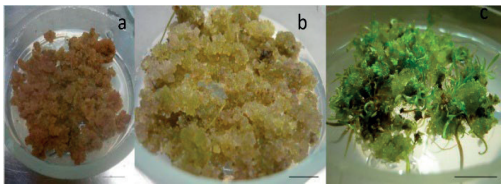


Figure 5. The effect of different media variants *in L. nivale*: a - nonregenerative callus; b - proembryogenic friable callus; c - high embryogenic cultures on MSL13

The number of recorded regenerants was over 50/initial explant. The ratio 2/1 of auxins (2.4-D or Dicamba combined with IBA) in MSL11 and MSL12 led to a high proembryogenic mass production with a lower rate of somatic embryos' conversion, therefore a transfer on hormone free MS medium was necessary.

Auxin treatment of explants was reported to be an indispensable inducer of somatic embryogenesis (SE) in a large number of plant species (Wójcik et al., 2020).

When NAA and kinetin were present in the growth medium, the callusing rate decreased and a nonregenerative callus was obtained (Figure 5 a) (Table 3). On the other hand, the higher level of kinetin combined with NAA did not have a positive impact on callogenesis, conducting to the browning of the culture.

On low auxin added variants (MSL 9, 10, 15, 16), all developmental stages of somatic embryos occurred from earlier to the later stages, even converting directly into plants.

On MSL11-14 variants, proembryogenic and early embryogenic stages developed, however, plantlet formation was favoured after the transfer on MS hormone free medium added with 0.5 g/l active charcoal (Figure 7 a, b).

The number of somatic embryos varied between 20 to over 50/initial explant after 8 weeks of culture, which was higher than the number of regenerants recorded through direct morphogenesis.

Considering the presence of root meristems, the developmental process of these structures was easier, faster and subculturing could be performed monthly, the rate of plant production being improved through somatic embryogenesis, compared to morphogenesis or shooting (Figure 7).

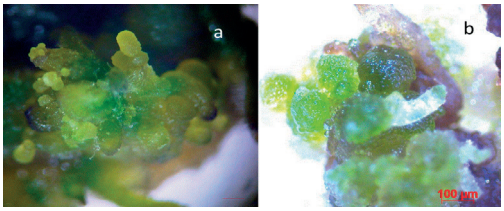


Figure 6. Somatic embryos developed on leaf (a) and root (b) explant on MSL 13 medium

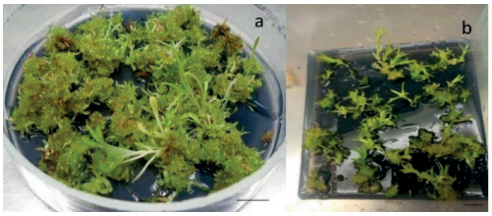


Figure 7. Highly regenerative cultures and plant development on MS medium supplemented with active charcoal (scale bar = 1 cm)

Table 3. *In vitro* response concerning callusing and somatic embryogenesis

Variants	Explant type	In vitro response	% of callusing	% of regeneration	Mean number of regenerants/ explant ± SD
MSL9	Root	Brown yellow embryogenic callus	80-100%	83.3%	21.1 ± 5.44^{b}
	leaf	Greenish friable embryogenic callus	100%	100 %	$\mathbf{54.9 \pm 8.54}^{t}$
MSL10	Root	Friable embryogenic callus	100%	90 %	19.8 ± 4.96^{b}
	leaf	Friable embryogenic callus	100%	100%	$\mathbf{56.2 \pm 15.75}^{t}$
MSL11	Root	Friable embryogenic callus	80-100 %	60%	11.5 ± 2.36^{c}
	leaf	Friable embryogenic callus	80-100%	50-60%	12 ± 3.26^{z}
MSL12	Root	Friable embryogenic callus	100%	60%	10.4 ± 2.75^{c}
	leaf	Green embryogenic callus	100%	50-60%	13.1 ± 3.90^{z}
MSL13	Root	Embryogenic callus	80-100%	80 %	20.9 ± 4.70^{b}
	leaf	Embryogenic callus	80-100%	80-100%	26.3 ± 4.87^{y}
MSL14	Root	embryogenic callus	80-100%	80%-100%	19.6 ± 3.53^{b}
	leaf	Embryogenic callus	80-100%	83%	29.2 ± 4.23^{y}
MSL15	Root	Embryogenic callus	80-100%	80%	36.3 ± 8.76^{a}
	leaf		80-100%	90-100%-	$\mathbf{45.6 \pm 7.98}^{tx}$
MSL16	Root	Embryogenic callus	80-100%	90-100%	38.2 ± 8.39^{a}
	leaf		80-100%	90-100%	$\mathbf{36.2 \pm 6.14}^{xy}$

Variants	Explant type	In vitro response	% of callusing	% of regeneration	Mean number of regenerants/ explant ± SD
MSL17	Root	Nonregenerative yellowish callus	80%	-	-
	leaf		80%	-	-
MSL18	Root	Yellow friable non-regenerative callus	60-70%	-	-
	leaf		80%	-	-
MSL19	Root	Brown non regenerative calli	60%	-	-
	leaf	Yellow Brown non regenerative callus	50-60%	-	-
MSL20	Root	Friable non regenerative callus	80%	-	-
	leaf	Friable non regenerative callus	100%	-	-

Values with different letters are significantly different ($p < 0.001$), using ANOVA and Multiple pairwise-comparison Tukey test

The positive effect of activated charcoal on embryo conversion was probably due to the adsorption of growth inhibitory substances, decreasing the accumulation of toxic metabolites and phenolic exudation (Weatherhead et al., 1978; Thomas, 2008).

The analysis performed using optical microscopy revealed different stages characteristic for somatic embryogenesis, from early stages of somatic embryos (globular, heart, torpedo) and even cotyledonary (Figure 8 a, b) with two apexes: apical meristem and root.

Histological samples showed no vascular connection with the origin explant or callus (Figure 8c), an aspect characteristic of somatic embryos (Merkle et al., 1995; Yeung, 1995).

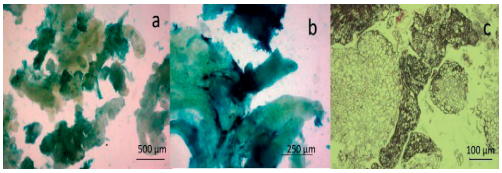


Figure 8. Optical microscopy analysis of embryogenic cultured induced on MSL9 variant with different developmental stages as globular, heart, torpedo, cotiledonary (a, b); c- histological samples revealing somatic embryos structures without vascular connections

The scanning microscopy also confirmed the structures described in optical microscopy (Figure 9).

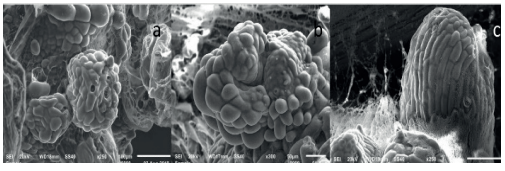


Figure 9. SEM analysis of embryogenic cultures showed somatic embryos (a, b, c)

Early embryogenic globular stage (Figure 9 a, b) and cotyledonary stage (Figure 9 c) were detected. Among different ways of regeneration, somatic embryogenesis is considered to reduce the time necessary for plant propagation, also minimizing genetic changes (Sivanesan & Jeong, 2016).

The culture media variants MSL17-MSL20 led to the formation of a non-regenerative callus, but only variants with auxin dominance and the 2/1 auxin/cytokinin ratio ensured a good growth and survival of the calli. This material can also be used as a supplementary source for secondary metabolites' production through establishment of stables cell lines.

Synthetic seeds or artificial seeds represent a tool with high practical importance, being alginate-encapsulated micropropagules, which can be meristems, apexes or somatic embryos that can behave similarly to classic seeds, being able to develop into plantlets after propagation under *in vitro* or *in vivo* conditions.

Artificial seeds can be used for cryopreservation approaches (Fabre, 1990), such as encapsulation-dehydration or encapsulation-vitrification techniques (Sakai et al., 2008) and represent an efficient strategy for long-term preservation (Kulus, 2016).

After different treatments (dehydration and/or vitrification) and cryostorage, artificial seeds can ensure plant production whenever necessary, based on previously established *in vitro* regeneration protocols for every taxon (Sharma, 2005; Sivanesan & Jeong, 2016).

Besides germplasm preservation, artificial seeds may be used for exchange of plant material between laboratories. Encapsulated material can effectively save many subcultures and eliminate the difficult stage of acclimatization of *in vitro* plants (Qahtan et al., 2019).

Owing to the large-scale production capacity of somatic embryos, this material can easily and successfully be used to prepare synthetic seeds and test different preservation protocols, instead of isolating meristems, a delicate and time consuming procedure.
Testing multiple dehydration procedures as a preliminary step for medium- and long-term preservation, our results showed that immersion for two days in sucrose at 0.25 M and 0.5 M led to an approximately 70% regeneration rate. After a subsequent treatment with 0.75 and 1 M sucrose, the rate decreased to 30%.
Similarly, a sucrose dehydration treatment was effective as a prerequisite for the survival of artificial seeds obtained from embryogenic cultures in *Dianthus nardiformis* exposed to ultralow temperatures (Holobiuc et al., 2023).
Once cultured on an induction medium mainly containing 2.4-D or Dicamba at levels of 0.5-1 mg/L, artificial seeds continued to produce somatic embryos.
In order to develop an appropriate treatment that can ensure plant material survival during ultralow temperatures exposure, further tests need to be performed.

CONCLUSIONS

Over decades, the conservative, qualitative and quantitative aspects of *in vitro* cultures of *L. nivale* have been studied.
Our results revealed that the addition of glutamine and a combination of BAP, kinetin and NAA can be beneficial for stimulating direct morphogenesis starting from aseptic seedling explants.
The growth of plantlets was improved on MS medium added with charcoal 0.5 g/l.
Secondly, our results proved that low levels of 2/4-D or Dicamba successfully induced somatic embryogenesis, further increasing the mass production of this valuable plant, for economic and conservative purposes.
In vitro cultures can efficiently provide biological material necessary for different basic studies without the need to use *ex vitro* plants, for the production of valuable compounds and plant multiplication, with low costs.
The high regeneration rate through somatic embryogenesis can supply a large number of propagules, useful for plant regeneration, for ornamental purpose and also to provide mass production for different important compounds, a process which can undergo all-round the year, independent of environmental factors and in a reduced space.

ACKNOWLEDGEMENTS

This research work was carried out with the support of the Institute of Biology, Romanian Academy, Project RO1567-IBB06/2024.

REFERENCES

Anis, M., & Ahmad, N. (2016). Plant tissue culture: a journey from research to commercialization. *Plant tissue culture: propagation, conservation and crop improvement*, 3-13.

Campiche, R., Le Riche, A., Edelkamp, J., Botello, A. F., Martin, E., Gempeler, M., & Bertolini, M. (2022). An extract of *Leontopodium alpinum* inhibits catagen development ex vivo and increases hair density in vivo. *International Journal of Cosmetic Science*, *44*(3), 363-376.

Chauhan, R. S. (2016). Biotechnological approaches for conservation of rare, endangered and threatened plants. *International Journal of Scientific and Research Publications*, *6*(12), 10-14.

Ciocan, A. G., Mitoi, E. M., Helepciuc, F. E., Neguț, D., Moldovan, R. C., Petrache, A. M., Iuga, C. A., Holobiuc, I. M., Maximilian, C. R., Radu, M., & Cogălniceanu, G. C. (2023). Is acute low-dose gamma irradiation an effective elicitor for secondary metabolism in *Leontopodium alpinum* (Cass.) callus culture?. *Industrial Crops and Products*, *197*, 116547.

Fabre, J. (1990). Encapsulation Dehydration-a new approach to cryopreservation of Solanum shoot-tips. *Cryo-lett.*, *11*, 413-426.

Fasciani, P., Pirone, G., & Pace, L. (2011, September). Plant biodiversity at high altitude: in vitro preservation. In *VII International Symposium on In Vitro Culture and Horticultural Breeding 961* (pp. 159-166).

Faure, O., Dewitte, W., Nougarède, A., & Van Onckelen, H. (1998). Precociously germinating somatic embryos of Vitis vinifera have lower ABA and IAA levels than their germinating zygotic counterparts. *Physiologia Plantarum*, *102*(4), 591-595.

Gamborg, O. L., Miller, R., & Ojima, K. (1968). Nutrient requirements of suspension cultures of soybean root cells. *Experimental cell research*, *50*(1), 151-158.

Hoagland, D. R., & Arnon, D. I. (1938). The water-culture method for growing plants without soil.

Holobiuc, I., Brezeanu, A., & Blindu, R. (2009). Somatic embryogenesis induction in presence of moderate osmotic stress, synthetic seeds production in rare

Dianthus species from Romanian flora as a tool for ex situ conservation.

Holobiuc, I., Mitoi, M., Catană, R., Helepciuc, F. & Carmen, M. (2023). Assessment of the threatened species Dianthus nardiformis Janka after slow-growth culture and cryopreservation as ex situ conservation approach.

Hook, I. (1994). Secondary metabolites in hairy root cultures of *Leontopodium alpinum* Cass.(Edelweiss). In Schripsema, J., Verpoorte, R. (eds), *Primary and Secondary Metabolism of Plants and Cell Cultures III: Proceedings of the workshop held in Leiden, The Netherlands, 4–7 April 1993* (pp. 321-326). Springer, Netherlands.

Hurdu, B. I., Coste, A., Halmagyi, A., Szatmari, P. M., Farkas, A., Puşcaş, M., Turtureanu, P. D., Roşca-Casian, O., Tănase, C., Oprea, A., Mardari, C., Răduţoiu, D., Camen-Comănescu, P., Sîrbu, I.-M., Stoie, A., Lupoae, P., Cristea, V., Jarda, L., Holobiuc, I., Goia, I., Cătană, C., & Butiuc-Keul, A. (2022). Ex situ conservation of plant diversity in Romania: A synthesis of threatened and endemic taxa. *Journal for Nature Conservation*, *68*, 126211.

Khela, S. (2013), *Leontopodium alpinum*. The IUCN Red List of Threatened Species 2013: e.T202984A2758405.

Kozuharova, E., Panayotov, M., & Spadaro, V. (2018). Autecology and ex situ growth of Leontopodium nivale subsp. nivale (Asteraceae) from North Pirin marbles (SW Bulgaria). *Flora Meditarrenea*, *28*, 187-206.

Kulus, D. (2016). Application of cryogenic technologies and somatic embryogenesis in the storage and protection of valuable genetic resources of ornamental plants. In Mujib, A. (eds), *Somatic embryogenesis in ornamentals and its applications* (pp. 1-25). Springer, New Delhi.

Merkle, S. A., Parrott, W. A., & Flinn, B. S. (1995). Morphogenic aspects of somatic embryogenesis. In Thorpe, T. A. (Ed.), *In vitro embryogenesis in plants* (pp. 155-203). Dordrecht: Springer Netherlands.

Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia plantarum*, *15*(3).

Pace, L. G., Bruno, A. A., & Spano, L. (2009). In vitro plant regeneration and clonal micropropagation of Leontopodium nivale (Ten.) Heut ex Hand.-Mazz. (Asteraceae). *Plant Biosystems-An International Journal Dealing with all Aspects of Plant Biology*, *143*(sup1), S12-S16.

Pralea, I. E., Moldovan, R. C., Ţigu, A. B., Petrache, A. M., Hegheş, S. C., Mitoi, M., ... & Iuga, C. A. (2021). Profiling of polyphenolic compounds of *Leontopodium alpinum* cass callus cultures using UPLC/IM-HRMS and screening of in vitro effects. *Plants*, *11*(1), 100.

Qahtan, A. A., Abdel-Salam, E. M., Alatar, A. A., Wang, Q. C., & Faisal, M. (2019). An introduction to synthetic seeds: Production, techniques, and applications. In Faisal, M., Alatar, A. (eds), *Synthetic seeds: germplasm regeneration, preservation and prospects* (pp. 1-20). Springer, Cham.

Reed, B. M., Sarasan, V., Kane, M., Bunn, E., & Pence, V. C. (2011). Biodiversity conservation and conservation biotechnology tools. *In Vitro Cellular & Developmental Biology-Plant*, *47*, 1-4.

Sakai, A., Hirai, D., Niino, T. (2008). Development of PVS-Based Vitrification and Encapsulation–Vitrification Protocols. In: Reed, B.M. (eds) Plant Cryopreservation: A Practical Guide. Springer, New York, NY. https://doi.org/10.1007/978-0-387-72276-4_3

Sarasan, V., Cripps, R., Ramsay, M. M., Atherton, C., McMICHEN, M. O. N. I. C. A., Prendergast, G., & Rowntree, J. K. (2006). Conservation in vitro of threatened plants—progress in the past decade. *In Vitro Cellular & Developmental Biology-Plant*, *42*, 206-214.

Sivanesan, I., & Jeong, B. R. (2016). Optimizing factors affecting somatic embryogenesis in Cineraria. *Somatic Embryogenesis in Ornamentals and Its Applications*, 55-65.

Shahzad, A., Parveen, S., Sharma, S., Shaheen, A., Saeed, T., Yadav, V., Akhtar, R., Ahmad, Z., & Upadhyay, A. (2017). Plant tissue culture: applications in plant improvement and conservation. In Abdin, M., Kiran, U., Kamaluddin, Ali, A. (eds), *Plant Biotechnology: principles and applications* (pp. 37-72). Springer, Singapore.

Tauchen, J., & Kokoska, L. (2017). The chemistry and pharmacology of Edelweiss: A review. *Phytochemistry Reviews*, *16*, 295-308.

Thomas, T. D. (2008). The role of activated charcoal in plant tissue culture. *Biotechnology advances*, *26*(6), 618-631.

Trejgell, A., Szczepanek, D., Domzalska, L., & Tretyn, A. (2010). In vitro propagation of *Leontopodium alpinum* Cass. From various explants of seedling. *Propagation of Ornamental Plants*, *10*(2), 81-87

Vasile, L., Zapartan, M., &Agud, E. (2011). *In vitro* conservation of certain endangered and rare species of Romanian spontaneous flora. *Analele Universităţii din Oradea, Fascicula Protecţia Mediului Vol. XVI, pp 251-26.*

Wójcik, A. M., Wójcikowska, B., & Gaj, M. D. (2020). Current perspectives on the auxin-mediated genetic network that controls the induction of somatic embryogenesis in plants. *International Journal of Molecular Sciences*, *21*(4), 1333.

Wawrosch, C., Schwaiger, S., Stuppner, H., & Kopp, B. (2014). Lignan formation in hairy root cultures of Edelweiss (*Leontopodium nivale* ssp. *alpinum* (Cass.) Greuter). *Fitoterapia*, *97*, 219-223.

Weatherhead, M. A., Burdon, J., & Henshaw, G. G. (1978). Some effects of activated charcoal as an additive to plant tissue culture media. *Zeitschrift für Pflanzenphysiologie*, *89*(2), 141-147.

Xu, Z., Zhang, C., Zhang, X., Liu, C., Wu, Z., Yang, Z., Zhou, K., Yang, X., & Li, F. (2013). T ranscriptome Profiling Reveals Auxin and Cytokinin Regulating Somatic Embryogenesis in Different Sister Lines of Cotton Cultivar CCRI24. *Journal of integrative plant biology*, *55*(7), 631-642.

Yeung, E. C. (1995). Structural and developmental patterns in somatic embryogenesis. *In vitro embryogenesis in plants*, 205-247.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

EX SITU CONSERVATION OF *SERRATULA BULGARICA* ACHT. & STOJ. IN THE REPUBLIC OF MOLDOVA

Olga IONIȚĂ, Elena TOFAN-DOROFEEV

"Al. Ciubotaru" National Botanical Garden (Institute),
Moldova State University, 18 Pădurii Street, Chisinău, Republic of Moldova

Corresponding author email: olgaionita.gbn@gmail.com

Abstract

The study refers to an Asteraceae taxa – Serratula bulgarica Acht. & Stoj., a very rare species in the flora of the Republic of Moldova, reported only in the phytocenoses of Quercus pubescens Willd. in the vicinity of the Batîr commune in the Cimişlia district, whose actual number does not exceed 50 phytoindividuals. This is a Critically Endangered taxa, protected by law, included in the Red Book of the Republic of Moldova, 3rd ed., the 1997 IUCN Red List of Threatened Plants. For the conservation and multiplication of the Serratula bulgarica species in ex situ conditions, fragments of rhizomes collected in natural habitat in the autumn of 2022 were planted in the experimental plot. Ex situ the species develops well, goes through all ontogenetic stages, forms a vigorous habitus. The seeds are viable and the germination rate in laboratory conditions is about 90%. Vegetative propagation by plantlets from renewal buds on rhizomes is high.

Key words*: Serratula bulgarica Acht. & Stoj., rare taxa, conservation, Republic of Moldova.*

INTRODUCTION

Serratula bulgarica Acht. & Stoj. (= *S. caput-najae* Zahar., *Klasea bulgarica* (Acht. & Stoj.) Holub) of the Asteraceae family is one of the most endangered species in the flora of the Republic of Moldova and requires the application of urgent conservation measures (Ionita and Tofan-Dorofeev, 2015). Species protected by law (Environmental legislation..., 1999), included in the Red Book of the Republic of Moldova, 3rd ed. (Ioniță, 2015), as well as in the Red Book of Vascular Plants in Romania (Dihoru and Negrean, 2009), Red Book of Bulgaria (Tzonev, 2015), listed in the IUCN Red List of Threatened Plants (1997). Balkan sub-endemic. The general distribution of the species includes north-eastern Bulgaria, south-eastern Romania, southern Republic of Moldova and south-eastern Ukraine (Figure 1).
In the Republic of Moldova, *S. bulgarica* grows only in the phytocenoses of xerophilic *Quercus pubescens* Willd. with *Iris variegata* L. (Pînzaru et al., 2022) in the vicinity of Batîr commune, Cimislia district. Almoust 50 years after the first attestation of the species in the country's flora (Gochu, 1979), the state of its population is unfavorable, threatened with extinction, with a very small number of individuals.



Figure 1. *Serratula bulgarica* Acht. & Stoj. distribution worldwide powo.science.kew.org

In such cases, the only way to support the conservation of species is to create artificial populations, grown *ex situ* in Botanical Gardens for a long time (Gorbunov et al., 2008), with the aim of propagating and restoring population conditions *in situ*. At the same time, the collection of living plants serves as a base for in-depth study of the biological and ecological peculiarities of the species.

MATERIALS AND METHODS

The research was conducted during 2022-2024 according to the generally accepted method (Akeroyd et Jackson, 1995; IUCN Guidelines for Re-introductions, 1998; Gorbunov et al., 2008; Godefroid et al., 2011). This study is part of the *ex situ* conservation and *in situ* reintroduction program of endangered plant species, carried out within the "Al. Ciubotaru" National Botanical Garden (Chisinau). The materials for the creation of the collection of living plants under *ex situ* conditions of the species *Serratula bulgarica* were taken within its *in situ* study.

The plants for conservation, multiplication and seed production were obtained vegetative, from fragments of rhizomes collected in natural habitat in the autumn of 2022. In the 2023 growing season, *ex situ* plants developed well, bloomed and fructifies, and the collected seeds were used for germination capacity research and seedling cultivation for *in situ* actions. The seed germination ratio was tested by placing on filter paper in Petri dishes at 18-20°C and watered with distilled water and recording the proportion of germinated seeds. Subsequently, germinated seeds were transferred from Petri dishes to pots of 10 cm diameter, which contained a peat and soil mixture.

The population assessment of *Serratula bulgarica* species was carried out, the degree of threat was estimated and the endangered category established, according to the requirements of the International Union for Conservation of Nature (IUCN, 2012), at the same time limiting factors and proposing protection measures were identified. The color photos are original, taken by Olga Ioniță with a Nikon D 3100 digital camera.

RESULTS AND DISCUSSIONS

In the flora of the Republic of Moldova *in situ,* the population of the species *Serratula bulgarica* grows in the clearings and edge of a stand of *Quercus pubescens* Willd. near the village of Batîr, Cimislia district, which represents an unprotected forest sector, subject to anthropogenic pressures. The size of the population and its number of individuals is extremely low, about 30 specimens, and seed production is practically absent (Figure 2).



Figure 2. *Serratula bulgarica* in natural habitat

Although *Serratula bulgarica* has an increased capacity to reproduce vegetatively, in the natural habitat the species faces severe regeneration problems. Being threatened with extinction, *ex situ* conservation is the most suitable option for conserving the species.

Serratula bulgarica Acht. & Stoj. in Bull. Soc. Bot. Bulgarian. 5: 111 (1932) (= *S. caput-najae* Zahar., Bull. Sect. Sci. Acad. Roumaine 28 (Nouv. Esp. Compos. 9): 318 (1946); *Klasea bulgarica* (Acht. & Stoj.) Holub, in Folia Geobot. Phytotax. 12(3): 305 (1977).

Morphology. Perennial plant, 35-100 cm tall. Blackish-brown horizontal rhizome, branched. Stem erect, sparsely foliated. Basal leaves subleathery, elliptical to lanceolate-ovate, long-petiolate, acute or obtuse, irregularly toothed or entire, glabrous, only on short rough-ciliated edges. Stem leaves variable: the lower similar to the basal ones; the middle attenuated and gradually shorter petiolate, lanceolate, the lamina towards the base hard toothed to the penate-fidate; the upper narrow-lanceolate to linear, sessile, deeply penate-partite or whole. Anthodium solitary, globular or campanulate, 30-35 mm long and 16-18 mm in diameter.

Involucral bracts polyseriate, coriaceous, glabrous, green, at the tip with yellowish, membranous, wide-ovate, ovate-triangular or subround appendages, wavy on the edges. Flowers tubular, actinomorphic, bisexual, purplish to pinkish-purple, 20-25 mm long, Achene glabrous, compressed, with papus of dentate hairs (Cannon et Marshall, 1976, Ioniță, 2022) (Figure 3).

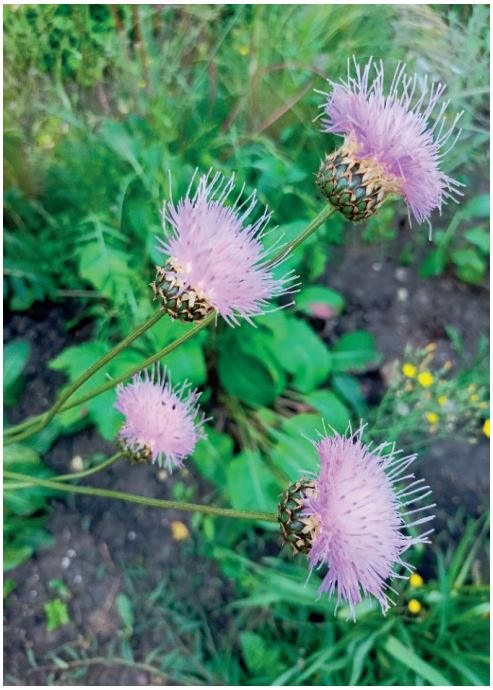


Figure 3. Flowering specimens of *Serratula bulgarica* in *ex situ* conditions

Biological and ecological peculiarities. Balkan hemicryptophyte. Mesophilous species. It blooms in May-June, fructifies in June-July. It propagates both generatively, through seeds, and vegetatively, through root rosette. An anemochore and zoochore plant. Decorative during flowering.

***Ex situ* conservation actions.** In August 2022, trips were made to the natural habitat of the *Serratula bulgarica* species, with the aim of both conducting floristic research and collecting the botanical material necessary for creating the collection of living plants preserved *ex situ*.

In the spring of 2023, 7 specimens sprouted in the experimental sector: 4 generative, floriferous and 3 vegetative (Figure 4).



Figure 4. *Serratula bulgarica* in experimental plot of the "Al. Ciubotaru" National Botanical Garden (I) (April, 2023)

Under *ex situ* conditions, the mature specimens of the *Serratula bulgarica* species have gone through all the phenological phases of the seasonal rhythm of development (Figure 3). In this way, frutify and produced viable seeds, which served as a basis for further research and the multiplication of the species generatively.

In the study, in March 2024 the ability to germinate seeds under laboratory conditions was tested.

To perform the experiment, 50 seeds were placed in the Petri dish on filter paper, moistened with distilled water, at a temperature of 18-20°C. On the fifth day from the beginning of the imbibition, the first seeds germinated, and germination was recorded over 5 days, until new germinations were not observed. According to the data obtained, the germination rate is high, about 90%, an important aspect for the propagation of endangered species that produce a low number of viable seeds (Figure 5).



Figure 5. *Serratula bulgarica* - seed germination

On the tenth day after the start of the experiment, the plantlets in the Petri dish were planted in pots with a diameter of 10 cm and transferred to the greenhouse for further growth and development (Figure 6).

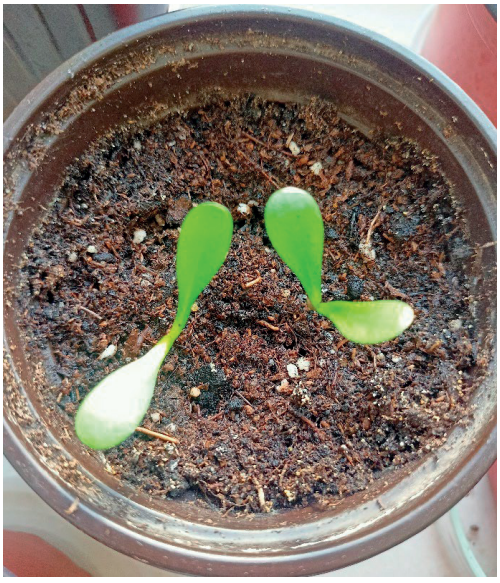


Figure 6. Plantlets in first week

After 4 weeks, the grown seedlings were transferred to the open space. Subsequently, after 5 weeks, in the second decade of May, the acclimatized seedling was planted directly in the soil, in open ground where they quickly adapted (Figure 7). Within 5 months of planting, significant increases were recorded. In October, the vegetative specimens obtained from seeds had rosettes of 15-20 cm high, with 9-18 leaves, 8-20 cm long and 3-7 cm wide. Well-developed root system, 10-15 cm long. Most plants, in addition to the main rosette, emitted from 2-3 to 6-7 seedlings from the root buds (Figure 8). As a result, for the first time, 40 vegetative specimens of the species *Serratula bulgarica* were generatively obtained, under cultivation conditions, multiplied in order to expand the population in the natural habitat, which is at a standstill.



Figure 7. Seedlings recently, planted in open ground (May, 2024)

Sozoological status. In the Republic of Moldova *Serratula bulgarica* is an endangered species, categorized, according to the guide developed by the International Union for Conservation of Nature as Critically Endangered [B2ab(ii,iii); C2a(i,ii); D], included in the Red Book of the Republic of Moldova, 3rd edition.

At the same time, *Serratula bulgarica* is a rare species in all the countries from which it has been reported so far, being included in the Red Book of Vascular Plants in Romania, as Vulnerable (Dihoru et Negrean, 2009) and in the Red Book of Bulgaria, within the Critically Endangered category (Tzonev, 2015). In Ukraine, where the species was most recently

discovered in 2001, it is also a rare taxon, proposed for inclusion in the Red Book of Ukraine (Kucherevskyi et al, 2009).

At the European level, *Serratula bulgarica* is included in the 1997 IUCN Red List of Threatened Plants, with category ”Rare” (Walter et Gillet, 1998).

As an additional conservation measure of the species, *S. bulgarica* is cultivated in the Botanical Garden of Kryvyi Rih, Ukraine (Kucherevskyi et al, 2009), the "D. Brandza" Botanical Garden in Bucharest, Romania (Dihoru et Negrean, 2009) and in the National Botanical Garden "Al. Ciubotaru" in Chisinau, Republic of Moldova.

As an efficient means of medium or long-term *ex situ* conservation, researchers from the Institute of Biology in Bucharest used *in vitro* culture systems, presenting for the first time efficient protocols for seed germination, *in vitro* seedling development, induction of somatic embryogenesis and complete regeneration, for the purpose of *ex situ* conservation of the endangered species *Serratula bulgarica* (Manole-Aiftimie et al, 2013).

Limitation factors. Small area, limited specific habitats, very small number of individuals. Weak natural regeneration. Inadequate forest management and destruction of individuals during forest improvement works. Anthropopression of forest sectors where the plant grows, mowing and grazing of forest glades. Ruderalization of habitats.

Necessary protection measures. Strict compliance with the protection regime for rare species. Monitoring the state of the population, size, number of individuals, growing conditions. Research of the biological and ecological peculiarities of the species, threats, presservation of germplasm in gene banks. To include in the Fund of State Protected Natural Areas of the Republic of Moldova the forest sector in the vicinity of Batâr commune, Cimislia district, the only place of growth of the species *S. bulgarica*. Developing an action plan aimed at identifying the measures and activities necessary to ensure the sustainable conservation of the population and habitat of the *Serratula bulgarica* species in the Republic of Moldova, an action plan that was successfully developed and implemented for this species in Bulgaria (Stoyanov et Goranova, 2014).



Figure 8. Seedlings at the end of the growing season (October, 2024)

CONCLUSIONS

Serratula bulgarica is an endangered subendemic species with a very restricted range, fragmented populations and a small number of individuals. Field research conducted in the natural habitat of the species has elucidated that the biological indicators of the populations are deteriorating more and more, and *Serratula bulgarica* is threatened with extinction in flora of Republic of Moldova. *Ex situ* conservation in the "Al. Ciubotaru" National Botanical Garden (I) is a meaningful way to preserve this endangered species, as well as to accumulate experience regarding its behavior in culture conditions.

As a result of this study, it can be concluded that in *ex situ* conditions the species manifests itself differently than *in situ*, mature specimens develop a vigorous habitus, bloom and fruit, producing viable seeds, with a high germination rate of 90%. Thus, it was possible to cultivate the plants generatively, the experience demonstrating very good results. Most of the plants, successfully reproduced *ex situ*, will be reintroduced into the natural habitat where the species grows for reinforcement and restore the *in situ* population, which is facing serious

propagation problems. This will represent an important stage in the efforts made to conserve the species on the territory of the Republic of Moldova.

AKNOWLEDGEMENTS

The research was supported by the Moldova State University through the Project "Research and *ex situ*, *in situ* conservation of the plant diversity of the Republic of Moldova", (010101).

REFERENCES

Akeroyd, J.R., Jackson, P.W. (1995). *A handbook for botanic gardens on the reintroduction of plants to the wild*. BGCI, UK.

Cannon, J.F.M. & Marshall J.B. (1976). Genus *Serratula* L. *Flora Europaea*. Vol 4. Cambridge University Press, 250–252.

Dihoru, Gh. & Negrean, G. (2009). *Cartea Roşie a plantelor vasculare din România*. Bucureşti: Ed. Acad. Române, 495–496.

Environmental legislation of Republic of Moldova (1996-1998). (1999). Chisinau, 233 p. Legea privind fondul ariilor naturale protejate de stat Nr. 1538 din 25.02.1998. In Monitorul Oficial din 16.07.1998, Nr. 066 art. Nr. 442

Gochu, D.I. (1979). Novyy dlya flory Sovetskogo Soyuza vid *Serratula caput-najae* Zahar. (Asteraceae). *Botan. Journ*., 64 (5), 727–728.

Godefroid S., Piazza, C., Rossi, G., Buord, S., Stevens, A., Aguraiuja, R., Cowell, C., Weekley, C., Vogg, G., Iriondo, J., Johnson, I., Dixon, B., Gordon, D., Magnanon, S., Valentin, B., Bjureke, K., Koopman, R., Vicens M., Virevaire, M., Vanderborght, T. (2011). How successful are plant species reintroductions? Biological Conservation, 144: 672-682. Disponible: https://www.researchgate.net/publication/229104055_How_successful_are_plant_species_reintroductions_Biol_Cons

Gorbunov, Yu. N., Dzybov, D.S., Kuzmin, Z.E., Smirnov, I.A. (2008). Methodological Recommendations for Botanic Gardens on the Reintroduction of Rare and Threatened Plants. Tula: Grif & Co, 53 pp. with photos. ISBN 978-1-905164-28-8 Disponible: https://www.bgci.org/files/Worldwide/Publications/reintroduction_manual_engl.pdf

Ioniţa, O., Tofan-Dorofeev, E. (2015). Species of *Serratula* L. (*Asteraceae* Dumort.) for the Red Book of the Republic of Moldova. *International Scientific Symposium "Conservation of Plant Diversity"*, 28-30 September (Chişinău), p. 26. Disponible: https://ibn.idsi.md/ro/vizualizare_articol/77034

Ioniţa, O. (2015). *Serratula bulgarica* Acht. & Stoj. In: *The Red Book of the Republic of Moldova*. Plants. 3rd ed. Chisinau, Î.E.P. Ştiinţa, p. 29.

Ioniţa, O. (2022). Genul *Serratula* L. In: *Flora Basarabiei*. Vol. V. Chişinău: Universul, 348–357.

IUCN (2012). Guidelines for Application of IUCN Red List Criteria at Regional and National Levels: Version 4.0. Gland, Switzerland and Cambridge, UK: IUCN

Klasea bulgarica (Acht. & Stoj.) Holub [online]. Plants of the World Online (POWO) [cited on 01.03.2025], from https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:227308-1#publications

Kucherevskyi V. V., Tashev, A. N., Sirenko, T.V., Shol, H. N. (2009). *Klasea bulgarica* (Acht. and Stoj.) Holub, a species new for Ukraine, and its distribution in Europe. *Ukr. Botan. Journ.*, 66 (6), 825–832.

Manole-Aiftimie, A., Mihai, R., Banciu, C. (2013). *In vitro* clonal propagation and whole plant regeneration for *Serratula bulgarica* Achtaroff et Stoj. germplasm conservation. *Buletinul AŞM. Ştiinţele vieţii.* Nr. 3(321), 60–68. Disponible: https://ibn.idsi.md/vizualizare_articol/98741

Pînzaru, P., Cantemir, V. Belous, S. (2022). *Iridio variegatae-Quercetum pubescentis* Pînzaru, Cantemir et Belous ass. nova ın vegetatia Republicii Moldova. In: *Journal of Botany*. Chisinau, vol. XIV, 2(25), 51–69. E-ISSN 2587-3814.

Tzonev, R. (2015). *Serratula bulgarica* Acht. et Stoj. In: *Red Data Book of the Republic of Bulgaria*. Plant and Fungi, vol. I, Sofia, P. 315. ISBN 978-954-974621-1

Stoyanov, S., Goranova, V. (2014.). Plan za deystvie za opazvane na rastitelniya vid Blgarski srpets (*Serratula bulgarica* Acht. & Stoj.) v Blgariya 2014-2023. Sofia, 43 p. Disponible: https://www.moew.government.bg/static/media/ups/tiny/filebase/Nature/Biodiversity/Protected_specie/Action_Plans/AP_PLANTS/*Serratula_bulgarica*_AP2014-2023_RD493-2014.pdf

Walter, K.S., Gillet, H.J. [eds.] (1998). 1997 IUCN Red List of Threatened Plants. Compiled by the World Conservation Monitoring Centre. IUCN. The World Conservation Union, Gland, Switzerland and Cambridge, UK, 862 p. Disponible: https://www.biodiversitylibrary.org/page/31085364

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

VARIATION AND CORRELATION OF TEXTURAL AND FRUIT MATURITY PARAMETERS DURING STORAGE OF DALINBEL, JONAFREE, AND REAL APPLE CULTIVARS

Ionuţ ISTRATE, Florin STĂNICĂ, Adrian ASĂNICĂ, Ana-Cornelia BUTCARU, Liliana TUDOREANU

University of Agronomic Sciences and Veterinary Medicine of Bucharest,
59 Mărăşti Blvd, District 1, Bucharest, Romania

Corresponding author email: istrateionut912@yahoo.com

Abstract

The paper aims to identify the influence of 82 days of storage of the Dalinbel, Jonafree, and Real apple cultivars on their textural parameters: hardness, fracture force, cohesiveness, and chewiness. It also aims to identify a possible correlation between the textural parameters and fruits' maturity, characterized by chlorophyll absorbance index IAD (index of absorbance difference), glucose, and fructose concentrations. Textural parameters were measured by puncture test and texture profile analysis (TPA). Dalinbel, Jonafree, and Real apples cultivars had particular variations of textural properties during storage. No significant differences were found for cortex (pulp) tissue's hardness, cohesiveness, chewiness, and fracture force (measured by TPA) for Dalinbel apples from day 47 to 82 of storage and from day 1 to day 82 of storage for the Jonafree apples. Linear correlation analysis over all apple cultivars revealed that after 47 days of storage, there was a negative correlation between glucose concentration and fracture force ($r = -0.9995$; $p = 0.0196$). Additionally, a linear correlation was observed for IAD and fruits hardness ($r = 0.9878$, $p = 0.0028$) measured by TPA.

Key words*: apple cultivars, storage, textural parameters, fruit maturity parameters, correlation.*

INTRODUCTION

Textural properties are among the most critical attributes of apples, alongside their appearance, aroma, and nutritional properties, playing a significant role in consumer acceptance and preference and play an important role in the development and selection of cultivars for commercialization as fresh fruit.

In addition to aroma and color, attributes such as juiciness and crispiness are essential factors influencing consumer acceptability for different apple varieties (Tudoreanu, 2014).

The primary textural characteristics of apples that are valued and expected by consumers are commonly described as "crisp," "juicy," and "firm."(Bourne, 2002).

'Dalinbel' (DL 11/Antares) apple cultivar originates from France and is characterized by high vigor, late flowering, and pink floral buds. The fruit reaches maturity at the end of September and has a medium-to-large size with a flattened-conical shape.

Its skin features a yellow background color with a red over-color (Branişte & Uncheaşu, 2011).

'Jonafree' (Coop 22) cultivar originates from the United States and exhibits medium vigor, late flowering, and pink floral buds. The fruit reaches maturity at the end of September, has a medium size, and a spherical shape. Its skin features a greenish-yellow background color with a red over-color (Branişte & Uncheaşu, 2011).

'Real' (V9/78-92) cultivar originates from Romania and is characterized by medium vigor, intermediate flowering, and pink floral buds. The fruit matures in September, has a large size, and an oblong shape. Its skin features a greenish-yellow background color with a red over-color (Branişte & Uncheaşu, 2011).

Fruit ripening is a complex process governed by physiological, biochemical, and genetic factors that influence apple's firmness, crispness, and juiciness. Firmness, often referred to as hardness, is a critical attribute of apple texture, reflecting the fruit's resistance to deformation and fracture. It is commonly measured using penetrometers, which evaluates the force required to puncture the fruit's flesh. Several studies (Bily et al., 2009) have shown that firmness is closely related to the fruit's storage

potential and consumer preference, with firmer apples generally being associated with longer shelf life and better texture retention. Postharvest treatments such as 1-methylcyclopropene (1-MCP) have been shown to maintain firmness by inhibiting ethylene action, thereby delaying softening (Li et al., 2019). Pre-harvest factors, including cultivar selection and growing conditions, also significantly impact firmness, as demonstrated by studies on 'Cripps Pink' apples treated with aminoethoxyvinylglycine (AVG) (Khan & Singh, 2009).

Cohesiveness refers to the degree to which the apple tissue withstands deformation before breaking. It is a measure of the fruit's intercellular adhesion strength. Cohesiveness is often evaluated using texture profile analysis (TPA), which simulates the compression and decompression of the fruit. Apples with high cohesiveness tend to have a more uniform and resilient texture, which is desirable for both fresh consumption and processing. Furthermore, the composition of cell wall polysaccharides, particularly pectin, plays a crucial role in determining cohesiveness. Studies have shown that changes in pectin composition during storage can significantly alter cohesiveness, with higher pectin content generally associated with better tissue integrity (Billy et al., 2008).

Multivariate statistical analyses of textural parameters have demonstrated that cohesiveness is influenced by the fruit's rheological properties, such as compression/extension strength, and is closely linked to texture attributes like firmness and chewiness (Ballabio et al., 2012).

Chewiness is a texture attribute that describes the energy required to masticate the apple flesh to a state ready for swallowing. It is a composite parameter derived from firmness, cohesiveness, and springiness. Apples with high chewiness are often perceived as having a denser and more satisfying texture. This attribute is particularly important for consumer acceptance, as it directly impacts the sensory experience during consumption. Studies have shown that chewiness is influenced by the fruit's structural integrity and the composition of cell wall polysaccharides, which can vary significantly among different apple cultivars (Zhao et al., 2017). Additionally, sensory evaluations have demonstrated a strong correlation between chewiness and instrumental measurements of texture, highlighting the importance of this attribute in determining overall fruit quality (Brookfield et al., 2011).

Fracture force is the maximum force required to break or rupture the apple tissue, reflecting its mechanical strength. This parameter is often measured using compression tests and is closely related to the fruit's structural properties, such as cell wall thickness and intercellular adhesion. Fracture force is a critical indicator of apple quality, as it affects both handling and processing. Apples with higher fracture force are less prone to bruising and mechanical damage during harvest and transport. Research has shown that fracture force is influenced by pre-harvest factors, including cultivar, growing conditions, and maturity at harvest (Musacchi & Serra, 2018). Additionally, postharvest storage conditions, such as temperature and humidity, can significantly impact fracture force, with improper storage leading to a rapid decline in mechanical strength (Johnston et al., 2002).

Glucose and fructose are the primary sugars in apples, contributing significantly to their sweetness and overall flavor profile. The ratio of glucose to fructose can vary among apple cultivars, influencing the fruit's taste and consumer preference. Studies have shown that glucose and fructose levels are influenced by factors such as cultivar, growing conditions, and post-harvest storage. Additionally, developmental changes in sugar content have been observed, and glucose and fructose levels increasing as the fruit matures (Zhang et al., 2010, Filip et al., 2016).

The aim of the present study is to study the key textural parameters relevant to understanding consumer response (first compression hardness, fracture force, and chewiness) and to examine how these parameters may be influenced by storage duration prior to commercialization. Furthermore, the study explores the potential relationship between textural properties and the ripening stage of the fruit, as indicated by chlorophyll b content (IAD absorbance index), as well as glucose and fructose concentrations.

MATERIALS AND METHODS

The samples consist of 150 apples (50 apples /variety) from three cultivars of “Malus domestica”: ‘Dalinbel,’ ‘Jonafree,’ and ‘Real’ which are cultivated in the experimental orchard

of the Faculty of Horticulture of the University of Agronomic Sciences and Veterinary Medicine of Bucharest (USAMVB).
After harvest the apples were stored for 82 days in the cold storage room of the Research Center for the Study of Agri-Food Products – HORTINVEST of USAMVB at a temperature between 1^0C and 3^0C. All analysis were made in day 1, day 47 and day 82 after harvest.
Textural analysis and statistical analysis were carried out in the Interdisciplinary Laboratory for the Study of Heavy Metal Accumulation and Transport in the Food Chain – HEVMETFOOD, within the Faculty of Veterinary Medicine of USAMVB. The analysis of chlorophyll, glucose and fructose concentrations were carried out in the Laboratory of Integrated Pomology of the Faculty of Horticulture of USAMVB.

Rheological Analysis
The universal testing machine Texture Analyser TAPlus, Lloyd Instruments and Nexigen Plus software were used to analyse apple's textural profile. Textural profile analysis (TPA) is a double compression test which was performed on peeled apple pulp. TPA analysis was made using 5 apples from each cultivar for each of the storage period.
The apples samples were cut as cylinders (approximatively 3cm height and 1 cm diameter). 4 cylindrical samples were cut from each fruit. The cutting was made radially, from the outer surface of the fruit towards the seed cavity. The working parameters for the TPA test were: compression speed 1 mm/s for a compression of 50% of the sample height. The height of each sample was measured using a calliper with a precision of 0.02mm. The following parameters were measured: hardness (firmness) at the first compression, chewiness, fracture force, and cohesiveness.

Chlorophyll, Fructose, and Glucose Concentrations Analysis
The chlorophyll concentration was measured by using the DA-meter on each apple, with the peel intact. The analysis was conducted at two distinct points on the fruit—one at the lightest-colored area and the other at the darkest-colored area—recording the average of these two measurements for each apple.
The analysis of glucose and fructose was performed using glucose and fructose refractometers. Chlorophyll, fructose, and glucose concentration analysis were made on three different fruits from each cultivar.

Statistical Data Analysis
Statistical data analysis was conducted using the MedCalc software.
Since multiple apple cultivars were compared for each textural parameter, the statistical analysis employed was either one-way ANOVA or its non-parametric equivalent, the Kruskal-Wallis test, with the storage period considered as the main factor for each cultivar. One-way ANOVA was applied when the data followed a normal distribution. In cases of ANOVA significant result ($p < 0.05$), post-hoc comparisons were performed using the Tukey-Kramer test to identify significantly different values of the parameters analysed. If the normality check indicated a non-normal data distribution, the Kruskal-Wallis test was used. When the Kruskal-Wallis test yielded a significant result ($p < 0.05$), post-hoc analysis was conducted using the Conover test.
The relationships between firmness and chlorophyll concentration, as well as between chewiness and glucose and fructose concentrations, were analysed using linear correlation analysis. Pearson's correlation coefficient was used when the data followed a normal distribution, while Spearman's rank correlation coefficient was used to study the correlation between non-normally distributed data.

RESULTS AND DISCUSSIONS

Dalinbel apples
It was observed that the first compression hardness (firmness) for the Dalinbel cultivar does not change significantly after 47 days of storage. Firmness is influenced by factors such as cell wall composition, turgor pressure, and the arrangement of parenchyma cells. Genetic factors also play a significant role in determining firmness, as highlighted by genome-wide association studies (GWAS) that identify specific loci associated with texture traits (Farneti et al., 2017).
For the Dalinbel cultivar, the fracture force at the cortex tissue level do not change significantly after 47 days. The chewiness of the cortex tissue in the Dalinbel cultivar does not change significantly between 47 and 82 days of storage.

Khan and Vincent (1993) showed that the cortex apples cells and intercellular spaces are radially oriented. They identified that if the apple sample is compressed radially, along the cell rows the fracture is generated by the collapse of a single layer of cells. In rheology toughness is a mechanical parameter measuring the ability of a material to absorb energy and plastically deform without fracturing, it is a measure of the strength with which the material opposes rupture (Villasenor-Ochoa, 2017). In apples rupture might occur through cell membrane and /or through the intercellular space (Ng et al., 2013). Due to the molecular cell arrangement in the apple cortex, a compression exerted radially on the cortex will generate a smaller toughness and requires a smaller deformation to fracture (Khan and Vincent, 1993) compared to a sample compressed tangentially.

For the apple cortex the stiffness (the ability of a material to return to its original form after being subjected to a force) of samples compressed radially is larger compared to samples compressed tangentially, (Khan and Vincent, 1993). The cohesiveness of the cortex tissue in the Dalinbel cultivar does not change significantly between 47 and 82 days of storage, indicating that the modifications at cellular level occur mainly in the first 47 days of storage.

Jonafree apples

The compressive mechanical properties of fruits parenchyma are related to the size, shape and orientation of cells and intercellular spaces, and cellular adhesion. Moreover apple parenchyma is mechanically very anisotropic due to their elongated cells and intercellular spaces (Khan and Vincent, 1993). There are no significant differences for hardness (Table 1) throughout the entire storage period for Jonafree apples, meaning that their hardness remains constant over the 82-day period (Table 1).

However, after 47 days, Jonafree apples exhibit a significant different cortex fracture force compared to day 82. (Table 1). Softening of apples is dependent of cortical microstructure and cell adhesion as well as of cell shape and size (Ng et al., 2014). Fracture may be generated by cell rupture or by cel-to-cell separation, the later being characteristic for apples with a softer behavior during the first bight and less juiciness (Ng et al., 2014). Modification of Jonafree fracture force after 82 days, but no firmness modification might be explained by a modification in cell adhesion after 47 days of storage.

Table 1. Variation of hardnes at first compression (firmness), Cohesiveness (Coh); Chewiness (Chew); Fracture force (Ff) in apples cultivars. P values for one-way ANOVA/ Khruskal-Wallis test. Data are presented as **mean** ±standard deviation. Comparison can be made only between values associated to storage period. No comparison between apple varieties can be made

Cultivar	Day	Hardness (N)	Coh x10 $^{-2}$	Chew (J) x10 $^{-2}$	Ff (N)
Dalinbel	1	**124.61a** ±26.80	**1.995a** ±1.132	**1.110a** ±0.869	**94.765a** ±62.899
	47	**52.922b** ±13.66	**0.95b** ±0.757	**0.26b** ±0.53	**21.516b** ±25.024
	82	**58.014b** ±10.264	**1.04b** ±1.24	**0.31b** ±0.64	**19.608b** ±27.455
	P	0.001	*0.005	*0.001	*0.004
Jonafree	1	**92.416a** ±48.637	**2.84a** ±1.97	**0.90a** ±0.82	**13.172a** ±14.877
	47	**65.110a** ±18.082	**4.59a** ±6.65	**1.96a** ±3.42	**17.580b** ±24.827
	82	**69.278a** ±14.062	**1.27b** ±1.10	**0.46a** ±0.62	**24.649a** ±31.823
	P	*0.001	*0.004	*0.001	*0.003
Real	1	**183.17a** ±31.291	**2.60a** ±1.16	**1.99a** ±1.10	**33.806a** ±76.203
	47	**92.119b** ±24.449	**4.85a** ±12.18	**1.26b** ±1.15	**28.208b** ±44.828
	82	**89.967b** ±12.852	**0.80b** ±1.15	**0.38c** ±0.60	**20.103b** ±30.642
	P	*0.001	*0.175	*0.001	*0.755

*Significant value for the Khruskal-Wallis test- data not normally distributed

The choice of performing the compression radially on the apples' cortex was in order to identify the possible modifications of the cell walls mechanical properties during storage.

The cohesiveness of the cortex in Jonafree apples does not change significantly after 47 days compared to day 1, but has a significant change after 82 days (Table 1). The intercellular links by which the apples' cortex mass is held together determine its cohesiveness. The cohesiveness value of a sample is directly related to the compression strength of the apple cortex. The Jonafree low cohesiveness after 82 day, which is significantly lower compared with the cohesiveness in day 1 and 47 (table 1) suggest that the intercellular links of the cortex cells becomes weaker after 82 days. The intercellular space is the continuum consisting of all voids or pores between the cells and may encompass as much as 30% of the total volume of an apple fruit depending on the cultivar (Herremans et al., 2015, Li et al., 2019, Li et al., 2023.). In the perception of cohesiveness, the

intracellular space is playing and important role alongside rheological properties of cell walls and membranes. The cohesiveness indicates how well a food retains its form between the first and the second chew. When stored at temperatures between 1^0C to 3^0C the Jonafree apples showed no cohesiveness modification which is an indicator that their crispiness is stable for at least 47 days. In this situation the stability of the cohesiveness might be explained by no modifications in cell adhesion during the 47 days of storage.
Data from table 1 show that there are no significant differences in chewiness throughout the 82 storage period for Jonafree apples.

Real apples
For the Real cultivar, first compression hardness does not differ significantly between days 47 and 82 of storage (Table 1).
The fracture force of the cortex does not differ significantly between days 47 and 82 of storage for the Real cultivar (Table 1).
The Table 1 shows that there are no significant differences in cortex cohesiveness between days 1 and 47 of storage for Real apples; in other words, Real apple cohesiveness changes after 47 days. The chewiness of the Real cultivar differs significantly between day 47 and day 82.
The variations of glucose and fructose concentrations (Table 2) may be due to the inter-individual differences and to a lesser extent to the storage period.

Table 2. Mean values and standard deviations (**mean** ±standard deviation) of Chlorophyll, Glucose, and Fructose. No comparison between cultivars or chemical parameters can be made

Cultivar	Day	Glucose (%)	Fructose (%)	Chlorophyll (AU)
Dalinbel	1	**12.7a** ±1.2	**13.1a** ±1.1	**1.16a** ±0.72
	47	**12.2a** ±0.8	**12.7a** ±0.7	**2.41b** ±1.20
	82	**14.3a** ±1.1	**14.36a** ±1.01	**0.86a** ±1.57
Jonafree	1	**12.46a** ±1.15	**12.7a** ±1.3	**4.22** ±0.87
	47	**13.43a** ±1.89	**13.16a** ±1.76	**4.52** ±1.96
	82	**10.16a** ±0.96	**9.8b** ±2.5	**3.58** ±2.42
Real	1	**11.73a** ±0.45	**11.7a** ±0.2	**5.08a** ±0.70
	47	**11.83a** ±1.32	**11.53a** ±1.49	**4.49a** ±2.32
	82	**11.3a** ±0.3	**11.4a** ±0.4	**3.7a** ±0.8

No significant variation of glucose and fructose was found over time for the three cultivars.
This result may be due to the small number of apples tested (only 3 fruit /cutivar).
The linear correlation study (Table 3) between selected quality parameters revealed that after 47 days, there is an inverse significant (p=0.0196) very good correlation between glucose concentration and fracture force (r =-0.9995).
This result should be interpreted with caution, as the correlation was based on a very small sample size (three cultivars). It serves as an indication for potential future research, therefor increasing the number of analyzed cultivars may either confirm or contradict the present results.

Table 3. Study of the Correlation Between Quality Parameters of Dalinbel, Jonafree, and Real Apple Cultivars. Pearson correlation coefficient (PCC) and significance level (P) are indicated. Clorophill (Cph); Glucose (Gl); Fructose (Fr). Cohesiveness (Coh); Chewiness (Chew); Fracture force (Ff)

Storage duration			Hardness	Coh.	Chew.	Ff
Day 1	Cph	PCC	0.19	0.51	0.98	-0.91
		P	0.87	0.65	0.12	0.26
	Gl	PCC	-0.71	0.062	-0.70	0.52
		P	0.48	0.96	0.50	0.64
	Fr	PCC	-0.67	-0.0005	-0.74	0.58
		P	0.52	0.99	0.46	0.60
Day 47	Cph	PCC	**0.98**	0.84	0.92	-0.33
		P	**0.002**	0.35	0.24	0.78
	Gl	PCC	0.30	-0.24	-0.07	**-0.99**
		P	0.80	0.84	0.95	**0.01**
	Fr	PCC	0.23	-0.71	-0.57	-0.83
		P	0.84	0.49	0.60	0.36
Day 82	Cph	PCC	0.79	-0.04	0.80	0.54
		P	0.41	0.97	0.40	0.63
	Gl	PCC	-0.57	-0.25	-0.94	-0.77
		P	0.61	0.83	0.21	0.43
	Fr	PCC	-0.50	-0.33	-0.96	-0.82
		P	0.66	0.78	0.16	0.38

After 47 days, a highly significant (p=0.0028) strong linear correlation (r=0.9878) and was observed between the IAD absorbance index and first compression hardness (table3). However, this correlation should also be validated by expanding the study to include a larger number of cultivars (10-15 cultivars).

CONCLUSIONS

The Jonafree apples cortex's firmness and chewiness showed no significant differences throughout 82 days of storage. Additionally, cortex cohesiveness remained unchanged after

47 days compared to Day 1 but showed a significant difference after 82 days.

The Dalinbel apples showed no statistically significant changes in firmness (hardness at first compression), cohesiveness, chewiness, and fracture force between 47 and 82 days of storage.

The Real apples' cortex firmness, and fracture force, was not significantly different between 47 and 82 days of storage however it significantly changed between day 1 and 47. Moreover no significant differences were observed in pulp cohesiveness between day1 and 47 of storage, but significant differences was observed after 82 days of storage.

Using data from the three cultivars studied, an inverse linear correlation was found between glucose concentration and fracture force ($r = -0.9995$; $p = 0.0196$) after 47 days of storage, a linear correlation between the IAD absorbance index and first compression hardness ($r = 0.9878$; $p = 0.0028$).

Although linear correlations were tested for 3 storage times and three quality parameters for all textural parameters, only 2 significant linear correlations were obtained, the rest of linear correlations were not significant. Further investigation is needed to identify other possible correlations between textural and quality parameters of apples and to confirm the present findings.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to Professor Florin Stănică, Vice-Rector of the University of Agronomic Sciences and Veterinary medicine of Bucharest (UASVMB), and Dr. Ana-Cornelia Butcaru for their invaluable support and permission to use samples from the experimental orchard of the Faculty of Horticulture of UASVMB. Additionally, I am deeply thankful to them for the opportunity to conduct analyses at the Integrated Pomology Laboratory of the Faculty of Horticulture Bucharest

I am also grateful to my colleagues, the students Ana-Antonia Moșteanu and Roxana Mihalache, from the University of Agronomical Sciences and Veterinary Medicine of Bucharest for helping me during the harvest period.

REFERENCES

Bourne, M. (2002). Food Texture and Viscosity: Concept and Measurement, 2nd Edition, (pp 1-12, 257-292) San Diego.

Braniște, N.& Uncheașu, G. (2011). Determinator pentru soiuri de mere, Editura CERES, Romania.

Ballabio, D., Consonni, V., & Costa, F. (2012). Relationships between apple texture and rheological parameters by means of multivariate analysis. Chemometrics and Intelligent Laboratory Systems, 111(1), 28–33.

Billy, L., Mehinagic, E., Royer, G., Renard, C. M. G. C., Arvisenet, G., Prost, C., & Jourjon, F. (2008). Relationship between texture and pectin composition of two apple cultivars during storage. Postharvest Biology and Technology, 47(3), 315–324.

Brookfield, P. L., Nicoll, S., Gunson, F. A., & Harker, F. R. (2011). Sensory evaluation by small postharvest teams and the relationship with instrumental measurements of apple texture. Postharvest Biology and Technology, 59(2), 179–186.

Costa, F., Cappellin, L., Zini, E., Patocchi, A., Kellerhals, M., & Komjanc, M. (2013). QTL dynamics for fruit firmness and softening around an ethylene-dependent polygalacturonase gene in apple (Malus domestica Borkh.). Journal of Experimental Botany, 64(9), 3029–3039.

Farneti, B., di Guardo, M., Khomenko, I., Cappellin, L., Biasioli, F., Velasco, R., & Costa, F. (2017). Genome-wide association study unravels the genetic control of the apple volatilome and its interplay with fruit texture. Journal of Experimental Botany, 68(7), 1467–1478.

Filip, M., Vlassa, M., Coman, V., & Halmagyi, A. (2016). Simultaneous determination of glucose, fructose, sucrose and sorbitol in the leaf and fruit peel of different apple cultivars by the HPLC–RI optimized method. Food Chemistry, 199, 653–659.

Harker, F. R., Redgwell, R. J., Hallett, I. C., Murray, S. H., & Carter, G. (1997). Texture of fresh fruit. Horticultural Reviews, 20, 121–224.

Johnston, J. W., Hewett, E. W., & Hertog, M. L. A. T. M. (2002). Postharvest softening of apple (Malus domestica) fruit: A review. Postharvest Biology and Technology, 24(3), 247–264.

Khan, S. A., & Singh, Z. (2009). Pre-harvest application of aminoethoxyvinylglycine (AVG) modifies ripening and enhances fruit quality of 'Cripps Pink' apple. Journal of the Science of Food and Agriculture, 89(5), 863–869.

Khan, A. A., & Vincent, J. F. (1993). Compressive stiffness and fracture properties of apple and potato parenchyma. *Journal of texture studies*, *24*(4), 423-435.

Konopacka, D., Jesionkowska, K., Kruczyńska, D., & Stehr, R. (2010). The effect of storage and processing on vitamin C content and sensory properties of apple puree. *Journal of Food Science*, 75(4), S264–S272.

Herremans, E., Verboven, P., Hertog, M. L., Cantre, D., Van Dael, M., De Schryver, T., ... & Nicolaï, B. M. (2015). Spatial development of transport structures in apple (Malus× domestica Borkh.) fruit. *Frontiers in plant science*, *6*, 679.

Li, M., Li, X., Han, C., Ji, N., Jin, P., & Zheng, Y. (2019). Effects of 1-methylcyclopropene on fruit quality and cell wall metabolism in cold-stored apples. Postharvest Biology and Technology, 151, 1–8.

Li, X. L., Su, Q. F., Jia, R. J., Wang, Z. D., Fu, J. H., Guo, J. H., ... & Zhao, Z. Y. (2023). Comparison of cell wall changes of two different types of apple cultivars during fruit development and ripening. *Journal of Integrative Agriculture*, *22*(9), 2705-2718

Musacchi, S., & Serra, S. (2018). Apple fruit quality: Overview on pre-harvest factors. Scientia Horticulturae, 234, 409–430.

Ng, J. K., Schröder, R., Sutherland, P. W., Hallett, I. C., Hall, M. I., Prakash, R., ... & Johnston, J. W. (2013). Cell wall structures leading to cultivar differences in softening rates develop early during apple (Malus x domestica) fruit growth. *BMC Plant Biology*, *13*, 1-17.

Ross, K., DeLury, N., Li, A., Fukumoto, L., & Zurowski-Tiffin, K. (2024). A Comparative Study of Five Apple Cultivars Linking Biochemical Changes at Different Developmental Stages with Storage Textural Quality. *Horticulturae*,*10*(4), 379.

Tudoreanu, L. (2014). Reologia si analiza texturala a produselor alimentare. Editura Tehnopress, Iasi. ISBN 978-606-687-088-7.

Varela, P., Salvador, A., & Fiszman, S. (2007). Shelf-life estimation of 'Fuji' apples: Sensory characteristics and consumer acceptability. Postharvest Biology and Technology, 45(2), 223–230.

Villasenor-Ochoa, H. (2017). Engineering fundamentals refresh: Strength vs stiffness vs hardness. *taken from https://www. fictiv. com/hwg/design/engineering-fundamentals-refresh-strength-vs-stiffness-vs-hardness*, *12*.

Zhang, Y., Li, P., & Cheng, L. (2010). Developmental changes of carbohydrates, organic acids, amino acids, and phenolic compounds in 'Honeycrisp' apple flesh. Food Chemistry, 123(4), 1013–1018.

Zhao, W., Fang, Y., Zhang, Q. A., Guo, Y., Gao, G., & Yi, X. (2017). Correlation analysis between chemical or texture attributes and stress relaxation properties of 'Fuji' apple. Postharvest Biology and Technology, 129, 45–51.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

TESTING BIOLOGICAL FERTILIZERS USEFUL FOR SUSTAINABLE AGRICULTURE

Oana-Alexandra LUȚU, Nicolae-Alin VINTILĂ, Liliana Cristina SOARE, Codruța Mihaela DOBRESCU

National University of Science and Technology Politehnica Bucharest, Pitești University Center, 1 Târgu din Vale Street, Pitești, Romania

Corresponding author email: liliana.soare@upb.ro

Abstract

Biofertilizers represent an ideal alternative for sustainable agriculture, ensuring, in addition to increasing crop productivity, low costs and easy handling. The aim of this work was to test commercialized biofertilizers (Symbiomyco Grow - organic product based on mycorrhizal fungi), as well as potential biofertilizers (algae culture - Chlorella sorokiniana UTEX 1230 - with Symbiomyco Grow) to highlight the possible beneficial influence on some physiological and biochemical parameters in cucumber, Cucumis sativus L. Symbiomyco Grow was tested in two concentrations (1 and 0.1 g L^{-1} respectively) and 4 variants (with and without algae culture) and applied to the seeds before germination (experiment 1) and at the seedling stage (experiment 2). At the end of the experiments, the root and stem lengths were measured, and the fresh weight, total phenolic content, and concentration of the assimilatory pigments were determined. Among the parameters significantly increased compared with the control, after applying the algae culture with Symbiomyco Grow, we mention: axial organ length, fresh weight, and chlorophyll b.

Key words*: biofertilizers, cucumber, growth, assimilatory pigments, polyphenols.*

INTRODUCTION

The challenges of providing food under conditions of food and nutritional security for a population of 7.8 billion that is growing at a fast pace seem impossible to manage.

According to Alabdallah and Hasan (2021), agricultural crop demand will increase by up to 70-100% by 2050.

At first sight, there are two options to resolve these challenges: using additional land to be cultivated - but the world's area used for cultivation is 1.5 billion ha and is affected by various factors (climate change, pollution, etc.) or increasing the productivity of land already cultivated by using large-scale chemical fertilizers.

However, the use of chemical fertilizers has negative effects on the environment by: reducing the water retention capacity and fertility of soil, increasing soil acidity, and reducing the number of microorganisms, resulting in nutritional imbalances in the soil (Nosheen et al., 2021).

To meet the 2030 UN Sustainable Growth Goal of "Zero Hunger", the development should not compromise the ability of future generations to meet their own needs (Calabi-Floody et al., 2018).

The main problem in agriculture, especially in developing countries, is the inaccessibility of essential plant nutrients, either due to their insufficient quantity or due to the use of fertilizers (Itelima et al., 2018).

In this regard, biofertilizers (microorganisms or their products) have been shown to be an essential alternative for increasing crop productivity while being environmentally friendly, economical, and easy to use, and they have long-lasting benefits. Ancient Romans and classical Greeks used the term "biofertilizer" for various agricultural techniques intended to increase plant yield (Zafar et al., 2024).

Zhao et al. (2024) analyzed 12,880 journal articles to highlight the history of biofertilizers over 4 decades: 1980-2022. They divided biofertilizer research into three stages:

– the first stage (1980-2005) - nitrogen fixation;
– the second stage (2006-2015) focused on mechanisms for increasing plant yield;
– the third stage (2016-2022) - the application of biofertilizers to improve soil characteristics.

The aim of this work was to test commercialized biofertilizers (Symbiomyco Grow - organic product based on mycorrhizal fungi), as well as potential biofertilizers (algae culture - *Chlorella sorokiniana* UTEX 1230 - with Symbiomyco Grow) to highlight the possible beneficial influence on some physiological and biochemical parameters in cucumber, *Cucumis sativus* L.

Symbiomyco Grow is a biostimulator (organic product based on mycorrhizal fungi) which increases the nutrient supply capacity of plant roots, ensures the development of a well-branched root system capable of assimilating water much more easily, induces increased resistance to stress factors (drought, excessive humidity, sudden temperature changes), and protects plants against attacks by diseases and pests.

Algae culture was obtained by inoculating the BG 11 (Blue Green Medium 11) culture medium with *Chlorella sorokiniana* UTEX 1230 (Culture Collection of Algae at the University of Texas at Austin), a green alga (Chlorophyceae) capable of mixotrophic growth in the presence of carbon or nitrogen sources (Ramanna et al., 2014; Bohutskyi et al., 2016; Lizzul et al., 2018).

MATERIALS AND METHODS

For the experiments, cucumber seeds (*Cucumis sativus* L.) were hydrated for 1 h in distilled water. Two types of experiments were carried out. Within each experiment, 6 variants were performed (Table 1) with 10 seeds each, and the experiments had 3 repetitions.

Table 1. Experimental variants

Variants	Content
C (control)	distilled water
S_1	0.1 g Symbiomyco Grow L^{-1}
S_2	1 g Symbiomyco Grow L^{-1}
S_1A	0.1 g Symbiomyco Grow L^{-1}+ algae culture
S_2A	1 g Symbiomyco Grow L^{-1}+ algae culture
A	algae culture

1st Experiment: After hydrating in distilled water, the seeds were immersed in test solutions for 1 h (Table 1). We placed them in Petri dishes with filter paper and watered them periodically with distilled water for 4 days until the roots, stems, and fresh weight were measured.

2nd Experiment: The hydrated seeds were allowed to germinate for 3 days, after which they were immersed in the test solutions (Table 1) for 5 min. The seeds were distributed in Petri dishes on filter paper and watered periodically for 5 days using the test solutions. At the end of the experiment, the root and stem lengths were measured, and the fresh weight was determined. Also, the total phenol content (Orţan et al., 2015) and concentration of the assimilatory pigments (Popoviciu, 2018) were determined.

The results obtained after 3 repetitions were statistically analyzed using IBM SPSS Statistics 23 software. The mean and standard error were calculated, and the averages were compared using Duncan's test (a, b, c, etc).

RESULTS AND DISCUSSIONS

The influence of biofertilizers on seedlings growth and biochemical parameters in Cucumis sativus L. in 1st Experiment

The results obtained after 5 days of starting the experiment indicate significant differences between the control variant (Figure 1) and those in which algae (A) or algae mixed with Symbiomyco Grow (S_1A, S_2A) were used (Figures 2-4). Thus, a slight inhibition of root growth was observed in S_1A, whereas in S_2A and A, the roots had a significant increase in length, these variants recording the best increase in root length. A slight stimulation of root growth was also observed in variants S_1 and S_2 exposed to 0.1 and 1g L^{-1} Symbiomyco Grow (Figure 5).



Figure 1. Control

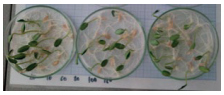


Figure 2. A variant

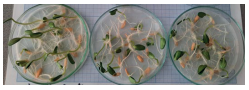


Figure 3. S_1A variant

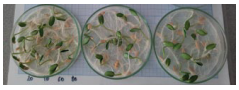


Figure 4. S_2A variant

The growth of the seedling stem, with the exception of variant S_1, which was exposed to 0.1g L^{-1} Symbiomyco Grow, was significantly influenced, with the best results obtained in variants S_2A and A (Figure 6). An analysis of the results obtained for the growth of the two axial organs showed that the most intense growth was determined by variants S_2A and A.

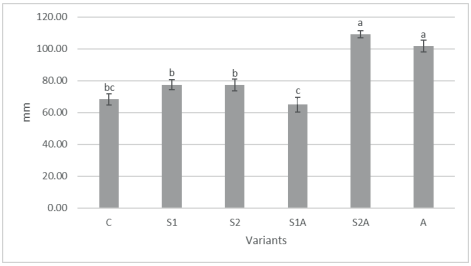


Figure 5. Influence of Symbiomyco Grow and algae culture on root growth in *Cucumis sativus* L. seedlings

The results for fresh biomass were correlated with those obtained for the growth of the seedling: all variants exposed to the tested fertilizer solutions, except for S_1, showed significant differences compared with the control (Figure 7).

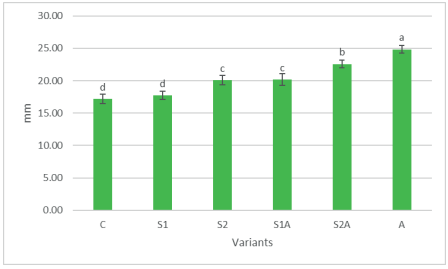


Figure 6. Influence of Symbiomyco Grow and algae culture on shoot growth in *Cucumis sativus* L. seedlings

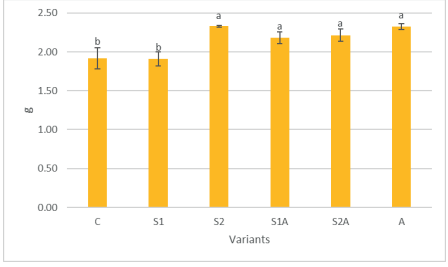


Figure 7. Influence of Symbiomyco Grow and algae culture on fresh weight in *Cucumis sativus* L. seedlings

Phenols represent a class of important compounds that act as biomarkers of stress induced by different biotic and abiotic factors. Analysis of the results indicates a significant decrease in the amount of total phenols (Figure 8), which can be interpreted as a lack of stress in the case of variants exposed to the tested fertilizer formulations.

Regarding the amount of pigments (Figure 9), only S_2A and A variants showed significant increases for chlorophyll *b* compared with the control, whereas in the rest of the variants, the decrease or increases were statistically insignificant.

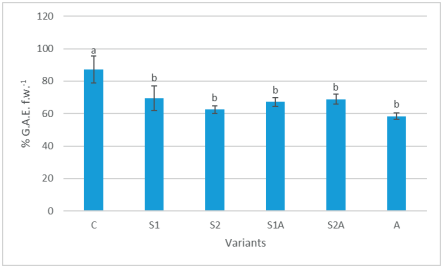


Figure 8. Influence of Symbiomyco Grow and algae culture on total phenolic content in *Cucumis sativus* L. seedlings

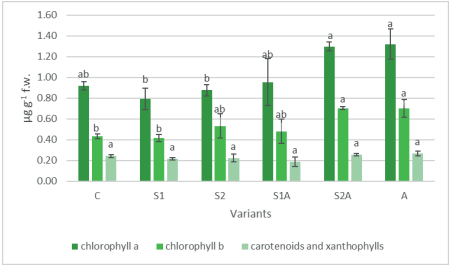


Figure 9. Influence of Symbiomyco Grow and algae culture on pigments content in *Cucumis sativus* L. seedlings

The influence of biofertilizers on seedlings growth and biochemical parameters in Cucumis sativus L. in 2nd Experiment

For the second experiment, the results indicate significant differences only between the control variant and the variant exposed to algae (A) variants in terms of the inhibition of root length growth (Figures 10-11). A slight stimulation of root growth was observed in S_1A, but the difference was not significant (Figure 14).

In the case of the stem and fresh weight (Figures 15-16), the results were similar to those obtained in 1st experiment, recording a more intense growth in length, with significant differences for variants S_2, S_1A, S_2A, and A, respectively, in weight in variants S_1A (Figure 12) and S_2A (Figure 13).

The total phenol content in the seedlings exposed to the fertilizer solutions did not vary significantly compared with the control variant (Figure 17), with slightly lower values recorded in the S_2, S_2A, and A variants.

The content of chlorophyll *a*, chlorophyll *b*, carotenoids, and xanthophylls did not significantly change. Only the S_2 and S_2A variants showed an increase above the value recorded in the control, but no increase was observed in chlorophyll *a* (Figure 18).



Figure 10. Control



Figure 11. A variant



Figure 12. S_1A variant



Figure 13. S_2A variant

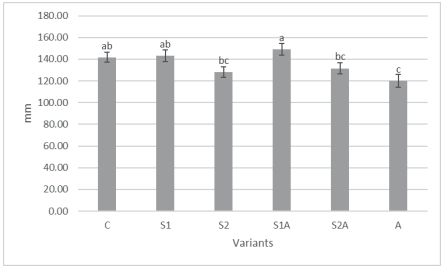


Figure 14. Influence of Symbiomyco Grow and algae culture on root growth in *Cucumis sativus* L. seedlings

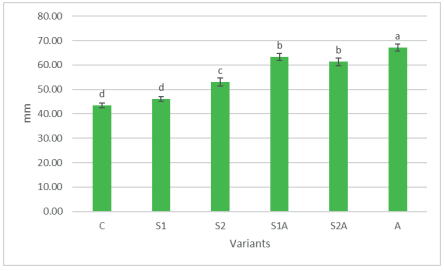


Figure 15. Influence of Symbiomyco Grow and algae culture on shoot growth in *Cucumis sativus* L. seedlings

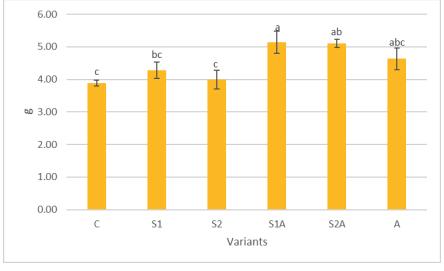


Figure 16. Influence of Symbiomyco Grow and algae culture on fresh weight in *Cucumis sativus* L. seedlings

Biofertilizers improve the physiological processes and health of plants, ultimately leading to increased productivity.

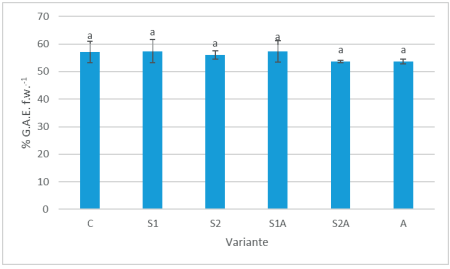


Figure 17. Influence of Symbiomyco Grow and algae culture on total phenolic content in *Cucumis sativus* L. seedlings

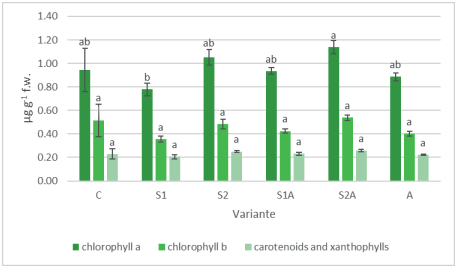


Figure 18. Influence of Symbiomyco Grow and algae culture on the assimilatory pigments content in *Cucumis sativus* L. seedlings

Biofertilizers currently represent an eco-friendly alternative for increasing productivity, as shown by various researches. Certain species of microorganisms (algae, fungi) or combinations thereof may determine superior results, similar to those reported by us for *Cucumis sativus*, exposed to certain concentrations of Symbiomyco Grow, *Chlorella sorokiniana* or combinations of the two.

So, the study conducted by Muscalu et al. (2020) regarding the effect of organic fertilizers in cucumber cultivation showed that the best results were obtained when using Rom-Agrobiofertil, which contains a mix of microorganisms (*Azospirillum lipoferum*, *Azotobacter chroococcum*, and *Bacillus megaterium*).

A series of research has indicated that algae stimulate plant growth, a fact also found for some of the variants tested in the two experiments (A, S_1A, S_2A). Thus, Lv et al. (2020) found that microalgae biomass significantly stimulated growth in height, the

number of leaves and flower buds, and the diameter of cucumber stems.

Algae suspensions (*Chlorella* sp.) of 0.17 and 0.25 g L^{-1} can stimulate root and stem length growth in cucumbers (Bumandalai & Tserennadmid, 2019), as we also obtained when using the algae *Chlorella sorokiniana* (A) or the algae and Symbiomyco Grow mixture (S_2A) for root growth, respectively A, S_1A, S_2A for stem growth

The presence of *Chlorella* species in the culture medium promotes cucumber seed germination (Abd Elhafiz et al., 2015).

Gitau et al. (2021) mention that the stimulation of plant growth in the presence of microalgae is due to the auxins and cytokines produced by them.Regarding the content of assimilatory pigments, various studies have indicated the beneficial effects of algal biostimulators (Gitau et al. 2021; Guedes et al., 2018; Faheed & Fattah, 2008; El-Naggar et al., 2005), as observed in our experiments, in some of the variants (A and S_2A). The importance of this aspect arises from the fact that studies are known for many species useful for food, agriculture, or medicinal purposes in the context of sustainable agriculture. Thus, in a study conducted by Gitau et al. (2021), the effects of two *Chlorella* species (MACC-360 and MACC-38) and the *Chlamydomonas reinhardtii* (cc124) on *Medicago truncatula* were monitored under greenhouse conditions. The application of algal cells influenced the pigment content of the plant; thus, in the variants with *C. reinhardtii*, the chlorophyll content increased remarkably (32% in the case of chlorophyll a, 35% chlorophyll b, 32% total chlorophyll). In contrast, the application of *Chlorella* MACC-38 cells had a negligible effect on chlorophyll a (0.3% increase), a moderate effect on chlorophyll b (15% increase), and a reduced effect on total chlorophyll (3.7% increase). In the treatment with *Chlorella* MACC-360, both chlorophyll a and total chlorophyll decreased by 5% and 4%, respectively, whereas chlorophyll b content increased by 1%.

The use of micro- and macroalgae extracts increases pigment content (Guedes et al., 2018). Thus, by applying *Chlorella vulgaris* powder, the dry and wet weight of *Lactuca sativa* shoots increased, as did the pigment content (Faheed & Fattah, 2008).

Chlorella kessleri extracts rich in phytohormones (auxins and gibberellins) stimulated seed germination and increased leaf area and pigment content in *Vicia faba* (El-Naggar et al., 2005).

Variant S_2 (1g Symbiomyco Grow L^{-1}) showed an increase in chlorophyll *b* levels above the value recorded in the control group in the first experiment and in the second experiment for chlorophyll *a*.

A similar situation was observed in *Arabidopsis thaliana* inoculated with *Azospirillum brasilense* (Cohen et al., 2015).

Also, *Pseudomonas* sp., *Bacillus lentus*, and *Azospirillum brasilense* strains alleviated water stress in basil plants by increasing the levels of photosynthetic pigments and antioxidant activity (Heidari & Golpayegani, 2012).

The present study demonstrated the efficiency of using the green algae *Chlorella* in a mixture with an organic product based on mycorrhizal fungi (variants S_1A and S_2A) in terms of improving fresh weight and chlorophyll content in *Cucumis sativus.*

Increases in weight and carotenoid content were observed in lettuce following the application of *Chlorella vulgaris*, *Bacillus* sp., *Azotobacter* sp. and *Azospirillum* sp. (Kopta et al., 2018).

Algae extracts (1%), phosphate-solubilizing bacteria biofertilizers (4%) and their combinations were tested by Azizi et al. (2024) under drought conditions in *Calendula officinalis*. The combination of the two types of biofertilizers led to the best results: the highest increase in relative water content (39.80%), chlorophyll a (45.19%), chlorophyll b (46.62%), total chlorophyll (45.49%), flavonoids (13.33%), flavonols (4.35%), proline (6.87%), and catalase (127.27%) compared with the control. In addition, plant photosynthesis is stimulated by mycorrhizae because they enhance the transport of inorganic elements from the soil to plants, causing an increase in chlorophyll content and, implicitly, a higher photosynthetic rate (Feng et al., 2002; Hend et al., 2007).

Maize (Zhu et al., 2010), sunflower (Abdallah et al., 2013), and wheat (Allah et al., 2015) had an increased chlorophyll content as a result of mycorrhizae inoculation. Other plant parameters positively influenced by the presence of mycorrhizal fungi are: increased water content

in leaves, turgor potential, increased root length, accumulation of nutrients in aerial parts (Ngwene et al., 2010), protection from pathogens (Elsen et al., 2008).

Dasgan et al. (2022) studied the effects of biofertilizers on the antioxidant content of basil, *Ocimum basilicum*. The highest phenol content was observed in the variants with bacteria-based biofertilizers, whereas the lowest content was observed in those with microalgae, which is similar to our results: the algae variant (A) exhibited the lowest concentration of polyphenol content in both experiments.

Babu et al. (2015) concluded that the presence of *Bacillus subtilis* and *B. cereus* promotes the accumulation of enzymes (peroxidase and polyphenol oxidase) involved in the metabolism of phenols and flavonoids.

CONCLUSIONS

Considering the results obtained for the influence of Symbiomyco Grow and algae culture on *Cucumis sativus* seedlings applied at the seed stage, the significant changes recorded (+ and -) indicate that the variants in which the seeds were immersed in the algae culture (A) and in the mixture of Symbiomyco Grow (1%) + algae (S_2A) had the most significantly increased parameters, compared to the control, respectively: growth in length of the axial organs of the seedling, wet biomass, and chlorophyll b. The summary of the influence of Symbiomyco Grow and algae culture on *Cucumis sativus* seedlings applied at the seedling stage indicates significant changes recorded (+ and -) in the variants in which the seedlings were immersed in the mixture of Symbiomyco Grow (0.1%) + algae (S_1A) and Symbiomyco Grow (1%) + algae (S_2A). The most significantly increased parameters compared to the control were the growth in stem length and wet biomass. The research carried out must be continued to evaluate the influence of biological fertilizers for a longer period of time and for more parameters.

ACKNOWLEDGEMENTS

The authors thank Dr. Cristina Moisescu from Institute of Biology Bucharest of Romanian Academy, who provided the algal inoculum (*Chlorella sorokiniana* UTEX 1230).

REFERENCES

Abd Elhafiz, A., Abd Elhafiz, A., Gaur, S.S., Hamdany, N., Osman, M., & Lakshmi, T.V.R. (2015). *Chlorella vulgaris* and *Chlorella pyrenoidosa* live cells appear to be promising sustainable biofertilizer to grow rice, lettuce, cucumber and eggplant in the UAE Soils. *Recent Research in Science and Technology*, 7, 14–21. https://doi.org/10.19071/RRST.2015.V7.2919.

Abdallah, M.M., Abd El-Monem, A.A., Hassanein, R.A., & El-Bassiouny, H.M.S. (2013). Response of sunflower plant to the application of certain vitamins and arbuscular mycorrhiza under different water regimes. *Austr. J. Basic. Appl. Sci.,* 7, 915–932.

Alabdallah, N.M., & Hasan, M.M. (2021). Plant-based green synthesis of silver nanoparticles and its effective role in abiotic stress tolerance in crop plants. *Saudi J. Biol. Sci., 28,* 5631–5639, 10.1016/j.sjbs.2021.05.081

Allah, M.M.-S.A., El-Bassiouny, H., Bakry, B.A, & Sadak, M. (2015). Effect of arbuscular mycorrhiza and glutamic acid on growth, yield, some chemical composition and nutritional quality of wheat plant grown in newly reclaimed sandy soil. *Research Journal of Pharmaceutical, Biological and Chemical Sciences. 6*(3)*,* 1038–1054.

Azizi, A., Bagnazari, M., & Mohammadi, M. (2024). Seaweed and phosphate-solubilizing bacteria biofertilizers ameliorate physiochemical traits and essential oil content of *Calendula officinalis* L. under drought stress, *Scientia Horticulturae 328*, 112653, https://doi.org/10.1016/j.scienta.2023.112653

Babu, N.A., Jogaiah, S., Ito, S., Nagaraj, K.A., & Tran, L. S. (2015). Improvement of growth, fruit weight and early blight disease protection of tomato plants by rhizosphere bacteria is correlated with their beneficial traits and induced biosynthesis of antioxidant peroxidase and polyphenol oxidase. *Plant science: an international journal of experimental plant biology*, *231*, 62–73. https://doi.org/10.1016/j.plantsci. 2014.11.006

Bohutskyi, P., Kligerman, D.C., Byers, N., Nasr, L.K., Cua, C., Chow, S., Su, C., Tang, Y., Betenbaugh, M.J., & Bouwer, E.J. (2016). Effects of inoculum size, light intensity, and dose of anaerobic digestion centrate on growth and productivity of *Chlorella* and *Scenedesmus* microalgae and their poly-culture in primary and secondary wastewater. *Algal Res,. 19*, 278–290. https://doi.org/10.1016/j.algal.2016.09.010

Bumandalai, O., & Tserennadmid, R. (2019). Effect of *Chlorella vulgaris* as a biofertilizer on germination of tomato and cucumber seeds. *Int. J. Aquat. Biol*., *7*(2), 95–99, https://doi.org/10.22034/ijab.v7i2.582

Calabi-Floody, M., Medina, J., Rumpel, C., Condron, L.M., Hernandez, M., Dumont, M., & de la Luz, M.M. (2018). Smart fertilizers as a strategy for sustainable agriculture. *Adv. Agron*., *147,* 119–157. https://doi.org/10.1016/bs.agron.2017.10.003

Cohen, A. C., Bottini, R., Pontin, M., Berli, F. J., Moreno, D., Boccanlandro, H., Travaglia, C. N., & Piccoli, P. N. (2015). *Azospirillum brasilense* ameliorates the response of Arabidopsis thaliana to drought mainly via enhancement of ABA levels. *Physiologia plantarum*, *153*(1), 79–90. https://doi.org/10.1111/ppl.12221

Dasgan, H.Y., Aldiyab, A., Elgudayem, F., Ikiz, B., & Gruda, N.S. (2022). Effect of biofertilizers on leaf yield, nitrate amount, mineral content and antioxidants of basil (*Ocimum basilicum* L.) in a floating culture. *Sci. Rep. 12,* 20917 https://doi.org/10.1038/s41598-022-24799-x

El-Naggar, A.H., Osman, M.E.H.E.-S., & Gheda S.F. (2005). Influence of the aqueous extracts of *Ulva lactuca* and *Chlorella kessleri* on growth and yield of *Vicia faba*, *Arch. Hydrobiol. Suppl. Algol*. *Stud., 116*, 213-229, 10.1127/1864-1318/2005/0116-0213

Elsen, A., Gervacio, D., Swennen, R., & De Waele, D. (2008). AMF-induced biocontrol against plant parasitic nematodes in *Musa* sp.: a systemic effect. *Mycorrhiza*, *18*(5), 251–256. https://doi.org/10.1007/s00572-008-0173-6

Feng, G., Zhang, F. S., Li, X. L., Tian, C. Y., Tang, C., & Rengel, Z. (2002). Improved tolerance of maize plants to salt stress by arbuscular mycorrhiza is related to higher accumulation of soluble sugars in roots. *Mycorrhiza*, *12*(4), 185–190. https://doi.org/10.1007/s00572-002-0170-0

Faheed, F.A., & Fattah, A.A.E. (2008). Effect of *Chlorella vulgaris* as bio-fertilizer on growth parameters and metabolic aspects of lettuce plant. *J. Agric. Soc. Sci., 4*, 165-169.

Guedes, W.A., Araújo, R.H.C.R., Rocha, J.L.A., de Lima, J.F., Dias, G.A., de Oliveira, Á.M.F., de Lima, R.F., & Oliveira, L.M. (2018). Production of papaya seedlings using *Spirulina platensis* as a biostimulant applied on leaf and root *J. Exp. Agric. Int., 28*, 1-9, https://doi.org/10.9734/jeai/2018/45053

Gitau, M. M., Farkas, A., Balla, B., Ördög, V., Futó, Z., & Maróti, G. (2021). Strain-Specific Biostimulant Effects of *Chlorella* and *Chlamydomonas* Green Microalgae on *Medicago truncatula*. *Plants*, *10*(6), 1060. https://doi.org/10.3390/plants10061060

Heidari, M., & Golpayegani, A. (2012). Effects of water stress and inoculation with plant growth promoting rhizobacteria (PGPR) on antioxidant status and photosynthetic pigments in basil (*Ocimum basilicum* L.). *Journal of the Saudi Society of Agricultural Sciences, 11*, 57-61.

Hend, S.M.F, Sakr, W.R.A., Sabh, A.Z., & Ragab, A.A. (2007). Effect of some chemical and biofertilizers on peppermint plants grown in sandy soil. 2. Effect on essential oil production, chemical composition and anatomical features. *Ann. Agric. Sci., 52*(2), 465–484 Ain shams Univ. Cairo

Itelima, J.U., Bang, W.J., Onyimba, I.A., & Oj, E. (2018). A review: biofertilizer; a key player in enhancing soil fertility and crop productivity. *J. Microbiol. Biotechnol. Rep.,* 2(1), 22–28.

Kopta, T., Pavlíková, M., Sękara, A., Pokluda, R., & Maršálek, B. (2018). Effect of Bacterial-algal Biostimulant on the Yield and Internal Quality of Lettuce (Lactuca sativa L.) Produced for Spring and Summer Crop. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, *46*(2), 615–621. https://doi.org/10.15835/nbha46211110

Lizzul, A. M., Lekuona-Amundarain, A., Purton, S., & Campos, L. C. (2018). Characterization of *Chlorella sorokiniana*, UTEX 1230. *Biology*, *7*(2), 25. https://doi.org/10.3390/biology7020025

Lv, J., Liu, S., Feng, J., Liu, Q., Guo, J., Wang, L., Jlao, X., & Xie, S. (2020). Effects of microalgal biomass as biofertilizer on the growth of cucumber and microbial communities in the cucumber rhizosphere, *Turk. J. Bot. 44*, 167–177, doi:10.3906/bot-1906-1.

Muscalu, S.-P., Munteanu, N., Brezeanu, P.M., Calara, M., & Bute, A. (2020). The influence of some biofertilizers on the production of cucumber seeds in ecological farming system, *Studii şi Cercetări: Biologie, Universitatea"Vasile Alecsandri" din Bacău, 29*(1), 45–48.

Ngwene, B., George, E., Claussen, W., & Neumann, E. (2010). Phosphorus uptake by cowpea plants from sparingly available or soluble sources as affected by nitrogen form and arbuscular mycorrhiza-fungal inoculation. *J. Plant. Nutr. Soil. Sci., 173*, 353-359. https://doi.org/10.1002/jpln.200900203.

Nosheen, S., Ajmal, I., & Song, Y. (2021). Microbes as biofertilizers, a potential approach for sustainable crop production. *Sustainability*, *13*(4), 1868. https://doi.org/10.3390/su13041868

Orţan, A., Fierăscu, I., Ungureanu, C., Fierăscu, R.C., Avramescu, S.M., Dumitrescu, O., & Dinu Pîrvu, C.E. (2015). Innovative phytosynthesized silver nanoarchitectures with enhanced antifungal andantioxidant properties. *Applied Surface Science*. Elsevier. London: 540–548.

Popoviciu, D.R. (2018). *Fiziologie vegetală caiet pentru lucrări practice de laborator*, Ovidius University Press, ISBN: 978-973-614-980-1

Ramanna, L., Guldhe, A., Rawat, I., & Bux, F. (2014). The optimization of biomass and lipid yields of Chlorella sorokiniana when using wastewater supplemented with different nitrogen sources. *Bioresource technology*, *168*, 127–135. https://doi.org/10.1016/j.biortech.2014.03.064

Zafar, S., Bilal, M., Ali, M.F., Mahmood, A., Kijsomporn J., Wong, L.S., Harsini, M., Kumar, V., & Alotaibi, S.S. (2024). Nano-biofertilizer an eco-friendly and sustainable approach for the improvement of crops under abiotic stresses. *Environmental and Sustainability Indicators, 25,* 100470, https://doi.org/10.1016/j.indic.2024.100470

Zhao, G., Zhu, X., Zheng, G., Meng, G., Dong, Z., Baek, J.H., Jeon, C.O., Yao, Y., Xuan, Y.H., Zhang, J., & Jia, B. (2024). Development of biofertilizers for sustainable agriculture over four decades (1980-2022). *Geography and Sustainability*, *5*(1), 19–28, https://doi.org/10.1016/j.geosus.2023.09.006.

Zhu, X., Song, F., & Xu, H. (2010). Influence of arbuscular mycorrhiza on lipid peroxidation and antioxidant enzyme activity of maize plants under temperature stress. *Mycorrhiza*, *20*(5), 325–332. https://doi.org/10.1007/s00572-009-0285-7

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

CARBON STABILIZATION AND SEQUESTRATION PROCESSES IN SOIL THROUGH SYNTHETIC MICROBIOMES

Sorin MATEI, Gabi Mirela MATEI, Sorina DUMITRU, Victoria MOCANU, Monica DUMITRAȘCU

National Research-Development Institute for Soil Science, Agrochemistry and Environment, 61 Mărăşti Blvd, District 1, Bucharest, Romania

Corresponding author email: so_matei602003@yahoo.com

Abstract

The soil microbial community plays a crucial role in the carbon (C) cycle, acting as a primary driver of variability in the soil's carbon storage potential. The taxonomic composition, through its structure and level of activities, ensures the regulation of nutrient delivery and the rate of decomposition of organic matter. The paper presents the results of the research carried out to assess the role of soil inoculation with synthetic microbiomes (M1, M4, M5, M7) containing performing heterotrophs, in priming the processes responsible for carbon sequestration. Specifically, the study evaluated the influence of these microbiomes on soil glycoprotein level, exopolysaccharide (EPS) production, dissolved organic carbon (DOC) and microbial biomass content. Significant differences were observed among experimental variants, particularly in microbial biomass accumulation. Among the tested microbiomes, M5 demonstrated superior performance, inducing the highest increases in glycoprotein content (1.73 mg·g^{-1}), EPS (594 µg·mL^{-1}), microbial biomass (363.2 mg C·kg^{-1}), and the lowest DOC content (10.13 mg·L^{-1}). These results indicate M5's enhanced integrative capacity and compatibility with soil conditions, supporting its potential role in improving carbon stability and sequestration.

Key words*: dissolved organic carbon (DOC), exopolysaccharides (EPS), glycoprotein, microbial biomass, synthetic microbiome.*

INTRODUCTION

Generally, synthetic communities (SynComs) of microbiomes have a created, controlled composition, with low complexity, an aspect that is reflected in the reduced capacities to manipulate the native microbiota in soils. Their taxonomic composition is important for maintaining soil ecosystem services, because the structure and level of activities regulate the delivery of nutrients and the rate of decomposition of organic matter in the soil. Carbon sequestration, through microbial contribution, is influenced by factors such as the structure of the microbial community, by-products of microbial secondary metabolism, microbial biomass and soil characteristics. Created synthetic communities can present biodiversity, can contain species with different ecological niches and sensitivities to soil pressures, can survive/colonize complex soils, relatively rich in resources, much better. Some of the basic soil microorganisms play key roles, essential in community organization and/or performing specific functions for maintaining communities, so that in the creation of synthetic microbiomes it appears necessary to protect and even adopt such key residents in their composition (Cheng et al., 2022; Finkel et al., 2020). The degree of microbial involvement in the process of carbon stabilization/ sequestration in the soil depends on the complexity of the interactions. Microorganisms selected from the composition of synthetic microbiomes constitute essential components for the presence of soil organic carbon (SOC) and carbon sequestration in terrestrial ecosystems. Thus, synthetic microbiomes together with endemic microbiota mediate the impact of these factors on the transformation of SOC through decomposition as well as the biosynthesis of new microbial C (Rillig et al., 2019; Yue et al., 2020; Li et al., 2023). Synthetic microbiomes can transform primary C derived from decomposition into persistent microbial metabolic products that gradually accumulate in the soil as stable forms of C, such as soil protein bound to glomalin and amino sugars (Irving et al., 2021; Singh et al., 2024; Agnihotri et al., 2022; Feng and Wang,

2023). Inoculated microbiotas attach to organic matter, other organisms, or mineral substrates, aggregating into biofilms with cells embedded in biosynthesized extracellular polymeric substances (EPS), generating adaptability to variable conditions and diverse habitats. EPSs produced by new microbiota in soils restore ecosystem health through the properties of mineral-associated organic carbon formation, mineral particle aggregation, and water retention (Liang 2020; Xiao et al., 2021; Witzgall et al., 2021; Craig et al., 2022; Dong et al., 2022; Sokol et al., 2022; Zhang et al., 2022). Microbiota uses new organic matter as a source of energy and C for growth and biomass formation. Dissolved organic carbon in soil represents only a small fraction of total carbon, but is deeply involved in interactions with microorganisms, minerals and plants. The microbial processes responsible for their dynamics ensure biotic/abiotic degradation and aggregation on/in microparticles. Restoring soil quality and health are key elements in recent research and the use of synthetic microbiomes, a relatively ecological way to improve the carbon cycle. Inoculation of soil with beneficial microorganisms, grouped in different consortia, selected according to exometabolic qualities, integrative capacities and compatibility, can influence the stability and sequestration of biosynthesized C in soil organic matter (SOM). This research aimed to assess glycoprotein, EPS, DOC, and microbial biomass contents following the inoculation of synthetic microbiomes into Mollic Gleysol (Salinic), and to evaluate their potential as indicators of changes in organic carbon stability and sequestration promoted by these microbiomes during microbial restoration of nutrient cycles - particularly the carbon cycle.

MATERIALS AND METHODS

Soil type and characteristics - The sampling site is the Danube Delta, the soil used represents a soil subtype of the soil type, Mollic Gleysol (WRB). The location, using the global positioning system (GPS), was at 45.034452° N latitude and 29.205589° E longitude for Mollic Gleysol (Salinic). The soil was collected in randomly extracted samples, divided into subsamples from the surface layer (0-20 cm), for incubation experiments or stored directly at 4°C, for subsequent soil characterization. The soil had the following parameters: pH value 6.46, organic C content 5.5%, organic matter content 32%, humus content 9.54%, clay content <1.002%.

The experiment - A laboratory experiment was conducted to evaluate the impact of inoculating synthetic microbiomes (M1, M4, M5, M7), previously selected and tested, on the dynamics of organic C compounds, the capacities to influence the processes of stabilization and carbon sequestration in Mollic Gleysol (Salinic). The influence of microbiomes was analyzed after a period of 120 days from the application of 10ml of inoculum/pot and the variants carried out had three repetitions each. Analyses were performed for a series of parameters considered potential indicators for monitoring the processes of stability and carbon sequestration, such as the content in glycoproteins, exopolysaccharides, dissolved organic carbon, water-soluble fulvic compounds.

Microbiomes - The selection and creation of the synthetic microbiomes used M1, M4, M5, M7 ensures the biodiversity, adaptability, integrative capacity and compatibility of the microbial species in their composition. The basic discriminatory criteria were the production of microbial biomass, exometabolites, the level of rhizospheric activity, fungal preponderance, the presence of ecto-, endosymbionts and antagonists. The microbiomes had 7-12 microorganisms, the component microbial isolates were grouped in relation to the biomass/metabolites/CO_2 relationship, and in the case of each microbiome the exometabolites influence on the biochemical parameters characterizing the soil organic matter was monitored.

Composition of synthetic microbiomes - The complex microbial composition of the M1, M4, M5, M7 microbiomes consists of bacterial, fungal, diazotrophic and mycorrhizal microflora. Synthetic microbiomes contain microorganisms belonging to soil microflora, to bacterial genera such as *Pseudomonas, Bacillus, Rhizobium, Azotobacter, Acetobacter, Serratia, Paenibacillus, Streptomyces*, and to the fungal genera *Trichoderma, Stachybotrys, Mortierella, Chaetomium, Arthrobotrys,*

Humicola, Cunninghamella, Myrothecium, Torula, Aspergillus, Penicillium, Penicillium, Boletus, Cladosporium, Glomus. The selected microbial species are kept in pure cultures, in various culture media (Topping, Czapek, YEM, MMN, LB, BHI, MRS, King), at a constant temperature of 4°C.

The Glycoprotein content - Glomalin-related soil protein (GRSP), as a glycoprotein, was extracted using 1g of dry soil, granulation <2 mm, mixed with sodium citrate, pH 7.0. The mixture autoclaved at 121°C, 30min. was cooled and centrifuged at 5000 rpm, 10 min. resulting in a supernatant, typical of glomalin. The content was quantified colorimetrically using bovine serum albumin (BSA) as a standard and the method of Bradford (1976) for detecting color variations, and the glomalin content in the samples was determined using a microplate reader at 595 nm. Using specific fluorochrome, photographic images of glomalin on the surface of the aggregates were obtained.

Exopolysaccharide (EPS) quantification - The EPS soil sample analyzed by the phenol-sulfuric acid method involved extraction by ultrafiltration with a 10-500 kDa (MWCO) membrane, dissolution in ddH_2O, addition of phenol, concentrated sulfuric acid and glucose, used as a standard. The tube with the reaction mixture is left for 10 min. at ambient temperature, shaken and incubated in a water bath at 25°C for 20 min. and the absorbance of the yellow-orange color measured at 490nm, using a spectrophotometer (DuBois et al., 1956).

Dissolved organic carbon (DOC) - The soil samples were air-dried according to Ghani et al. (2003), deionized water was added and the suspensions obtained were subsequently sonicated with an ultrasonic power of 27W, for 10min, at a temperature below 30°C and under continuous stirring. After sonication, the samples were centrifuged for 10 minutes at 10°C, the suspensions filtered through a cellulose acetate membrane with Ø 0.45 µm. The samples were analyzed spectrometrically with UV-Vis, at spectral absorption at 254-400 nm, 1 cm quartz cuvette. The amount of DOC was calculated for substances with absorption in the UV range using the methods of Brandstetter et al. (1996).

Water-soluble fulvic subfraction - A separation of the water-soluble fulvic sub-fraction was achieved from Mollic Gleysol (Salinic) by extraction and subsequent fraction, by adsorption on activated carbon, serial elutions with acetone, NaOH and distilled water (Votolin et al., 2022).

Specific ascending chromatograms - To assess the changes occurring in the water-soluble fulvic sub-fraction, an adapted method of specific chromatograms was used. Through them, the contribution of exometabolites of the microbiomes formed images whose distribution pattern, shape, and dimensions highlighted the intensity of metabolic activities, variations in the composition of the fulvic sub-fractions, as well as changes in specific biochemical characteristics.

Dissolved Organic C Fluorescence - DOC from the water-soluble fulvic sub-fractions of Mollic Gleysol (Salinic) inoculated with microbiomes was treated with fluorochrome and the distribution revealed by specific chromatography (Wang et al., 2016). Photographic images were obtained under the influence of 350 nm UV illumination and revealed qualitative differences such as the distribution, density of biosynthesized components and their complexity, through the degree of affinity for fluorescence.

Microbial biomass - After 120 days of inoculation of synthetic microbiomes in bioassay pots, soil samples were collected, and the soil microbial biomass was determined by the fumigation-extraction method. Soil samples were fumigated with $CHCl_3$ for 24 h at 20°C, extracted with K_2SO_4 and filtered through cellulose ester. The carbon in the soil microbial biomass was calculated according to Standard-SR-ER-ISO-14240-1-(2012), as a means of three replicates.

Data analysis - Results were interpreted by one way analysis of variance (ANOVA). The value $p<0.05$ was considered statistic significant (Student test).

RESULTS AND DISCUSSIONS

Glycoprotein content in soil - Generally, the glycoprotein content of the soil is considered an indicator of the quality of organic matter, of soil fertility, and a series of recent studies have

also highlighted the fact that its values correlate significantly with the content in the microflora. Considering these aspects, the influence of synthetic microbiomes M1, M4, M5, M7 inoculated in Mollic Gleysol (Salinic) on the evolution of the content of glycoproteins, respectively of glomalin-related soil protein (GRSP), was analysed, in terms of a potential impact on the stability and sequestration of carbon. Thus, in Mollic Gleysol (Salinic) inoculation with the M4 and M5 microbiomes determined a GRSP content of 1.35 mg x g^{-1}, and respectively 1.73 mg x g^{-1}. The glycoprotein contribution appears lower after the introduction of the M7 and M1 microbiomes, the glycoprotein content reaching 1.27 mg x g^{-1}, and respectively 1.12 mg x g^{-1}, in the analyzed soil (Figure 1).

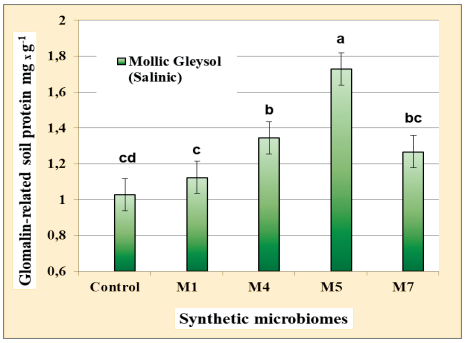


Figure 1. GRSP content generated by inoculating microbiomes in Mollic Gleysol (Salinic)

The GRSP content changed during the experiment, in relation to the analysed microbiome variant, presenting a stability and evolution similar to the intermediate assessments, an involvement in carbon dynamics, but also in the evolution of other carbon evolution indicators (soil respiration, microbial biomass) (Matei et al., 2015; Matei et al., 2017).

GRSP accumulations influence soil properties and compositional features, and for the stability of its optimal properties, GRSP could intervene through quantitative/qualitative adjustments, in guiding the way of managing them in the soil.

The GRSP content in soils, evaluated in numerous recent studies, can be considered an indicator of the quality of organic matter in arable soils (chernozems, luvisols, cambisols, fluvisols, etc.) or under different crops (wheat, barley, rapeseed, alfalfa, clover, potatoes, beet, corn, etc.). In these studies, the results of long-term monitoring and different soil-climate conditions revealed that the GRSP content significantly correlates with the carbon content of soil organic matter, with the ratio of carbon content in humic and fulvic acid (CHA/CFA), with the clay content (Ø < 0.002 mm), with particles smaller than 0.01 mm, with the type and texture of the soil. The GRSP content was significantly higher in chernozems compared to other soils (luvisols, cambisols, fluvisols, etc.). The results of other studies did not reveal significant correlations between GRSP and pH $CaCl_2$, bulk density, porosity, plant-available phosphorus content, the amount of organic matter applied (straw, manure, compost) or the type of organic fertilizer. Research has shown that GRSP has a dual contribution to the storage of soil organic carbon (SOC) through its long residence time (6-42 years) in which it directly improves SOC, through its sticky nature that leads to the formation and stabilization of soil aggregates, both of which protect labile C from microbial decomposition. The persistent composition of microbial biosynthesized products allows them to gradually accumulate in the soil as stable forms of C. Also, the contribution of GRSP to SOC stocks is considered to vary between 5-25%, to which is added the biomass and the microbial necromass. It has also been observed in some experiments that arbuscular mycorrhizal fungi (AMF), which are part of some microbiomes, produce GRSP in close correlation with the intensity of root colonization. Infrared thermogravimetric analyses showed that GRSP is thermostable, contains Fe and can form flocs with soil particles and has high adsorption capacity (76-95%) for metals such as Cu, Zn, Pb, Cd (He et al., 2020; Wang et al., 2021; Hartmann & Six, 2022; Li et al., 2023; San-Emeterio et al., 2024; Singh et al., 2024; Philippot et al., 2024).

The fluorescence affinity of the GRSP structure was analysed after inoculation with synthetic microbiomes, revealing the presence of biosynthesized microbial organic compounds on the surface of the aggregates. The fluorescent components highlight the degree of formation and influence of new biosynthesized compounds on the aggregate structures,

adjacent organic matter, in a short period of time (Figure 2).

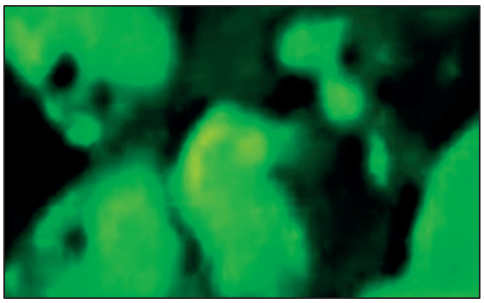


Figure 2. GRSP appear on the surface of aggregates as a green, fluorescent material, after M5 inoculation

The distribution of biosynthesized GRSP was different in the specific case of the type of microbiome applied to the soil. The organic component generated by endemic microflora of the un-inoculated soil allowed the comparison with the contribution of microbiomes and the possibility of preserving biosynthesized microbial C after inoculation in the soil. Through the dynamics of microbial processes and activities, through the composition of the extracellular organic component introduced into the soil, the direct impact on the organic component can be highlighted in the case of the M5 microbiome through the influence on the aggregate structures, generating organo-mineral protection.

Exopolysaccharides content in soil - Soil microbial exopolysaccharides (EPSs) are involved in the colonization and survival of microbiota, in the formation and stabilization of aggregates, in the enzymatic activity of organic matter, in the aggregation and degradation of minerals, in the water retention capacity, in the storage and capture of nutrients, in the sequestration and transformation of contaminants. The cells appeared incorporated in the self-produced extracellular biopolymeric substances, as a result of the strategy of adaptation and resistance to the conditions in Mollic Gleysol (Salinic). Their qualitative and quantitative content in the soil appeared possibly in close agreement or complementary to the composition of the exometabolites excreted by the endemic microbiota of the inoculated soil and thus helping the assessments regarding the improvement of ecological functions, through extracellular products. Thus, the biochemical protection offered to carbon compounds by the components of the synthetic microbiome M5, through the increased EPS biosynthesis capacity (594 $\mu g \times ml^{-1}$), was more efficient than that offered by the components of the microbiome M4 (548 $\mu g \times ml^{-1}$). This aspect perhaps suggests a more intense involvement in carbon sequestration/stabilization in Mollic Gleysol (Salinic), possibly through a direct adsorption of exometabolized organic molecules, as well as possibly through an intensification of aggregation with soil minerals. Also, the synthetic microbiomes M7 and M1 inoculated into the soil influenced the distribution of carbon compounds in the fulvic sub-fractions. Biochemical protection was provided less effectively by EPSs metabolites biosynthesized by the M7 and M1 microbiomes (487 $\mu g \times ml^{-1}$, and respectively 436 $\mu g \times ml^{-1}$), which generated a complexity and a reduced influence on the stability and sequestration of organic carbon in the soil (Figure 3).

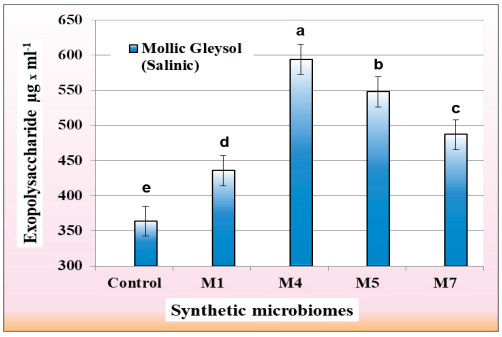


Figure 3. EPS content biosynthesized by microbiomes inoculated into the soil

The composition of EPSs is improved by the contribution of the new microbiota inoculated in Mollic Gleysol (Salinic) which intervenes in maintaining soil health by increasing the stability of aggregates and retaining extracellular enzymes to ensure the metabolic stability of the soil. The exopolysaccharide content biosynthesized in the soil by synthetic microbiomes, differed in the inoculated experimental variants, correlated with the changes in structure and complexity of the organic carbon content in the water-soluble fulvic sub-fractions of Mollic Gleysol (Salinic).

The bacterial microflora of the synthetic communities (SynCom) contributed substantially to maintaining the stability of C compounds in both macro-aggregates and micro-aggregates, and the fungal microflora had a strong impact predominantly on macro-aggregates. Thus, recent studies have highlighted the role of the *Bacillus* and *Pseudomonas* genera that secrete EPSs or their components in the formation of biofilms. The ability of microflora to improve soil aggregate formation is influenced in particular by the ability of the selected strains to secrete EPSs, the composition and biosynthesized structure of EPSs (Lehmann et al., 2017; Bérard et al., 2020; Cheng et al., 2020; Costa et al., 2020; Wei et al., 2024).

In studies on the biodegradation of polycyclic aromatic hydrocarbons in soils, bioremediation is effective because EPSs create bioavailability and accelerate their biodegradation, the dense matrix of EPSs being involved in the biodegradation of benzo-pyrene through external electron transfer, enzymatic catalysis, photo-degradation, etc. Also, quantitative quantifications, analyses of the composition and structure of EPSs have allowed estimates of the response of soil microbiota to stress, as well as the involvement of biosynthesized EPSs in soil functions and in ensuring its health (Flemming et al., 2023; Costa et al., 2020; Nkoh et al., 2020; Guhra et al., 2019).

Microorganisms in synthetic microbiomes can release stress-sensitive EPSs in difficult environments, supporting minimal nutrient concentrations and prosperity in oligotrophic conditions, constituting a resource of hydrocolloids, biodegradable and biocompatible biopolymers, bio-sorbents and soil improvers (Mahmoud et al., 2021; Kant et al., 2023; Ibrahim et al., 2022).

Studies on the taxonomic composition of microbiomes include bacterial and fungal species that produce EPSs and belong to the genera *Rhizobium, Pseudomonas, Bacillus, Azotobacter, Acetobacter, Azospirillium, Aureobasidium, Schizophyllum, Sclerotium, Botryosphaeria, Lentinus, Grifola*, etc. (Bourles et al., 2019; Deka et al., 2019; Luláková et al., 2019; Ali et al., 2020; Almansoory et al., 2020; Aksenov at al., 2021; Osińska-Jaroszuk et al., 2021; Jeong et al., 2022; Liu et al., 2022; Sagar et al., 2022; Balducci et al., 2023; Stoica et al., 2023; Liebmann et al., 2024).

The microbiota of microbiomes inoculated in Mollic Gleysol (Salinic) produce EPSs, as metabolic by-products or as extracellular polysaccharides, initially important in biofilm formation and microbial interactions.

Dissolved organic carbon content in soil - DOC, as a relatively labile fraction, actively participates in the soil in the processes of stimulating microbial activity and composition, co-metabolism, the passage of soil organic carbon into labile fractions, promoting the formation of aggregates, introducing stable components, inhibiting microbial activities, sorption of soil organic carbon and stimulating specific microorganisms involved in the C cycle belonging to the genera *Pseudomonas, Acidobacter, Acidimicrobium* etc.

Given its complex role in soil carbon dynamics, the evolution of the dissolved organic carbon content under the influence of inoculated microbiomes was analysed in the laboratory experiment in order to evaluate the stability and sequestration of carbon.

Thus, Mollic Gleysol (Salinic) inoculated with the M5 microbiome had a DOC content of 10.13 mg x L^{-1}. After inoculation of the M4 microbiome, at the end of the experiment, the dissolved organic carbon content determined was 11.89 mg x L^{-1} in Mollic Gleysol (Salinic). The highest dissolved organic carbon content was obtained after inoculation of the M7 and M1 microbiomes, 12.64 mg x L^{-1} and 14.06 mg x L^{-1}, respectively.

In the case of the M1 and M7 microbiomes, the dissolved organic carbon content in the soil decreased by 10.62% and 19.82%, respectively, compared to the control, and under the influence of the M5 microbiome, the dissolved organic carbon content decreased by up to 35.69%, compared to the control (15.76 mg x L^{-1}) (Figure 4). In the controlled environment, the variation of dissolved organic carbon concentrations did not depend on other disturbing factors such as the increase/decrease of average nitrate (NO^{-3}), sulphate (SO_2^{-4}), pollutants, climatic variations, etc., their magnitude depending on the specific influence of the microbiomes. The trends over the 120 days of DOC in the soil solution reflected the

interactions between soil properties and the specific characteristics of the microbiomes.

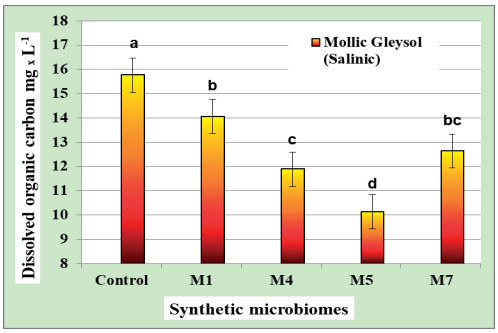


Figure 4. Dissolved organic carbon content in Mollic Gleysol (Salinic) inoculated with synthetic microbiomes

The amount of C stored in the soil depends on the metabolism of the microbial community and its physico-chemical structure, on the interactions or chemical absorption of carbon molecules to the mineral component, through which the accessibility of carbon can be modified. Extracellular enzymes produced by the synthetic microflora of the microbiomes may have a role in the production of consumable carbon compounds involved in the growth and activities of the microbiota. Through turnover, taxa from synthetic microbiomes contribute to the generation of necromass composed of melanins, chitins, hydrophobins, glycoproteins, lipids, β-glucans, etc., which enter the composition of DOC (Albright et al., 2020).

Recent studies have also identified in the early stages of soil decomposition of organic material, up to 42 bacterial and 9 fungal taxa, including protein compounds and tannins formed as a result of the process, which positively correlated with DOC concentrations. Changes in DOC concentration in the soil solution indicate the links of taxa with microbial protein consumption, and the observed correlations probably indicate their role as interconnecting factors between DOC concentration and microbial production, the decomposition of specific metabolites and carbon persistence (Campbell et al., 2022).

In some studies, DOC accumulation in soil has been influenced by the chemical profile of carbon generated by synthetic microbiomes acting on the substrate and on the endemic soil microbiota, such that some compounds and even groups of compounds can be predictive of increased soil DOC concentration and carbon storage (Thompson et al., 2019; Albright et al., 2020; Ghannam & Techtmann, 2021; Campbell et al., 2022).

Water-soluble fulvic sub-fractions - In the water-soluble fulvic sub-fractions were highlighted by fluorescence the changes in the composition structuring induced under the influence of the M1, M4, M5, M7 microbiomes inoculated in the soil. The results may suggest that the microbial carbon pumps in the analysed soil were stimulated differently depending on the microbiome, respectively the microbial composition and their metabolic and biosynthetic characteristics.

Thus, in Mollic Gleysol (Salinic) inoculated with the synthetic microbiomes, the extracted and isolated water-soluble fulvic sub-fractions were analysed by ascending chromatography in terms of the molecular distribution of the compounds, the formation of more stable molecular structures, of complex organic carbon compounds, highlighted by their fluorescence affinity. The analysed water-soluble fulvic sub-fractions presented the highest density of organic compounds, after 120 days from the inoculation with the M5 microbiome, in Mollic Gleysol (Salinic). In comparison, the synthetic microbiome M4 inoculated in the same soil had a water-soluble fulvic sub-fraction with organic carbon structures highlighted by the presence of dense areas with a lower molecular weight, as well as those with a lower density, represented by more complex compounds with a higher molecular weight. The stability of organic carbon (OC) compounds was lower in the analysed fulvic sub-fraction, after inoculation with M4, because the biochemical protection offered by exometabolites secreted by the components of the own microbiome was weaker compared to the stability of C compounds offered by the amount/diversity of exometabolites biosynthesized by the components of the M5 microbiome. In comparison, the synthetic microbiome M7 inoculated into the soil influenced the water-soluble fulvic sub-fraction by enriching it with new structures of biosynthesized organic C highlighted by the presence of dense areas dominated by

compounds with lower molecular weight, the increase in the share of those with reduced density, respectively with higher complexity and molecular weight. The stability of organic C in the soil inoculated with M7 is increased compared to M1, but lower than that provided by the microbiomes M5 and M4. In addition, even under conditions of reduced protection of C compounds, compared to other microbiomes, the exometabolites of the M7 microbiome provide a higher efficiency for the stability of organic C, than that provided by the microbiome M1 (Figure 5).

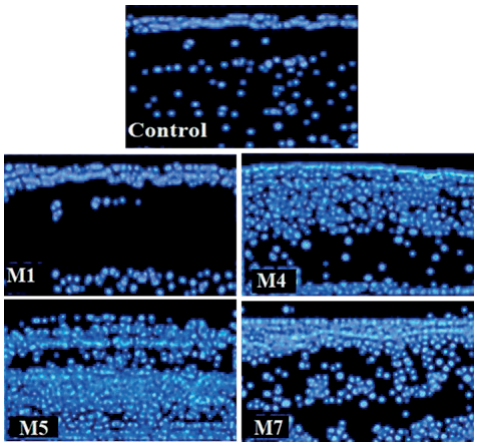


Figure 5. Water-soluble fulvic sub-fraction of soil after 120 days of microbiomes inoculation

Microbial biosynthesis of carbon in soil, for short periods of time and under standard conditions, generated labile substances, biosynthesized by endemic and inoculated microflora, extractable in water but also with fluorescence. To these was added the fluorescence of organo-mineral, humic and protein substances from the organo-mineral reserve of the soil. Monitoring their distribution and their visualization allowed estimates regarding the short-term influence of different microbiomes, with a biosynthetic role and possible options for enhancing C storage. Imaging, fluorescent compounds highlighted the degree of formation, distribution and organization of newly biosynthesized carbon, compared to uninoculated soil, under the influence of synthetic microbiomes. The distribution of biosynthesized carbon was specific to each type of microbiome applied to the soil. The dynamics of the processes and the composition of exometabolites introduced into the soil allowed highlighting the direct impact on the organic component. Biosynthesized exometabolites, detectable by fluorescence, revealed an abundance of fulvic materials of different molecular weights and degree of condensation, closely related to the number of species in the inoculum composition. Biosynthesized microbial C complements DOC in the soil, can stabilize on mineral surfaces, but if their concentration in the soil solution causes competition for sorption sites, desorption can also occur, with an impact on fluorescence. In addition, as a result of microbiota-mediated processes, the amounts of protons and the anion exchange capacity may increase in the soil, which allows a good highlighting of biosynthesized organic molecules.

Microbial biomass content in soil - The biostimulatory influence of selected synthetic microbiomes (M1, M4, M5, M7) on the evolution of total microbial biomass was analysed on Mollic Gleysol (Salinic).

The inoculated synthetic microbiomes contained different microbial species, present in different concentrations, compositions and ratios.

The biomass produced by the synthetic microbiomes inoculated in Mollic Gleysol (Salinic) reached values of up to 363.2 mg C x kg^{-1} soil (M5), after 120 days from inoculation and represented the experimental variant with an increase of up to 35.14% in the amount of microbial biomass. In the variants inoculated with the M4 and M7 microbiomes, the microbial biomass content increased by 25.28% (M7) to 31.28% (M4), reaching in its case a biomass of 352.8 mg C x kg^{-1} soil.

Under experimental conditions, biomass accumulations induced by inoculation showed variations ranging between 14.64-35.14% for the microbiome variants. The activity and diversity of the synthetic microbiomes correlated positively with microbial biomass. The biomass level reflected the intensity of activities generated by the inoculated microbiomes in the soil, maintaining constant conditions allowed the evaluation of their influence on the analysed parameter. The biomass accumulations reflected, in addition to the bio-stimulatory effect of the 4 synthetic

microbiome variants, both the integrative capacities of the new communities of microorganisms introduced into the soil, and the functional compatibilities with the endemic soil microflora (Figure 6).

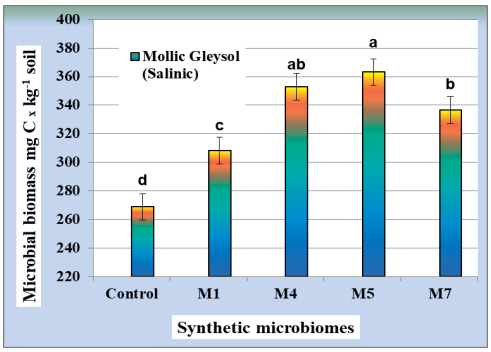


Figure 6. Microbial biomass of the Mollic Gleysol (Salinic) inoculated with synthetic microbiomes

In inoculated microbiomes, the fungal microflora, in addition to biosynthetic activity, is subsequently transformed into necromass, constituting the main microbiological contributing factor for the creation of stable soil organic matter in a much higher proportion, compared to the bacterial necromass originating from microbiomes. Also, the mycorrhizal fungi in the composition of synthetic microbiomes contribute significantly to the sequestration of carbon in the soil by transferring photosynthates to intraradicular hyphae, then to extraradicular hyphae and subsequently, to their release into the soil matrix, involving in obtaining these results the amount of hyphal biomass produced and the stabilized soil aggregates. Microbial growths, in the initial phase of decomposition, contain mainly bacterial taxa of the phylum Proteobacteria and fungi of the phylum Ascomycota, which is a particularly useful aspect regarding the involvement in the formation of soil organic matter (SOM). The relationships between natural/synthetic microbial communities in soil, organic matter and external factors manifest their impact on carbon storage, on the variability of available carbon in the soil. Synthetic communities (SynCom) can intervene to improve the microbial composition of soils and present themselves as a sustainable solution in restoring soil qualities.

Thus, synthetic microbiomes are not only participating elements in the soil, but have a facilitating role in the optimal functioning of ecosystems through interventions on: increasing the content of organic matter, through secondary metabolism compounds, organic nitrogen fixation, increasing the availability and acquisition of nutrients, inhibiting pathogens, secretion of extracellular biopolymers through which they bind soil particles into aggregates, creating stable structures, improving soil fertility, and plant growth (Asiloglu et al., 2021; Wang et al., 2021; Hartmann & Six, 2022; Zhang et al., 2022; Hao et al., 2024).

CONCLUSIONS

Synthetic microbiomes selected for their increased exogenous metabolic capacities for biosynthesis of organic compounds, compatibility, viability, intervened in the stabilization and sequestration of carbon in the soil.

Comparative studies and experimental data validated, through process indicators, the role of synthetic microbiomes with performing heterotrophs in priming soil processes and in carbon sequestration.

The analysed water-soluble fulvic sub-fraction in the soil inoculated with synthetic microbiomes allowed highlighting the different molecular distribution of the biosynthesized ones, the formation of more stable molecular structures of organic carbon, through changes in the distribution and molecular weight.

In the analysed soil, the endemic microbial communities as well as those from the inoculated microbiomes underwent quantitative growths, aggregated into biofilms in which the cells appeared incorporated in self-produced EPS substances, as a strategy of adaptation and resistance into the soil conditions.

The biosynthesized EPSs content determined by laboratory analyses correlated with the changes in the structure and complexity of the organic carbon content in the fulvic sub-fraction of the soil.

The GRSP content varied in relation to the type of inoculated microbiome, presented a stable evolution, influenced the carbon dynamics and

correlated with other indicators of carbon evolution.

The determined biosynthesized EPSs content varied in the case of the microbiomes and correlated with changes in the complexity of the organic carbon content.

GRSP, EPSs and DOC content in the soil varied significantly depending on the type of inoculated microbiome and were sensitive in detecting the amounts of bio-generated carbon after inoculations, as potential indicators of the evolution of carbon stability/sequestration.

The efficiency of synthetic microbiomes was validated by their specific contribution to the differential increase in biomass accumulations in the soil.

The results demonstrated that microbial carbon pumps in the soil were stimulated differently in the edaphosphere depending on the composition and characteristics of the microbiomes.

The results of the comparative study on the efficiency of selected synthetic microbiomes in carbon stability/sequestration processes highlighted the M5 and M4 microbiomes, for the composition and functional efficiency in the newly formed microbial communities.

ACKNOWLEDGEMENTS

This work was supported by the Ministry of Education and Research, Project number PN 23 29 03 01/2023 and Scientific Research Contract 78/2024 (NEC).

REFERENCES

Agnihotri, R., Sharma, M.P., Prakash, A., Ramesh, A., Bhattacharjya, S., Patra, A.K., Manna, M.C., Kurganova, I. & Kuzyakov, Y. (2022). Glycoproteins of arbuscular mycorrhiza for soil carbon sequestration: Review of mechanisms and controls. *Science Total Environment*, *806*, 150571. https://www.sciencedirect.com/science/article/pii/S0048969721056485

Aksenov, A.S., Shirokova, L.S., Kisil, O.Y., Kolesova, S. N., Lim, A. G., Kuzmina, D., Pouillé, S., Alexis, M.A., Castrec-Rouelle, M., Loiko, S.V. & Pokrovsky, O.S. (2021). Bacterial Number and Genetic Diversity in a Permafrost Peatland (Western Siberia): Testing a Link with Organic Matter Quality and Elementary Composition of a Peat Soil Profile. *Diversity*, *13*(7), 328. https://www.mdpi.com/ 1424-2818/13/7/328

Albright, M.B.N., Johansen, R., Thompson, J., Lopez, D., Gallegos-Graves, L.V., Kroeger, M.E., Runde, A., Mueller, R.C., Washburne, A., Munsky, B., Yoshida, T. & Dunbar, J. (2020). Soil bacterial and fungal richness forecast patterns of early pine litter decomposition. *Frontiers in Microbiology*, *11*, 542220. https://doi.org/10.3389/ fmicb.2020.542220

Ali, P., Shah, A.A., Hasan, F., Hertkorn, N., Gonsior, M., Sajjad, W. & Chen, F. (2020). A Glacier Bacterium Produces High Yield of Cryoprotective Exopolysaccharide. *Frontiers in Microbiology*, *10*, 3096. https://doi.org/10.3389/ fmicb.2019.03096.

Almansoory, A.F., Al-Baldawi, I.A. & Hazaimeh, M. (2020). Optimization of the EPS production of a bacterial floc consortium using different parameters. *Biocatalysis and Agricultural Biotechnology*, *23*, 101466, https://doi.org/10.1016/j.bcab.2019.101466.

Asiloglu, R., Kenya, K., Samuel, S.O., Sevilir, B., Murase, J., Suzuki, K. & Harada, N. (2021). Top-down effects of protists are greater than bottom-up effects of fertilisers on the formation of bacterial communities in a paddy field soil. *Soil Biology and Biochemistry*, *156*, 108186. https://doi.org/10.1016/ j.soilbio.2021.108186

Balducci, E., Papi, F., Capialbi, D. E., & Del Bino, L. (2023). Polysaccharides' Structures and Functions in Biofilm Architecture of Antimicrobial-Resistant (AMR) Pathogens. *International Journal of Molecular Sciences*, *24*(4), 4030, https://doi.org/10.3390/ ijms24044030

Bérard, A., Clavel, T., Le Bourvellec, C., Davoine, A., Le Gall, S., Doussan, C. and Bureau, S. (2020) Exopolysaccharides in the rhizosphere: A comparative study of extraction methods. Application to their quantification in Mediterranean soils. *Soil Biology and Biochemistry, 149*, 107961, https://doi.org/10.1016/j.soilbio.2020.107961

Bourles, A., Guentas, L., Chalkiadakis, E., Majorel, C., Juillot, F., Cavaloc, Y., Burtet-Sarramegna, V., Medevielle, V., Jourand, P. & Amir, H. (2019). New Caledonian ultramafic conditions structure the features of Curtobacterium citreum strains that play a role in plant adaptation. *Canadian Journal of Microbiology*, 65, 880–894.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein14 utilizing the principle of protein-dye binding, Analytical Biochemistry, 72, 248–254.

Brandstetter, A., Sletter, R.S., Mentler, A. & Wenzel, W.W. (1996). Estimating dissolved organic carbon in natural water by UV-absorbance (254 nm). *Z Pflanz Bodenkunde*, 159, 605–607.

Campbell, T.P., Ulrich, D.E.M., Toyoda, J., Thompson, J., Munsky, B., Albright, M.B.N., Bailey, V.L., Tfaily, M.M. & Dunbar, J. (2022). Microbial Communities Influence Soil Dissolved Organic Carbon Concentration by Altering Metabolite Composition. *Frontiers in Microbiology, 12*, 799014. https://doi.org/10.3389/fmicb.2021.799014.

Cheng, A. G., Po-Yi, H., Andrés, A.D., Sunit J., Feiqiao B. Y., Xiandong M., Min W., et al. (2022). Design, Construction, and in Vivo Augmentation of a Complex Gut Microbiome. *Cell*, *185*, 3617–3636, https://doi.org/10.1016/j.cell.2022.08.003.

Cheng, C., Shang-Guan, W., He, L. & Sheng, X. (2020). Effect of exopolysaccharide-producing bacteria on water-stable macro-aggregate formation in soil. *Geomicrobiology Journal*, 37, 738–745.

Costa, O.Y.A., Pijl, A. & Kuramae, E.E. (2020). Dynamics of active potential bacterial and fungal interactions in the assimilation of acidobacterial EPS in soil. *Soil Biology and Biochemistry*, *148,* 107916. https://doi.org/10.1016/j.soilbio.2020.107916

Craig, M.E., Geyer, K.M., Beidler, K.V., Brzostek, E.R., Frey, S.D., Stuart Grandy, A., Liang, C. & Phillips, R.P. (2022). Fast-decaying plant litter enhances soil carbon in temperate forests but not through microbial physiological traits. *Nature Communications, 13,* 1229. https://www.nature.com/articles/s41467-022-28715-9

Deka, P., Goswami, G., Das, P., Gautom, T., Chowdhury, N., Boro, R.C. & Barooah, M. (2019). Bacterial exopolysaccharide promotes acid tolerance in Bacillus amyloliquefaciens and improves soil aggregation. *Molecular Biology Reports*, 46, 1079–1091.

Dong, H., Huang, L., Zhao, L., Zeng, Q., Liu, X., Sheng, Y., Shi, L., Wu, G., Jiang, H., Li, F., Zhang, L., Guo, D., Li, G., Hou, W. & Chen, H. (2022). A critical review of mineral–microbe interaction and co-evolution: mechanisms and applications, *National Science Review, 9*, nwac128. https://doi.org/10.1093/nsr/nwac128

Dubois, M., Gilles, K.A., Hamilton, J.K., Pebers, P.A. & Smith, F. (1956). Colorimetric method for determination of sugar and related substances. *Analytical Chemistry*, 28, 350–356.

Feng, X. & Wang, S. (2023). Plant influences on soil microbial carbon pump efficiency. *Global Change Biology*, 29 (14), 3854–3856.

Finkel, O. M., Isai S.G., Gabriel, C., Jonathan, M. C., Theresa, F. L., Paulo, J. P. L. T., Ellie, D. W., Connor R.F., Corbin, D.J. & Jeffery D.L. (2020). A Single Bacterial Genus Maintains Root Growth in a Complex *Microbiome. Nature* 587, 103–108. https://doi.org/10.1038/s41586-020-2778-7

Flemming, H-C., van Hullebusch, E.D., Neu, T.R., Nielsen, P.H., Seviour, T., Stoodley, P., Wingender, J. & Wuertz, S. (2023). The biofilm matrix: Multitasking in a shared space. *Nature Reviews Microbiology*, 21, 70–86.

Ghani, A., Dexter, M. & Perrott, K.W. (2003). Hot-water extractable carbon in soils: a sensitive measurement for determining impacts of fertilisation, grazing and cultivation. *Soil Biology and Biochemistry*, 35 (9):1231-1243.

Ghannam, R. B.& Techtmann, S. M. (2021). Machine learning applications in microbial ecology, human microbiome studies, and environmental monitoring. *Computational and Structural Biotechnology Journal*, *19,* 1092–1107. https://doi.org/10.1016/ j.csbj. 2021.01.028.

Guhra, T., Ritschel, T. & Totsche, K.U. (2019). Formation of mineral-mineral and organo-mineral composite building units from microaggregate-forming materials including microbially produced extracellular polymeric substances. *European Journal of Soil Science*, 70, 604–615.

Hao, X., Gu, Y., Zhang, H., Wang, X., Liu, X., Chen, C., Wang, C., Zhang, X., Liu, X., & Shen, X. (2024). Synthetic Microbial Community Promotes Bacterial Communities Leading to Soil Multifunctionality in Desertified Land. *Microorganisms, 12*(6), 1117. https://doi.org/10.3390/microorganisms12061117.

Hartmann, M. &, Six, J. (2022). Soil structure and microbiome functions in agroecosystems. *Nature Reviews Earth & Environment*, 4, 4–18.

He, J.D., Chi, G.G., Zou, Y.N., Shu, B., Wu, Q.S., Srivastava, A.K. & Kuča, K. (2020). Contribution of glomalin-related soil proteins to soil organic carbon in trifoliate orange. *Applied Soil Ecology, 154*, 103592 https://doi.org/10.1016/j.apsoil.2020.103592.

Ibrahim, H.A., Abou Elhassayeb, H.E. & El-Sayed, W.M. (2022). Potential functions and applications of diverse microbial exopolysaccharides in marine environments. *Journal of Genetic Engineering and Biotechnology, 20* (1), 151, https://doi.org/10.1186/ s43141-022-00432-2.

Irving, T.B., Alptekin, B., Kleven, B. & Ané, J.M. (2021). A critical review of 25 years of glomalin research: a better mechanical understanding and robust quantification techniques are required. *New Phytologist*, 232 (4), 1572–1581.

Jeong, J.P., Kim, Y., Hu, Y. & Jung, S. (2022). Bacterial Succinoglycans: Structure, Physical Properties, and Applications. *Polymers, 14*(2), 276, https://doi.org/10.3390/polym14020276.

Kant, G., Pandey, A., Shekhar, H. & Srivastava, S. (2023). Enhanced bio-synthesis of isoprene via modifying mevalonate and methylerythritol phosphate pathways for industrial application: a review, *Process Biochemistry*, 130, 256–2171.

Lehmann, A., Zheng, W. and Rillig, M.C. (2017) Soil biota contributions to soil aggregation. *Nature Ecology & Evolution*, 1, 1828–1835.

Li, M., Peng, J., Lu, Z. & Zhu, P. (2023). Research progress on carbon sources and sinks of farmland ecosystems. *Resources, Environment and Sustainability*, *11*, 100099. https://doi.org/10.1016/j.resenv.2022.100099

Liang, C., (2020). Soil microbial carbon pump: Mechanism and appraisal, *Soil Ecology Letters*, 2, 241–254.

Liebmann, P., Bárta, J., Vogel, C., Urich, T., Kholodov, A., Varsadiya, M., Mewes, O., Dultz, S., Waqas, M., Wang, H., Shibistova, O., Guggenberger, G. (2024a). Permafrost degradation and its consequences for carbon storage in soils of Interior Alaska. *Biogeochemistry*, 167, 199–223.

Liu, X., Chai, J., Zhang, Y., Zhang, C., Lei, Y., Li, Q. & Yao, T. (2022). Halotolerant rhizobacteria mitigate the effects of salinity stress on maize growth by secreting exopolysaccharides. *Environmental and Experimental Botany, 204*, 105098, https://doi.org/10.1016/j.envexpbot.2022.105098

Luláková, P., Perez-Mon, C., Šantrůčková, H., Ruethi, J. & Frey, B. (2019). High-Alpine Permafrost and Active-Layer Soil Microbiomes Differ in Their Response to Elevated Temperatures. *Frontiers in*

Microbiology, 10, https://doi.org/10.3389/fmicb.2019.00668.

Mahmoud, Y.A., El-Naggar, M.E., Abdel-Megeed, A. & El-Newehy, M. (2021). Recent advancements in microbial polysaccharides: synthesis and applications. *Polymers, 13* (23), 4136, https://doi.org/10.3390/polym13234136

Matei, G.M., Dumitru, S., Matei, S., Butcaru, A. & Stănică, F. (2017). Microbiological aspects in soil microbiome composition and activity under the influence of organic mulches. *International Multidisciplinary Scientific GeoConference: SGEM* 17, 813–820.

Matei, S., Matei, A., Matei, G.M. & Cornea, C.P. (2015). Utilization of lactic acid bacteria and extracellular compounds in biological control of fungal species. *Research Journal of Agricultural Science, 47*(3), 122–132.

Nkoh, N.J., Liu, Z.-D., Yan, J., Cai, S.-J., Hong, Z.-N. & Xu, R.-K. (2020). The role of extracellular polymeric substances in bacterial adhesion onto variable charge soils. *Archives of Agronomy and Soil Science*, 66, 1780–1793.

Osińska-Jaroszuk, M., Sulej, J., Jaszek, M. & Jaroszuk-Ściseł, J. (2021). Applications of Fungal Polysaccharides. *Encyclopedia of Mycology*, 2, 613–628.

Philippot, L., Chenu, C., Kappler, A., Rillig, M.C. & Fierer, N. (2024). The interplay between microbial communities and soil properties. *Nature Reviews Microbiology*, 22, 226–239.

Rillig, M.C., Ryo, M., A. Lehmann, A., Aguilar-Trigueros, C.A., Buchert, S., Wulf, A., Iwasaki, A., Roy, J. & Yang, G. (2019). The role of multiple global change factors in driving soil functions and microbial biodiversity. *Science, 366* (6467), 886–890.

Sagar, A., Rai, S., Ilyas, N., Sayyed, R. Z., Al-Turki, A. I., El Enshasy, H. A., & Simarmata, T. (2022). Halotolerant Rhizobacteria for Salinity-Stress Mitigation: Diversity, *Mechanisms and Molecular Approaches. Sustainability, 14*(1), 490. Retrive from https://doi.org/10.3390/su14010490.

San-Emeterio, L.M., Lozano, E., Arcenegui, V., Mataix-Solera, J., Jiménez-Morillo, N.T. & González-Pérez, J.A. (2024). Soil-Easily Extractable Glomalin: An Innovative Approach to Deciphering Its Molecular Composition under the Influence of Seasonality, Vegetation Cover, and Wildfire. *Environmental Science & Technology, 58*(51), 22624–22634. https://doi.org/10.1021/acs.est.4c10036

Singh, A.K., Chen, C., Zhu, X., Yang, B., Khan, M.N., Zakari, S., Jiang, X.J., del Mar Alguacil, M. & Liu, W. (2024). Unraveling the impact of global change on glomalin and implications for soil carbon storage in terrestrial ecosystems. Resources, *Environment and Sustainability, 18*, 100174, https://doi.org/10.3389/fmicbdoi.org/10.1016/j.resenv.2024.100174.

Sokol, N.W., Slessarev, E., Marschmann, G.L., Nicolas, A., Blazewicz, S.J., Brodie, E.L., Firestone, M.K., Foley, M.M., Hestrin, R., Hungate, B.A., Koch, B.J., Stone, B.W., Sullivan, M.B., Zablocki, O. & Pett-Ridge, J. (2022). Life and death in the soil microbiome: how ecological processes influence biogeochemistry, *Nature Reviews Microbiology*, 20, 415–430.

Stoica, R. M., Moscovici, M., Lakatos, E. S., & Cioca, L. I. (2023). Exopolysaccharides of Fungal Origin: Properties and Pharmaceutical Applications. *Processes, 11*(2), 335, https://doi.org/10.3389/fmicbdoi.org/ 10.3390/pr11020335.

Thompson, J., Johansen, R., Dunbar, J. & Munsky, B. (2019). Machine learning to predict microbial community functions: an analysis of dissolved organic carbon from litter decomposition. *PLoS One 14*, e0215502, https://doi.org/10.1371/journal.pone.0215502

Votolin, K.S., Zherebtsov, S.I., Shpakodraev, K.M., Malyshenko, N.V. & Ismagilov, Z.R., (2022). Composition of Humic and Fulvic Acids from Lignite. *Coke Chemistry*, 65, 191–200.

Wang, Y. T, Shi, H. & Liu, X. F. (2016). Spectrofluorometric characterization of soil dissolved organic matter under different vegetation in Loess Hilly Region. *Journal of Plant Nutrition and Fertilizer, 22*(1): 171–179.

Wang,Q., Hong, H., Liao, R., Yuan, B., Li, H., Lu, H., Liu, J. & Yan, C. (2021). Glomalin-related soil protein: The particle aggregation mechanism and its insight into coastal environment improvement. *Ecotoxicology and Environmental Safety, 227*, 112940.https://doi.org/10.1016/j.ecoenv.2021.112940

Wei, Z., Niu, S., Wei, Y., Liu, Y., Xu, Y., Yang, Y., Zhang, P., Zhou, Q. and Wang, J.J. (2024). The role of extracellular polymeric substances (EPS) in chemical-degradation of persistent organic pollutants in soil: A review. *Science of The Total Environment, 912*, 168877. Retrieve from https://www.mdpi.com/2073-4441/16/19/2812

Witzgall, K., Vidal, A., Schubert, D.I., Höschen, C., Schweizer, S.A., Buegger, F., Pouteau, V., Chenu, C. & Mueller, C.W. (2021). Particulate organic matter as a functional soil component for persistent soil organic carbon, *Nature Communications, 12,* 4115. https://www.nature.com/articles/s41467-021-24192-8

Xiao, K.Q., Ge, T.D., Wu, X.H., Peacock, C.L., Zhu, Z.K., Peng, J., Bao, P., Wu, J.S. & Zhu, Y.G. (2021). Metagenomic and 14C tracing evidence for autotrophic microbial CO2 fixation in paddy soils. *Environmental Microbiology*, 23, 924–933.

Yue, C., Ciais, P., Houghton, R.A., & Nassikas, A.A. (2020). Contribution of land use to the interannual variability of the land carbon cycle. *Nature Communications, 11*(1), 3170. https://www.nature.com/articles/s41467-020-16953-8

Zhang, M., Cai, P., Wu, Y., Gao, C., Liu, J. & Huang, Q. (2022). Bacterial Extracellular Polymeric Substances: from the Perspective of Soil Ecological Functions, *Acta Pedologica Sinica,* 59, 308–323.

Zhang, Z., Lu, Y., Wei, G. & Jiao, S. (2022). Rare species-driven diversity-ecosystem multifunctionality relationships are promoted by stochastic community assembly. *mBio, 13,* e00449-422. Retrived from https://doi.org/10.1128/mbio.00449-42.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

CLIMATE TRENDS IN OLTENIA. CASE STUDY: DĂBULENI WINE CENTRE

Cristian MĂRĂCINEANU[1], Maria BĂJENARU[2], Aurelia DIACONU[2], Nicolae GIUGEA[1], Ramona CĂPRUCIU[1]

[1]University of Craiova, Faculty of Horticulture, 13. A. I. Cuza Str, Craiova, Romania
[2]Research and Development Station for Plant Culture on Sandy Soils Dăbuleni, Romania

Corresponding author email: mariaciuca92@yahoo.ro

Abstract

The Dăbuleni viticultural centre differs from other centres in Oltenia by the nature of the ecological factors. Located near the extreme south of Romania, it is characterized by sandy soils, high temperatures and a reduced rainfall regime. Under these conditions, viticulture acquires a series of particularities about culture technology and the choice of varieties for planting. All of these can be influenced by climate change. Meteorological data provided by the Research and Development Station for Plant Culture on sandy soils in Dăbuleni (Romania) for 10 years were interpreted to show the current viticultural climate and its trend. For this, several statistical indices were calculated (e.g. arithmetic mean, standard deviation, coefficient of variation), the Martonne aridity index was calculated, and the climagram of the period was made. The results were included in tables and represented graphically and show the evolution of the climate that characterizes viticulture on sands, in the Oltenia region (Romania).

Key words*: climate, monitoring, trend, agroecology.*

INTRODUCTION

Climate change directly or indirectly affects society, with significant risks for state or private enterprises; most targeted are the tourism, energy, agriculture, and food sectors, with direct repercussions on the national and global economy (Cheval et al., 2022; Falcescu et al., 2024). The annual instability of agricultural production due to climate change may threaten local and global food security (Lesk et al., 2016; Piticar et al., 2024). The yield of agricultural products would be higher if climate change had not intensified over the last decade, climate factors being the main environmental elements influencing the evolution and productivity of crops (Popescu et al., 2023). In 2018, Northern and Eastern Europe experienced high crop losses associated with extremely low rainfall and high temperatures between March and August (Brillouin et al., 2020). For example, low or erratic rainfall during the tuber development phase can lead to yield losses (Oseni & Masarirambi, 2011).

Understanding the regional dynamics of terrestrial water resources depends on several factors: snow phenology (timing of snow onset and snow melt) studied by Amihăesei et al., 2024, shows different dynamics between altitude bands and Koeppen-Geiger climatic regions in Romania with impact on water resources distribution and hydrological cycle, precipitation regime, orography complexity (Necula et al., 2024).

In the Oltenia region, there is a clear altitudinal zonation of vegetation as altitudes increase from south (about 3 m) to north (2519 m), which means a decrease in temperatures and, to some extent, an increase in precipitation (Vlăduț et al., 2017). Climate change in recent years in southern Romania stands out as persistent heat waves and prolonged droughts. Heat waves are extreme phenomena amplified by climate change, with critical implications for the human and environmental systems they affect (Pardo & Paredes-Fortuny, 2024). Changes in precipitation, evapotranspiration, and thus changes in the climatic water balance, are imminent effects of climate warming (Prăvălie et al., 2019).

Average temperatures in Romania have increased by more than 1.5ºC in the last thirty years, with industrial pollutants being the main driving causes (Șmuleac et al., 2020; Ciornei et al., 2023; van Daalen et al., 2024). The

European Union (EU) is taking significant measures to face these challenges. The Green Deal Association aims to reduce greenhouse gas emissions in the EU by 55% by 2030 and achieve carbon neutrality by 2050 (Rant, 2024). If global temperatures reach +2°C, Central and Northern Europe will also become more prone to vegetation fires during droughts (El Garroussi et al., 2024). In particular, fire seasons are hypothesized to become more extreme and extend into more temperate northern latitude regions (Miller et al., 2024). Changes in global temperature and wind speed have varied in different months during 1989-2021, with their average rates of change being about 0.34℃/10a and 0.02 m/(s·10a), but they are much larger in some local regions (Vitasse et al., 2021; Fei et al., 2023). With growth rates of 0.003, 0.5°C and 5.5 mm per year, respectively, the interannual variation of NDVI (normalized difference vegetation index), temperature and precipitation showed a visible increasing trend (Nasiri et al., 2024). To assess the current climate, a large ensemble of multi-decadal simulations is needed to adequately sample internal climate variability, robustly identify model deficiencies, and convincingly demonstrate progress between climate model generations (Barbu et al., 2016; Jain et al., 2023). This variability stems from processes within the ocean-atmosphere-cryosphere-atmosphere-earth-biosphere system and external influences such as solar and orbital cycles, volcanic eruptions, and anthropogenic greenhouse gas emissions (Deser & Phillips, 2023).

The changes in Romania's hydropneumatic regime since 1960 have been more or less significant regarding the annual flow regime. Thus, increasing trends in winter correlated with the increase in air temperature and liquid precipitation at the expense of snowfall, decreasing trends in summer based on general warming increased evaporation and increasing trends in fall flow (Zaharia et al., 2020). To identify the anomalies and trends associated with high (annual and seasonal) flows of rivers in eastern Romania, two methods are applied: quantile perturbation method (QPM) and partial perturbation trend method (PMT) (Minea et Chelariu, 2021). The use of interpolation techniques and statistical tools in the determination of various climatic data (temperature, precipitation and reference evapotranspiration) to explore the impact of climate change on Romanian forests after 1987 was fast by Prăvălie et al., 2022, the results highlighting the general trends of forest greening at the national level.

Many studies have linked climate change to urban development over the last century: rising urban air temperature, energy consumption rates, increased use of raw materials, pollution and waste generation, conversion of agricultural land to developed land, loss of biodiversity and water scarcity (Shahmohamadi et al., 2011). An analysis of the effects of temperature change on heating and cooling demand in Europe up to 2050 is carried out by Larsen et al., 2020, investigating trend changes (10-year average) and extremes (10-year min./max.). And the results of Micu et al., 2021, show a significant warming trend, well characterized by a sharp decrease in frost days and a significant increase in absolute maximum temperatures, summer days and the duration of drought periods. The impact of climate change on offshore wind resources in Northern Europe in the 21st century is analyzed based on the latest societal development and land use scenarios, with results showing that reducing emissions as shown by current climate targets would mitigate the declining trend and also lead to a more stable resource (Martinez et al., 2023).

The increasing intensity and fecundity of extreme weather events in recent years are increasing the risks of multiple crop losses at regional, national or global levels (Tigchelaar et al., 2018; Furtak & Wolińska, 2023). Hydric and thermal stress in the context of climate change affects the agricultural and horticultural sectors, and an in-depth knowledge of the phenomenon is topical. Increasing evidence on the significant impacts of climate change on viticulture (Alikadic et al., 2019; Cichi et al., 2021; Costea & Capruciu, 2022; Keller, 2023; Andrade et al, 2024), pomiculture (Cosmulescu et al; 2010; Milošević & Milošević, 2023; Jamal et al, 2024), vegetable growing (Mekonnen et al., 2022; Dumitru et al., 2023; Soare et al., 2024), etc., requires scientific research to investigate the evolution of these effects and the measures that can be taken

shortly (Droulia et al., 2021). Establishing the relationship between climatic resources, soil and vine variety, and the biochemical processes occurring under their influence confirms the viticultural vocation of the area (Căpruciu et al., 2023).

The impact of climate change on crop pathogen biogeography, disease incidence and severity, and their effects on natural ecosystems, agriculture and food production are topical studies (Trebicki et al., 2020; Singh et al., 2023).

Climate change mitigation strategies, such as afforestation, reforestation, and sustainable forest management, offer promising solutions for climate change mitigation while yielding substantial co-benefits for human health (van den Bosch et al., 2024).

In this study, meteorological data from the last 10 years in the Dăbuleni area were interpreted to characterize sandy sands viticulture in the context of current climate change.

MATERIALS AND METHODS

The analysis of climate trends in recent years indicates steady warming at the global and world levels with clear repercussions on quality in the wine sector and beyond. Although the vine is highly adaptable to different environmental conditions, extremely high temperatures (over 35°C) in recent years or heat waves often associated with drought can affect vine physiology and yield. The increase in average temperatures in Romania (more than 1.5°C in the last thirty years, Ciornei et al., 2023) is unfavourable from an industrial point of view, and the wine sector is also being affected. This study aims to assess the current climate that characterizes sandy soil viticulture in Oltenia (Romania), the viticultural centre of Dăbuleni (Figure 1), using some climatic indicators relevant to viticulture (Martonne aridity index, Dantin-Ravenga index, other climatic indicators). They were calculated based on meteorological data recorded by RDSPCSS Dăbuleni in the period 2012-2023 and were interpreted by the information in the literature (Mărăcineanu et al., 2011; Patriche, 2009; Satmari, 2010). A series of statistical indices (arithmetic mean, amplitude, relative amplitude, standard deviation, coefficient of deviance) were calculated and graphs were realized to highlight the trend of the studied climatic factors.

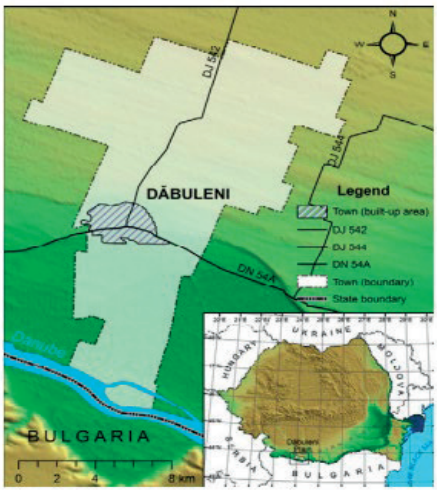


Figure 1. Location of the wine-growing center Dăbuleni (Achim et al., 2012)

The Excel program ensured fast and correct processing of meteorological data.

RESULTS AND DISCUSSIONS

Oltenia, a region located in the south-west of Romania, is important from an agricultural point of view due to the crops established (cereals, vines, vegetables), the climate evolution being defined for the quality of these crops (Mărăcineanu et al., 2021).

From a thermal point of view, the 2012-2023 period is characterized by a continuous upward trend in mean annual temperature. The maximum was recorded in 2020 with a value of 13.6ºC, and the minimum in 2012 with a value of 11.6ºC. The annual average calculated based on the 12 values indicates 12.7°C, which classifies the viticultural centre in the temperate type climate. A decreasing trend in the annual temperature amplitude values was observed (Figure 2). Basically, the difference between the annual maximum and minimum temperature has narrowed over the years of the study, due to warmer winter temperatures. Climatic observations also show a high number of tropical days (tmax ≥ 30oC), on average 79. In terms of mean temperature in July, values

are high, averaging 24.9°C, with extremes of 26.8°C (2012) and 23.1°C (2014), but dominant values fall between 25 and 26°C.

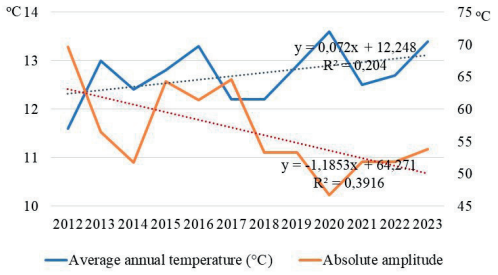


Figure 2. Annual mean temperature and amplitude evolution 2012-2023

Based on the excess heat factor and Antonescu et al. (2023) assess climate change for the near future 2021-2050, using the historical range 1971-2000 as a reference, the result of the study indicates that human exposure to heat waves will increase soon. The average temperature in July is a climatic indicator that orients the viticultural centre towards producing quality wines regarding carbohydrate accumulation. This is confirmed by interpreting the temperature and humidity values regarding the Seleaninov hydrothermal coefficient, the average for the period being 1. However, the quality of the wine must be proven by biochemical and organoleptic analysis, since excessive temperatures can negatively affect the quality indices of grape production and the wines. The evolution of another indicator, the overall heat balance, confirms the trend of increasing temperatures in this viticultural centre (Figure 3).

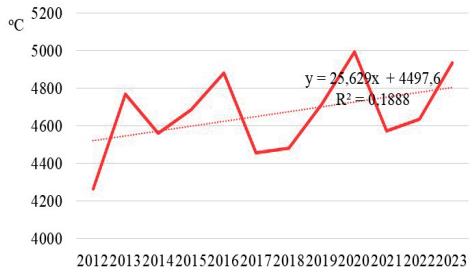


Figure 3. Heat Balance Evolution 2012-2023

A similar collection of information to that conducted in the study in relation to the extent to which organizations use climate services, their perception of the benefits of using the services, the technical characteristics of the services and the future needs of stakeholders was conducted in Romania by Falcescu et al., 2024.

Existing Mediterranean viticultural areas have been found to respond to these climatic changes by redistributing vine-growing areas, probably as a result of a progressive shift towards the NNV from their original areas, concluding that increased warming and drought will most likely lead in the future to an eventual overall loss of viticultural suitability in the Mediterranean climate zones of southern Europe. In contrast, warming in central and northern Europe will potentially benefit vine growth (Droulia & Charalampopoulos, 2021).

Another limiting factor is soil. In terms of annual precipitation, it averages 649 l/m^2. From a climatic point of view, this value corresponds to the temperate climate, but there are exceptions, such as 2012 and 2013, when the recorded precipitation fell below 500 l/m^2 (dry climate). During the growing season, an average of 331.8 l/m^2 is distributed, but the trend is decreasing (Figure 4).

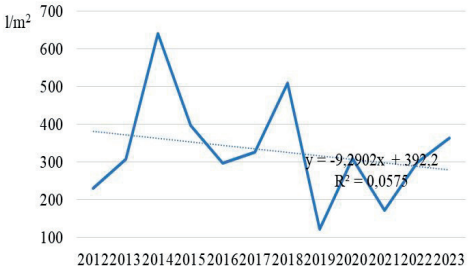


Figure 4. Annual Precipitation Evolution 2012-2023

As a result, the water supply to the vines is satisfied with a minimum of 250 l/m^2, but there are also situations when the precipitation volume falls below this critical value. Sometimes it reaches values below 150 l/m^2 (e.g. 2019 and 2021). Therefore, these sandy soils must monitor soil moisture status and supplement the water deficit.

The integration of the two important factors for the climatic characterization of a region resulted in Dăbuleni, the average of the 12 values of the Martonne aridity index, having a value of 28.6 (semi-humid climate). The

distribution of values indicates 2 years with a semi-arid climate, 5 years with a semi-humid climate and 5 years with a humid climate (Table 1). The climate characterization using the Dantin-Ravenga index confirms the name of Oltenia Sahara given to this area with sandy soil. Thus, 6 out of 12 years are semi-arid, 1 year is arid, and the rest have a humid climate.

Table 1. Evolution of climatic indicators in the wine-growing centre Dăbuleni

Martonne aridity index	Climate	Dantin-Ravenga index	Climate
17.8	semi-arid	3.02	Arid
19.6	semi-arid	2.88	semi-arid
44.4	humid	1.25	Humid
32.3	humid	1.74	Humid
30.8	humid	1.85	Humid
33.4	humid	1.64	Humid
41.6	humid	1.32	Humid
23.2	semi-humid	2.43	semi-arid
24.7	semi-humid	2.33	semi-arid
24.2	semi-humid	2.30	semi-arid
24.1	semi-humid	2.32	semi-arid
27.0	semi-humid	2.12	semi-arid
Average 28.6	semi-humid	Average 1.96	Humid

The mean value (1.96) is close to the value indicating a semi-arid climate. The values confirm the conclusion provided by the precipitation analysis regarding the danger of water deficit in sandy soil conditions.

The climatogram for 2012-2023 shows a period of drought between summer and fall. Moderate drought during this period is useful from this point of view. This corresponds to the stage of grapes scorching and ripening (Figure 5).

The statistical analysis of the main climatic parameters recorded in the Dăbuleni viticultural centre (Table 2) shows a more pronounced precipitation volume variation than the mean annual temperature. For this, the calculated coefficient of variation shows a homogeneous series with a small variation.

The situation is different if we refer to the annual precipitation amount, whose variation coefficient indicates a highly variable parameter. The amount of precipitation during the growing season has the highest value of the coefficient of variation, the series is heterogeneous. And Fonseca et al. (2024) conclude that understanding the spatial variability of microclimate is essential for sustainable and optimized grape production within vineyard plots. The impact on the wine sector of warmer climatic conditions, with a higher frequency of extreme temperatures and a decreasing trend in precipitation, in recent years has been assessed by Faralli et al, 2024.

The viticultural sector of the viticultural centre of Dăbuleni, one of southern Oltenia's most profitable agricultural sectors, is significantly affected by the ongoing climate changes (excessive temperature); the sandy soil is also a limiting factor.

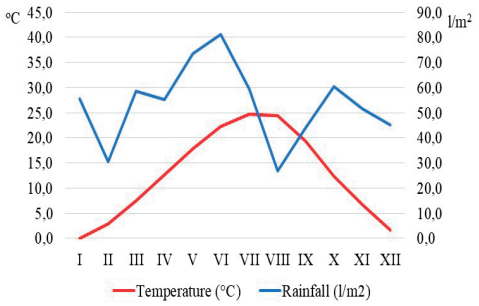


Figure 5. Dăbuleni Climagram (2012-2023)

Table 2. Statistical results on the main climatic parameters recorded at Dăbuleni

Climate parameter / Statistical indicator	Annual average temperature (°C)	Annual precipitation (l/m²)	Precipitation during the growing season (l/m²)
Arithmetic average	12.7	649.0	331.8
Absolute amplitude	2.0	610.5	518
Relative amplitude (%)	15.7	94.1	156.1
Standard deviation	0.57	182.12	139.74
Coefficient of variation (%)	4.52	28.06	42.11

CONCLUSIONS

The ecological offer that characterizes the Dăbuleni viticultural centre has evolved in the last 12 years in the sense of increasing average annual temperature. Also, the higher temperatures in winter have steadily reduced the annual thermal amplitude and the amount of precipitation during the growing period of the vines has a decreasing trend. In this area, the limiting factors for vine-growing are water

scarcity, excessive temperatures and sandy, permeable soil with low fertility. Under these conditions, economically positive results can be obtained using a technology adapted to the existing ecological conditions.

ACKNOWLEDGEMENTS

To realise this work, we benefited from the support of the University of Craiova through the internal competition for awarding the results of scientific research, Research Gala.

REFERENCES

Achim, E., Manea, G., Vijulie, I., Cocoș, O., & Laura, T. (2012). Ecological reconstruction of the plain areas prone to climate aridity through forest protection belts. Case study: Dăbuleni town, Oltenia Plain, Romania. *Procedia Environmental Sciences*, 14, 154-163.

Alikadic, A., Pertot, I., Eccel, E., Dolcia, C., Zarbo, C., Caffarra, A., De Filippi, R., Furlanello, C. (2019). The impact of climate change on grapevine phenology and the influence of altitude: A regional study. *Agric. For. Meteorol*, 271, 73–82.

Amihăesei, V. A., Micu, D. M., Cheval, S., Dumitrescu, A., Sfîcă, L., & Bîrsan, M. V. (2024). Changes in snow cover climatology and its elevation dependency over Romania (1961-2020). *Journal of Hydrology: Regional Studies,* 51, 101637.

Andrade, C., Fonseca, A., Santos, J. A., Bois, B., & Jones, G. V. (2024). Historic changes and future projections in Köppen–Geiger Climate classifications in Major Wine regions Worldwide. *Climate*, 12(7), 94.

Antonescu, B., Ene, D., Boldeanu, M., Andrei, S., Mărmureanu, L., Marin, C., & Pîrloagă, R. (2023). Future changes in heatwaves characteristics in Romania. *Theoretical and Applied Climatology*, 153(1), 525-538.

Barbu N, Cuculeanu V, Stefan S (2016b). Investigation of the relationship between very warm days in Romania and large-scale atmospheric circulation using multiple linear regression approach. *Theor Appl Climatol*, *126*(1-2): 273–284.

Beillouin, D., Schauberger, B., Bastos, A., Ciais, P., & Makowski, D. (2020). Impact of extreme weather conditions on European crop production in 2018. *Philosophical Transactions of the Royal Society B*, *375*(1810), 20190510.

Cheval, S., Bulai, A., Croitoru, A. E., Dorondel, Ș., Micu, D., Mihăilă, D., ... & Tișcovschi, A. (2022). Climate change perception in Romania. *Theoretical and applied climatology, 149*(1), 253-272.

Cichi, D.D., Căpruciu, R., Gheorghiu, N., & Stoica, F. (2023). Agrobiological and technological characteristics of table grapes varieties, grown in the temperate-continental climate from southwestern Romania. *Scientific Papers. Series B. Horticulture, 67*(1).

Ciornei, L., Udrea, L., Munteanu, P., Simion, P. S., & Petcu, V. (2023). The Effects of Climate Change. Trends Regarding the Evolution of Temperature in Romania. Annals of "Valahia" University of Târgoviște. *Agriculture, 15*(2), 50-57.

Căpruciu, R., Cichi, D. D., Mărăcineanu, L. C., & Stoica, F. (2023). Analysis of some biochemical compounds involved in adaptation mechanisms of vine to the minimum temperatures during the dormant season. *Scientific Papers. Series B. Horticulture, 67*(1).

Cosmulescu, S., Baciu, A., Cichi, M., & Gruia, M. (2010). The effect of climate changes on phenological phases in plum tree (*Prunus domestica* L.) in South-Western Romania. South Western *Journal of Horticulture, Biology and Environment, 1*(1), 9-20.

Costea, D. C., & Căpruciu, R. (2022). The influence of environmental resources specific to the cultivation year over the grapevine growth and yield. *Annals of the University of Craiova-Agriculture Montanology Cadastre Series, 52*(1), 95-100.

Deser, C., & Phillips, A. S. (2023). A range of outcomes: the combined effects of internal variability and anthropogenic forcing on regional climate trends over Europe. *Nonlinear Processes in Geophysics, 30*(1), 63-84.

Dragotă CS, Dumitra cu M, Grigorescu I, Kucsicsa G. (2011). The Climatic Water Deficit in South Oltenia Using the Thornthwaite Method. *Forum geografic*; 10(1): 140-148.

Droulia, F., & Charalampopoulos, I. (2021). Future climate change impacts on European viticulture: A review on recent scientific advances. *Atmosphere*, *12*(4), 495.

Dumitru, E. A., Berevoianu, R. L., Tudor, V. C., Teodorescu, F. R., Stoica, D., Giucă, A., ... & Sterie, C. M. (2023). Climate Change impacts on Vegetable crops: a systematic review. *Agriculture, 13*(10), 1891.

El Garroussi, S., Di Giuseppe, F., Barnard, C., & Wetterhall, F. (2024). Europe faces up to tenfold increase in extreme fires in a warming climate. npj *Climate and Atmospheric Science, 7*(1), 30.

Falcescu, V., Cheval, S., Micu, D. M., Dumitrescu, A., Roznovieţchi, I., Dumitrașcu, M., & Damian, N. (2024). Climate services in Romania - an analysis of stakeholders' perceptions and needs. *Climate Services*, 34, 100476.

Faralli, M., Mallucci, S., Bignardi, A., Varner, M., & Bertamini, M. (2024). Four decades in the vineyard: the impact of climate change on grapevine phenology and wine quality in northern Italy. *OENO One*, *58*(3).

Fei, Y., Leigang, S., & Juanle, W. (2023). Monthly variation and correlation analysis of global temperature and wind resources under climate change. *Energy Conversion and Management*, 285, 116992.

Fonseca, A., Cruz, J., Fraga, H., Andrade, C., Valente, J., Alves, F., Neto, A.C., Flores, R., Santos, J. A. (2024). Vineyard Microclimatic Zoning as a Tool to Promote Sustainable Viticulture under Climate Change. *Sustainability*, *16*(8), 3477.

Furtak, K., & Wolińska, A. (2023). The impact of extreme weather events as a consequence of climate change on the soil moisture and on the quality of the soil environment and agriculture - A review. Catena, 231, 107378.

Jain, S., Scaife, A. A., Shepherd, T. G., Deser, C., Dunstone, N., Schmidt, G. A., ... & Turkington, T. (2023). Importance of internal variability for climate model assessment. npj *Climate and Atmospheric Science, 6*(1), 68.

Jamal, M. U., Bilal, M., Durani, A., Waseem, M., & Jabarkhil, A. K. (2024). Effects of Climate Change on Horticulture Sector Productivity: A Review. *Nangarhar University International Journal of Biosciences*, 231-236.

Keller, M. (2023). Climate Change Impacts on Vineyards in Warm and Dry Areas: Challenges and Opportunities: From the ASEV Climate Change Symposium Part 1–Viticulture. *American Journal of Enology and Viticulture, 74*(2).

Larsen, M. A., Petrović, S., Radoszynski, A. M., McKenna, R., & Balyk, O. (2020). Climate change impacts on trends and extremes in future heating and cooling demands over Europe. *Energy and Buildings*, 226, 110397.

Lesk C, Rowhani P, Ramankutty N. 2016 Influence of extreme weather disasters on global crop production. *Nature*, 529, 84-87.

Martinez, A., Murphy, L., & Iglesias, G. (2023). Evolution of offshore wind resources in Northern Europe under climate change. *Energy*, 269, 126655.

Mărăcineanu L.C. (2011). *Aplicații ale ecologiei în viticultură*. Ed. Universitaria Craiova.

Mărăcineanu, C., Giugea, N., Mărăcineanu, E., & Căpruciu, R. (2021). Climate trends in Oltenia. Case study: Craiova-Banu Mărăcine, *Scientific Papers. Series B, Horticulture. Vol. LXV*, No. 1, 762-766

Mekonnen, T. W., Gerrano, A. S., Mbuma, N. W., & Labuschagne, M. T. (2022). Breeding of vegetable cowpea for nutrition and climate resilience in Sub-Saharan Africa: progress, opportunities, and challenges. *Plants, 11*(12), 1583.

Micu, D. M., Amihaesei, V. A., Milian, N., & Cheval, S. (2021). Recent changes in temperature and precipitation indices in the Southern Carpathians, Romania (1961-2018). *Theoretical and Applied Climatology, 144*(1), 691-710.

Miller, J., Böhnisch, A., Ludwig, R., & Brunner, M. I. (2024). Climate change impacts on regional fire weather in heterogeneous landscapes of central Europe. *Natural Hazards and Earth System Sciences, 24*(2), 411-428.

Milošević, N., & Milošević, T. (2023). Impact of climate change on plum (*Prunus domestica* L.). In *Cultivation for Climate Change Resilience, Volume 2* (pp. 257-278). CRC Press.

Minea, I., & Chelariu, O. E. (2021). Anomalies and trends of high river flow under temperate climatic conditions in north-eastern Romania. *Journal of water and climate change, 12*(2), 552-565.

Nasiri, V., Rahmanian, S., Aslani, Z. H., Maftei, C., & Vaseashta, A. (2024). Spatial Trend Analysis of Vegetation Dynamics and Their Responses to Climate Change on Black Sea Coasts, Romania From 2000 to 2021. In *Modeling and Monitoring Extreme Hydrometeorological Events* (pp. 162-184). IGI Global.

Necula, C., Stefan, S., Birsan, M. V., Barbu, N., & Nita, I. A. (2024). Maximum winter temperature over Romania in connection to atmospheric circulation. Theoretical and Applied Climatology, 1-10.

Oseni T and Masarirambi M. (2011) Effect of climate change on maize (*Zea mays*) production and food security in Swaziland. Change, 2: 3.

Pardo, S. K., & Paredes-Fortuny, L. (2024). Uneven evolution of regional European summer heatwaves under climate change. *Weather and Climate Extremes*, 43, 100648.

Patriche, C.V. (2009). *Metode statistice aplicate în climatologie*. Ed. Terra Nostra, Iași.

Piticar, A., Andrei, S., & Tudor, A. (2024). Spatiotemporal Variability of Convective Events in Romania Based on METAR Data. *Sustainability, 16*(8), 3243.

Popescu, A., Dinu, T. A., Stoian, E., & Șerban, V. (2023). Climate change and its impact on wheat, maize and sunflower yield in Romania in the period 2017-2021. *Scientific Papers Series Management, Economic Engineering in Agriculture & Rural Development, 23*(1).

Prăvălie, R., Sîrodoev, I., Nita, I. A., Patriche, C., Dumitrașcu, M., Roșca, B., ... & Birsan, M. V. (2022). NDVI-based ecological dynamics of forest vegetation and its relationship to climate change in Romania during 1987-2018. *Ecological Indicators*, 136, 108629.

Prăvălie, R., Piticar, A., Roșca, B., Sfîcă, L., Bandoc, G., Tiscovschi, A., & Patriche, C. (2019). Spatio-temporal changes of the climatic water balance in Romania as a response to precipitation and reference evapotranspiration trends during 1961-2013. *Catena*, 172, 295-312.

Rant, V. (2024). Regulating Sustainable Finance and the Green Transition in the EU. In *Handbook of Environmental and Green Finance: Toward a Sustainable Future* (pp. 359-405).

Satmari A. (2010). *Lucrări practice de biogeografie*. Edit.
Eurobit. Timișoara

Shahmohamadi, P.; Che-Ani, A.I.; Maulud, K.N.A.; Tawil, N.M.; Abdullah, N.A.G. (2011). The Impact of Anthropogenic Heat on Formation of Urban Heat Island and Energy Consumption Balance. *Urban Stud. Res.*, 497524.

Singh, B. K., Delgado-Baquerizo, M., Egidi, E., Guirado, E., Leach, J. E., Liu, H., & Trivedi, P. (2023). Climate change impacts on plant pathogens, food security and paths forward. *Nature Reviews Microbiology, 21*(10), 640-656.

Soare, R., Dinu, M., Babeanu, C., Niculescu, M., Soare, M., & Botu, M. (2024). Quantitative and Qualitative Production of Species *Cucumis metuliferus* and the Potential for Adaptation in the Context of Current Climate Change. *Plants, 13*(13), 1854.

Șmuleac, L., Rujescu, C., Șmuleac, A., Imbrea, F., Radulov, I., Manea, D., ... & Pașcalău, R. (2020).

Impact of Climate Change in the Banat Plain, Western Romania, on the Accessibility of Water for Crop Production in Agriculture. *Agriculture, 10*(10), 437.

Tigchelaar M, Battisti DS, Naylor RL, Ray DK. 2018 Future warming increases probability of globally synchronized maize production shocks. *Proc. Natl Acad. Sci. USA*, 115, 6644-6649

Trebicki, P. (2020). Climate change and plant virus epidemiology. *Virus research*, 286, 198059.

van Daalen, K. R., Tonne, C., Semenza, J. C., Rocklöv, J., Markandya, A., Dasandi, N. & Lowe, R. (2024). The 2024 Europe report of the Lancet Countdown on health and climate change: unprecedented warming demands unprecedented action. The Lancet Public Health.

van den Bosch, M., Bartolomeu, M. L., Williams, S., Basnou, C., Hamilton, I., Nieuwenhuijsen, M., ... & Tonne, C. (2024). A scoping review of human health co-benefits of forest-based climate change mitigation in Europe. *Environment International*, 108593.

Vitasse, Y., Ursenbacher, S., Klein, G., Bohnenstengel, T., Chittaro, Y., Delestrade, A., ... & Lenoir, J. (2021). Phenological and elevational shifts of plants, animals and fungi under climate change in the European A lps. *Biological Reviews, 96*(5), 1816-1835.

Vlăduţ, A., Nikolova N., Licurici M. (2017). Influence of climatic conditions on the territorial distribution of the main vegetation zones within Oltenia region, Romania. Muzeul Olteniei Craiova. Oltenia. *Studii şi comunicări. Ştiinţele Naturii*, 33, 1, ISSN 1454-6914, 154-164.

Zaharia, L., Ioana-Toroimac, G., & Perju, E. R. (2020). Hydrological impacts of climate changes in Romania. *Water Resources Management in Romania*, 309-351.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

UMBRELLA EFFECT OF EUROPEAN PROTECTED SPECIES OVER NATIONAL RED LISTED *BETULA HUMILIS* SCHRANK - "MESTECĂNAȘUL DE LA COȘNA" AS A NEW NATURA 2000 SITE

Roxana NICOARĂ, Constanța-Mihaela ION, Gabriela TAMAS, Ana-Maria MOROȘANU, Florența-Elena HELEPCIUC, Tiberiu SAHLEAN, Constantin-Ciprian BÎRSAN, Minodora MANU, MIHAELA CIOBOATĂ, Sanda MAICAN, Viorel-Dumitru GAVRIL, Miruna-Maria ȘTEFĂNUȚ, Daniel Ioan MAFTEI, Mirela-Mădălina MOLDOVEANU, Anca MANOLE, Sorin ȘTEFĂNUȚ

Institute of Biology Bucharest of Romanian Academy,
296 Splaiul Independenței, District 6, Bucharest, Romania

Corresponding author email: mihaela.ion@ibiol.ro

Abstract

Betula humilis Schrank is a rare, glacial relict species primarily found in eutrophic peatlands. In Romania, it is limited to the Carpathian depressions of Transylvania and Bukovina, marking the southernmost boundary of its European distribution. Due to drainage and land-use changes, Betula humilis populations have declined and are nationally endangered. Despite these threats, a population of over 50 shrubs was mapped from Coșna area (Suceava County), making it one of the largest known populations in Romania. As part of a peatland restoration project, our efforts aimed at restoring the optimal hydrological regime to protect Betula humilis. During the area's flora and fauna inventory, two European protected species were discovered: Lycaena helle (an endangered butterfly) and Ligularia sibirica (a rare glacial relict plant). These findings highlight the site`s exceptional conservation value, leading to an expansion of the initial proposal for a Botanical Reserve of local interest to a broader Natura 2000 site covering 21 hectares. A management plan is needed to protect both Betula humilis and its umbrella species.

Key words*: Betula humilis, endangered species, Natura 2000, peatland restoration.*

INTRODUCTION

Peatlands are among the most valuable terrestrial ecosystems, covering approximately 3% of the Earth's land surface, yet storing about one-third of global soil carbon (Harenda et al., 2018; Leifeld & Menichetti, 2018). They play a critical role in climate regulation and provide essential ecosystem services, including water filtration, flood mitigation, nutrient cycling, resources for local communities' livelihoods and cultural heritage (Joosten & Clarke, 2002; Limpens et al., 2008; Bonn et al., 2016). Additionally, peatlands host a distinctive assemblage of plant and animal species, many of which are rare, endangered, or specifically adapted to acidic, waterlogged, and nutrient-poor conditions (Joosten et al., 2017). Globally, an estimated 12% of peatlands have been degraded (UNEP, 2022), while in Europe, this figure rises to 25%, and up to 50% within the European Union (Tanneberger et al., 2021). In the last two centuries, many European peatlands have been drained for agriculture, forestry, and peat extraction, leading to severe habitat loss (Verhoeven, 2014; Swindles et al., 2019). To counteract these impacts, efforts must focus on implementing restoration measures, including close collaboration between scientists, local policymakers, and community stakeholders to ensure sustainable land-use planning and peatland management practices (Pop et al., 2025).

The 2030 Conservation Goals, established by the EU Biodiversity Strategy and the Global Biodiversity Framework, aim to protect 30% of land and marine areas by 2030, with a strong focus on habitat preservation and ecological restoration (European Commission, 2020; CBD, 2022). This objective is particularly crucial for *relict species*, whose survival depends on conserving peatland ecosystems, increasingly threatened by land-use changes, and climate shifts. Given their vital role in climate change

mitigation, peatlands are a priority for ecological restoration initiatives.

Since peatland distribution is primarily governed by climate, bedrock composition, and topography, their extent in Southeastern Europe remains limited (Joosten et al., 2017). According to Tanneberger et al. (2017), organic soils with a peat layer (exceeding 20 cm and more than 20% organic matter content) cover approximately 7,690 km², representing 3.23% of Romania's surface. However, only 1347 ha of peatlands (oligotrophic acidic mires, locally known as *tinov*) are included in protected areas (Joosten et al., 2017).

According to Pop (1960), who extensively studied Romania's peat bogs, Bukovina is country's most representative and floristically rich peatland regions, characterized by extensive marshy areas and thick peat deposits. Within this landscape, the upper Dorna Basin, particularly the Coșna and Poiana Stampei areas, represents the core zone where oligotrophic peatlands reach their highest abundance and ecological authenticity. Between 2022 and 2024, the Institute of Biology Bucharest of Romanian Academy implemented the PeatRO 2 Project, focusing on the restoration of 12 degraded wetland ecosystems across 719.4 ha in Suceava, Brașov, and Sibiu Counties. Among these, the peatland near Coșna school (Suceava) emerged as a site of exceptional conservation importance, supporting one of the largest known populations of the rare shrubby birch, *Betula humilis* Schrank in Romania, along with other protected species of European interest.

These findings initiated plans to propose the area hosting *B. humilis* as a 1 ha local reserve, with the aim of extending its protection to 21 ha as a Natura 2000 site.

B. humilis is a glacial relict shrub adapted to cold, moisture-rich environments (Pop, 1976; Szatmari & Hurdu, 2022), thriving in diverse wetland habitats such as oligotrophic and eutrophic peatlands, transition mires, and wet meadows (Załuski et al., 2001; Jabłońska, 2012; Shaw et al., 2014).

It has an extensive yet fragmented global distribution across subarctic and subalpine regions of Eurasia, stretching eastward as far as Korea. In Europe, its distribution ranges primarily through the boreal regions of Scandinavia, continuing through Central and Eastern Europe, with its southernmost boundary in the Romanian Carpathians (POWO, 2025).

In Romania, the distribution is restricted to the Carpathian depressions of Transylvania and Bukovina, (Pop, 1928, 1960; Georgescu, 1952; Oprea, 2005).

According to the IUCN Red List global assessment (Shaw et al., 2014), *B. humilis* is currently classified as Least Concern (LC), with a decreasing population trend. Across Europe, the absence of conservation measures has resulted in severe declines, rendering the species vulnerable or rare in several countries. In Poland, some populations have drastically declined (Chrzanowska et al., 2016), while in the Czech Republic, it has been declared extinct (Kaplan, 2017). It is also critically endangered in the Carpathians (Witkowski et al., 2003) and Romania (Dihoru & Negrean, 2009). Table 1 presents various assessments of its endangered status in Romania.

Table 1. Conservation status in Romania, according to some authors (LC - Least concern; CR - critically endangered; EN - endangered; E/R - endangered/rare; V - vulnerable, ML - local monument)

Authors	Category of threat
Oltean et al., 1994	E/R
Boșcaiu et al. 1994	E
Dihoru & Dihoru, 1994	V
Ciocârlan, 2009	R
Witkowski et al., 2003	CR - in Carpathians EN - in Romania
Dihoru & Negrean, 2009	CR
OUG 57/2007	ML
IUCN Red list	LC

While most Romanian peatlands benefit from some form of legal protection, either as reserves (Borsaros-Sâncrăieni Nature Reserve, Beneș Nature Reserve) or as part of Natura 2000 sites (ROSAC0247 Tinovul Mare Poiana Stampei, ROSAC0245 Tinovul de la Românești), several populations of *B. humilis* remain unprotected or insufficiently monitored.

One of the most significant populations of *B. humilis* in Romania was recorded in Borsaros-Sâncrăieni Nature Reserve, previously considered the largest in the country (Goia & Farcaș, 1997). This site also marks the southernmost point of the species' Romanian and European distribution. However, in the late decades, human activities, including deforestation, drainage, and infrastructure

development, have led to a significant decline in the *B. humilis* population, reducing its habitat and threatening its long-term survival. Much of the population has been accidentally cut down, leaving only tens of individuals remaining (Pricop et al., 2019).

A broader assessment of *B. humilis* populations in Romania, summarized by Dihoru & Negrean (2009), highlights similarly critical declines across other reserves: Borsec - a single, fenced specimen remains; Remetea, Mlaștina cea Mare - 7 shrubs recorded in 1954; Honcsok Reserve - 8 shrubs recorded in 1952; Beneș Reserve - 14 shrubs recorded in 1952; Hármas Liget Reserve (Dumbrava Întreită) - 2 shrubs recorded in 1934. These observations show the ongoing decline of the species throughout its historical range, reinforcing the urgent need for targeted conservation measures. A recent study on peatland conservation revealed that only 17% of peatlands are protected worldwide, significantly less than many other high-value ecosystems (Austin et al., 2025).

Thus, the designation of "Mestecănașul de la Coșna" as a local reserve and a Natura 2000 site represents a significant step forward in securing the long-term conservation of *B. humilis*, addressing gaps in protection at both the national and European levels.

This study aims to assess the conservation importance of the Coșna peatland for *B. humilis* and other protected species; evaluate the role of EU-listed species (*Lycaena helle*, *Ligularia sibirica*) in strengthening site protection through the "umbrella effect" and provide a scientific basis for the designation of "Mestecănașul de la Coșna" as a Natura 2000 site, ensuring long-term protection of its habitats and species.

MATERIALS AND METHODS

Study area

The "Mestecănașul de la Coșna" site (47.372837° N, 25.174266° E) is located within the administrative territory of Coșna locality, Suceava County.

Biogeographically, it belongs to the alpine region. Geographically it lies in the northern sector of the Eastern Carpathians, within the southwestern part of the Suhard Mountains, inside the Dorna Depression (857 m a.s.l.).

Geologically, the area lies within a region of crystalline schists and Eocene sandstones. The peat deposits are separated from the underlying bedrock by a layer of siliceous alluvium, which forms the bog floor (Pop, 1960). The climate in the area is temperate-boreal montane, subalpine with influences from both the continental east and the sub-Baltic boreal north. The mean annual temperature is 5.1°C with an average annual precipitation of 657.4 mm, water supply and low temperatures being crucial for sustaining the site as a viable ecological component (Anonymus, 2015).

The study area is located within the Teșna River basin, with its main left tributaries, Tocila and Bancu, shaping the local hydrological system. The site is practically enclosed on three sides by water, as these streams flow around it before converging near the village of Coșna. Following their confluence, the Teșna flows into the Dorna River, which serves as the region's primary drainage system (Figure 1).

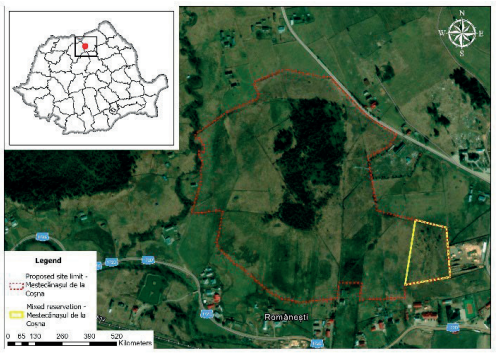


Figure 1. Proposed limit of Natura 2000 site Mestecănașul de la Coșna within the Coșna region (red dotted line), Suceava County, Romania; Reserve area (yellow) and close surroundings

The substrate consists of acidic peat, with soils classified as histosols (Doniță et al., 2005). The region falls within the montane vegetation zone, characterized by exceptionally compact spruce forests. In terms of nearby protected areas, the proposed site is situated in close proximity to the Natura 2000 site ROSAC0245 Tinovul de la Românești. In the old literature the site is known as the peaty terrain behind Coșna School (Pop, 1960).

Field survey

The biodiverisity survey within the proposed Natura 2000 site "Mestecănașul de la Coșna" began with an exploratory assessment in spring 2022, to identify key habitats and conservation priorities, in order to develop a restoration strategy. The evaluation focused on determining priority areas for intervention, mapping drainage channels, identifying species and habitats of conservation interest, and evaluating existing threats to the site.

Subsequent field investigations, followed floristic inventories, with species identification in situ and supplemented by herbarium specimens when necessary. Vegetation structure and composition were analyzed using relevé sampling, with plots placed across different microhabitats to ensure comprehensive coverage of the site's peatland, wet meadows, and transition zones.

A DJI Mini 2 drone was used to capture aerial imagery to assess specific habitat distribution, which was then integrated with ground-based botanical surveys.

Field campaigns were carried out in multiple phases to capture the seasonal dynamics of the vegetation. The botanical data collection was conducted between March-November 2022-2023 and January-April 2024, ensuring a comprehensive inventory of plant diversity across different phenological stages.

Additionally, plant material was collected to establish an herbarium collection deposited in the BUCA Herbarium, preserving a reference for future studies. Plant species were identified in the field using the botanical identification key provided by Sârbu et al. (2013). The plant nomenclature for the listed taxa follows the classification established by Flora Europaea and updated through Euro+Med PlantBase, which provides the latest information on species present in the European and Mediterranean regions. For *B. humilis*, the GPS coordinates of each individual shrub were recorded to track population size and spatial distribution.

The phytosociological nomenclature is based on comprehensive syntheses of Romania's vegetation (Sanda et al., 2008; Doniță et al., 2005). Habitat assessments were also performed according to EU Habitats Directive classification, evaluating conservation status and potential threats.

Regarding butterfly fauna, field investigations were carried out on-site, usually in May, at the beginning of the *L. helle* adult flight season. Surveys involved direct visual observation, transect counts, and photographic documentation, with GPS coordinates recorded for each observation. Butterfly species were identified in the field using identification keys (Tolman & Lewington, 2008; Rákosy, 2024).

Data on bird diversity was gathered using the point counts method (Bibby et al., 1992).

RESULTS AND DISCUSSIONS

Challenges of a glacial relict plant in the Anthropocene world

The last glaciation significantly reshaped Europe's vegetation, forcing many species to retreat into refugia, where they could survive in colder and wetter conditions. *B. humilis* (Figure 2) has a long evolutionary history in Europe, multiple lines of evidence suggesting that it survived the Last Glacial Maximum (LGM) in periglacial wetland habitats at higher latitudes. Fossil and pollen records confirm its presence in Germany, Ukraine, and Hungary in Weichselian cold-stage environments (Jadwiszczak et al., 2012).



Figure 2. *Betula humilis* shoot in early vegetative stage, showing young leaves and developing catkins. Photo taken at the Mestecănașul de la Coșna site (Suceava County), June 2023

Unlike other *Betula* species that expanded from Southern European refugia, genetic analyses reveal that *B. humilis* persisted in northern periglacial zones before recolonizing suitable postglacial habitats (Jadwiszczak et al., 2014). Modern relict populations indicate that *B. humilis* had a much broader range in the past, likely extending into both the Alps and the Carpathians during the LGM (Załuski et al., 2001).

In Romania, particularly in the Southeastern Carpathians, peat bogs are not of glacial origin (Pop, 1960). Paleostratigraphic studies (Pop, 1929; Tantau et al., 2003) indicate that glacial-age peat deposits are eutrophic, while the oligotrophic peatlands of today only began forming in the postglacial period. According to these studies, peat bogs in Romania are not a direct result of glaciation but rather of the warmer postglacial climate, which allowed their development. As the climate warmed following the LGM, forests expanded, gradually replacing tundra landscapes. Some of the current glacial relict species found in these peat bogs, including *B. humilis*, originally thrived in tundra-like landscapes or cold, acidic mires with *Pinus sylvestris* (Pop, 1960; Szatmari & Hurdu, 2022). As their original habitats disappeared or transformed, *B. humilis* is now restricted to scattered peat bogs and fens, which became their last refugia. These habitats provide stable conditions for millennia, allowing the species to persist despite climate fluctuations. However, in the Anthropocene, these refuges are increasingly threatened by habitat fragmentation, drainage, and climate change.

The presence of *Betula humilis* in the Coșna area was first recorded by the geologist László (1915). Later, Pop (1928) visited the site in August 1927 and described the entire region as a once-extensive peatland, now severely degraded due to drainage by locals aiming to convert the area into hay meadows. He noted that only three remnants of the original bog persisted, located on the left side of the Teșna stream, between the Jinu stream and the parish house. In these areas, *B. humilis* still grew abundantly, together with *Salix repens* subsp. *rosmarinifolia*, including along the edges of drainage ditches and in repeatedly mown meadows, where it had resisted extirpation. By 1960, however, he observed that the peatland behind the Coșna school no longer resembled a typical bog, lacking the characteristic *Pinus sylvestris* cover seen in other regional sites. Instead, livestock grazing had severely impacted it, with vegetation degraded.

Peterschilka (1928) also surveyed the area, reporting *B. humilis* in the "Putredu" bog (Pilugani) and as a dominant species within the meadow complexes underlain by peat substrates in the Coșna region.

Infrastructural development further impacted the habitat: the construction of the Ilva Mică-Vatra Dornei railway, which traverses the Teșna Valley, began in 1923 and caused substantial alteration of the local landscape. Although severely damaged during World War II, the railway was rebuilt and reopened in 1947 (Botez, 1977). The region was also affected by the later construction and expansion of County Road DJ 172D, which cuts through the Coșna peatland and contributed to fragmentation and hydrological disruption. Combined with increased housing development and the need to extend grazing areas, these pressures led to further drainage and habitat loss, shrinking what was once a large, continuous bog complex.

Although the Tinovul Mare Coșna Natura 2000 site was designated as a protected area in 2017, comprising two bog fragments, one near Coșna and another in Românești, *B. humilis* was no longer present at the time of designation and is not included in the site's management plan (Anonymus, 2015). Currently, the only known surviving population of *B. humilis* in Bukovina is found within the proposed nature reserve "Mestecănașul de la Coșna". The present-day fragmented nature of the peatland meadows is a remnant of a once-continuous wetland ecosystem that formerly covered much of the Teșna Valley. According to elderly locals from Coșna, the original peatland ("Tinovul cel Mare de la Coșna") was much larger, estimated at over 50 ha. Although only isolated patches of peatland remain today, locals still refer to non-forested areas as "tinov", reflecting the past extent of this ecosystem.

Present vegetation structure

The "Mestecănașul de la Coșna" peatland is a complex wetland mosaic consisting of wet meadows, pastures, eutrophic marshes, and oligotrophic bogs, with patches of pine-

dominated bog woodland characteristic of the Dorna Basin peatlands (Figure 3).
The vegetation of the site was partially documented in a previous study focusing on the rediscovery of conservation concern *Lycaena helle* (Ion et al., 2023), particularly describing the specific habitats used by this butterfly.



Figure 3. Aerial view of the proposed Natura 2000 site Mestecănașul de la Coșna and the *B. humilis* Reserve

The local nature reserve is located in the eastern part of the proposed Natura 2000 site, while the *B. humilis* population is concentrated in the central sector of the designated reserve area. A total of 55 individuals were recorded and are represented in the distribution map (Figure 4).
The spatial aggregation of individuals indicates a relatively compact population reflecting a reduced suitable microhabitat following past land-use changes and hydrological alterations.

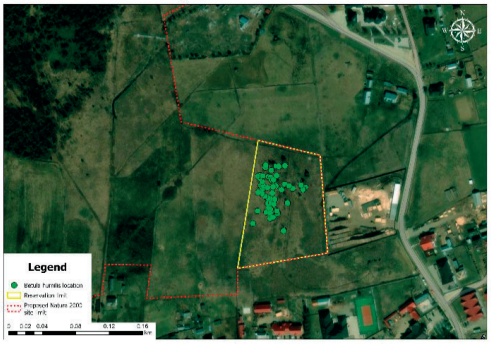


Figure 4. Location of the individuals of *B. humilis* (green circles) in Mestecănașul de la Coșna proposed local reservation

The aspect of the area is characterized by an open wetland with scattered individuals of *Picea abies*, *Betula pendula*, *Frangula alnus*, and *Salix caprea*, interspersed with dense shrub formations dominated by *B. humilis* and *Salix repens* subsp. *rosmarinifolia* (Figure 5). The herbaceous layer is structurally diverse and floristically rich and includes species such as *Bistorta officinalis*, *Filipendula ulmaria*, *Comarum palustre*, *Cirsium rivulare*, *Dryopteris cristata*, *Epilobium palustre*, *Valeriana officinalis*, *Equisetum palustre*, *Crepis paludosa* and *Rumex acetosa, Galium palustre*, *Caltha palustris*, *Plantago lanceolata*, *Potentilla erecta*, *Viola declinata*, *Galeopsis speciosa*, *Cardamine pratensis*, *Agrostis canina*, *Campanula abietina*, *Carex diandra*, and *Vaccinium oxycoccos*.

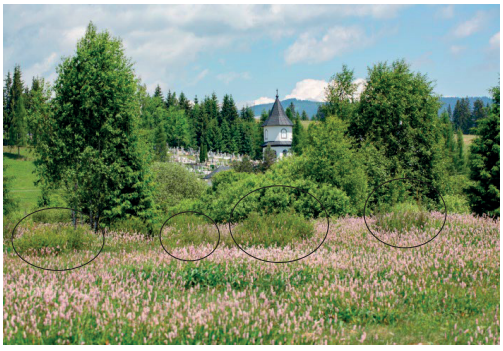


Figure 5. *B. humilis* shrubs (marked with circles) growing in a wet meadow habitat within the Mestecănașul de la Coșna site

Several species recorded within this herbaceous community are of conservation interest. *Comarum palustre* is listed as vulnerable in the Romanian flora (Dihoru & Dihoru, 1994), while *Dryopteris cristata* is classified as vulnerable and rare (Dihoru & Dihoru, 1994; Oltean et al., 1994). *Vaccinium oxycoccos* (Figure 7 c) is considered endangered (Witkowski, 2003) and rare at the national level (Oltean et al., 1994; Dihoru & Dihoru, 1994). *Salix repens* subsp. *rosmarinifolia* (Figure 7 d), a characteristic element of oligotrophic wetlands, is also regarded as rare (Oltean et al., 1994). Additionally, the presence of *Dactylorhiza majalis* (Figure 7 b), a rare orchid species in Romania (Oltean et al., 1994; Dihoru & Dihoru, 1994), further underlines the botanical significance of the site.
The reserve's eastern edge borders a local timber depot, which poses potential ecological pressures through edge disturbance and changes

in hydrology, highlighting the need for buffer zones.

In the northern section of the site, a bog woodland community is clearly delineated, corresponding to the *Vaccinio-Pinetum sylvestris* Kleist 1929 em. Matuszk. 1962 association. This habitat has been identified as 91D0* (*Bog woodland*) under the EU Habitats Directive, a priority habitat type that plays a crucial role in maintaining peatland biodiversity. It occupies approximately 17% of the total site area and is characterized by a species-poor composition due to the oligotrophic conditions of the wetland.

The tree layer is dominated by *Pinus sylvestris*, with scattered occurrences of *Betula pendula* Roth, *Sorbus aucuparia*, and *Rhamnus frangula*, contributing to a total canopy cover ranging from 50% to 70%. The pines are typically stunted, reaching a maximum height of 3–4 meters, and are frequently covered with lichens. Beneath the canopy, the shrub layer is sparsely developed, consisting mainly of juvenile *Pinus sylvestris*, along with occasional individuals of *B. pendula* and *B. humilis*. The herbaceous and dwarf shrub layer is well represented, with *Vaccinium vitis-idaea* being dominant, alongside *Vaccinium myrtillus*, *Vaccinium oxycoccos*, and *Eriophorum vaginatum*. In areas where the tree layer is less developed, this vegetation achieves a high degree of coverage, ranging from 70% to 80%.

The moss layer is highly developed, forming a dense carpet dominated by *Sphagnum* species: *Sphagnum angustifolium* (Russow) C.E.O. Jensen, *Sphagnum capillifolium* (Ehrh.) Hedw. *Sphagnum divinum* Flatberg & K. Hassel, *Sphagnum medium* Limpr. At the margins of the bog woodland, where groundwater infiltration is more pronounced, additional species such as *Salix repens* subsp. *rosmarinifolia*, *Salix caprea*, *Rhamnus frangula* and *Carex brizoides* in the ground layer become more frequent.

The area at the margins of the forested bog, represents a transition zone between the wooded peatland and open wet meadows. The site lacks mature trees, with only a few scattered saplings of *Picea abies, Salix capraea* and isolated occurrences of *B. humilis*. The surface is characterized by numerous hummocks, which create a microtopographic mosaic that influences plant distribution. *Vaccinium oxycoccos* is abundant on the elevated hummocks, sometimes accompanied by *Andromeda polifolia*. The latter is considered endangered in the Carpathians (Witkowski, 2003) and rare at the national level (Oltean et al., 1994; Dihoru & Dihoru, 1994).

The natural mesophilic meadows (Figure 6) are covering approximately one-quarter of the site's surface, forming a distinct vegetation complex within the *Molinio-Arrhenatheretea* class. These fenced and ungrazed meadows are characterized by a dense and compact herbaceous layer, 80-90% coverage.

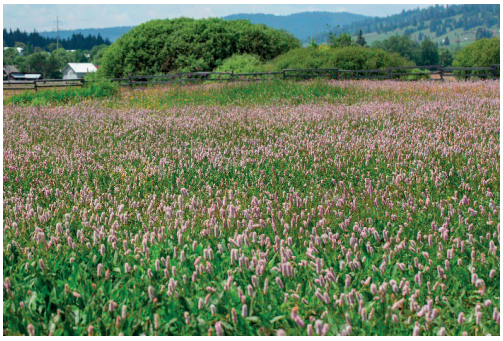


Figure 6. Mesophilic meadows dominated by *Bistorta officinalis*

The floristic composition is characterized by the dominance of *Bistorta officinalis*, along with other hygrophilous species, such as *Rumex acetosa, Cirsium rivulare, Ranunculus repens, R. acris, Succisa pratensis, Epilobium palustre, Veronica chamaedrys, Equisetum palustre, Poa palustris, Crepis paludosa*, *Cardamine pratensis*, *Geum rivale*, interspersed with scattered shrubs of *Salix caprea*.

In the north-eastern extremity of the protected area, the vegetation is characterized by a hygrophilous meadow community comprise by *Carex rostrata*, *Carex acutiformis*, *Ranunculus acris*, *Mentha longifolia*, *Cirsium palustre*, *Lysimachia vulgaris*, *Agrostis capillaris*, *Anthoxanthum odoratum*, *Phleum pratense*, *Briza media*, *Molinia caerulea*, *Lathyrus pratensis*, *Equisetum palustre*, *Euphrasia* sp., *Angelica sylvestris*, *Rumex acetosa*, *Parnassia palustris*, and scattered *Salix capraea* shrubs.

Within this meadow, a population of *Ligularia sibirica* was identified (Figure 7 a), consisting of approximately 70 individuals - 55 of which were recorded in a fruiting state at the time of the field

investigation and around 15 in a vegetative state. *Ligularia sibirica* is a rare glacial relict species of significant conservation value, listed in Annex II of the EU Habitats Directive (92/43/EEC) as a species of community interest requiring the designation of Special Areas of Conservation, and under Appendix I of the Convention on the Conservation of European Wildlife and Natural Habitats (Bern Convention). It is also classified as Critically Endangered (CR) in the Carpathians (Witkowski, 2003), Data Deficient (DD) on the IUCN Red List (Bernhardt et al., 2025), and Rare (R) in the Romanian flora (Oltean et al., 1994; Dihoru & Dihoru, 1994).



Figure 7. Rare and protected plant species characteristic of the peatland habitat at Mestecănașul de la Coșna: *Ligularia sibirica* (a); *Dactylorhiza majalis* (b); *Vaccinium oxyccocos* (c); *Salix repens* subsp. *rosmarinifolia* (d)

Its presence further emphasizes the ecological significance of the proposed protected area and highlights the necessity for effective conservation and habitat management measures.

The entire area is intersected by several drainage ditches, excavated to remove the excess water and convert it into pasture. Some of these channels are deep and well-defined, supporting characteristic hygrophilous vegetation, including *Typha angustifolia, Carex rostrata,* and *Carex nigra, Mentha aquatica, Caltha palustris.* An aquatic plant community is established in sections where water depth exceeds 40-50 cm, dominated by *Potamogeton natans, Callitriche palustris* and rare *Menyanthes trifoliata* (Dihoru & Dihoru, 1994; Oltean et al., 1994).

Aspects of local fauna

In addition to its botanical relevance, the proposed area for the Natura 200 site, in particularly the mesophilic meadows, plays a crucial role in maintaining a viable population of the Violet Copper butterfly *Lycaena helle* (Denis & Schiffermüller, 1775), a species of community interest listed in the Habitats Directive (92/43/EEC).

Lycaena helle (Figure 8) is a glacial relict with a Eurosiberian range, generally associated with the habitat types identified at Coșna. In Europe, it occurs from 0 to 2000 m a.s.l., inhabiting wetlands such as fens, *Sphagnum* bogs, wet meadows, swampy fallow lands, wet tussocky grasslands, as well as woodlands and forests (Tolman & Lewington, 2008). However, while the known populations in Romania live in clearings in oak woods (Craioveanu et al., 2014; Rákosy, 2024), the population in Coșna prefers open semi-natural habitats and meadows, moving towards the bog woodland but seldom venturing into the forest.



Figure 8. *Lycaena helle* feeding on a *Cardamine pratensis* flower in the wet meadows of Mestecănașul de la Coșna site

The larva of *L. helle* develops and feeds exclusively on the leaves of *Bistorta officinalis* Delarbre (Rákosy, 2024). Meanwhile, the adults

have a more varied diet, primarily feeding on *B. officinalis* in the first generation of lowland Romanian populations (Craioveanu et al., 2014). Additionally, they feed on nectar from *Myosotis* sp., *Cardamine pratensis*, *Anemone nemorosa*, *Ranunculus acris*, *Ligustrum vulgare*, *Mentha longifolia*, and *Eupatorium cannabinum*. In contrast, at Coșna, where *B. officinalis* is not in bloom in May, the fresh adults primarily feed on *Cardamine pratensis*, and occasionally on *Ranunculus acris*, *R. repens*, and *Viola sp.* (Ion et al., 2023).

In the Romanian Red Book of Invertebrates, this species is classified as Endangered, approaching Critically Endangered (Rákosy, 2021), primarily due to the loss of lowland populations. The population from Coșna, located at a higher altitude and surrounded by rapidly growing human settlements, is of particular conservation and scientific interest, further highlighting the necessity of legal protection for its habitat.

The conservation of these wet meadows is therefore, crucial for maintaining both habitat integrity and the persistence of *L. helle* populations within the site.

During the field studies in the perimeter of the proposed Natura 2000 site we identified five species of amphibians: the Carpathia newt (*Lissotriton montandoni*), the alpine newt (*Ichthyosaura alpestris*), the yellow-bellied toad (*Bombina variegata*), the common toad (*Bufo bufo*), and the common frog (*Rana temporaria*), and one species of reptile – the European adder (*Vipera berus*). All species of amphibians and reptiles identified are protected by national and European legislation. The Carpathian newt and the yellow-bellied toad are listed in Annex II of the Habitats Directive. Moreover, the Carpathian newt is endemic to the Carpathian Mountains; in Romania it is locally abundant, but its distribution is restricted to the Eastern Carpathians and parts of the Southern Carpathians. The proposed site is used by the species for breeding (where it takes advantage of the temporary and permanent water puddles), as well as for feeding and hibernation during the terrestrial phase.

In terms of bird diversity, the area seems to cater for several species. We recorded the presence of members belonging to 7 families represented through 16 species, out of which 5 are of community interest: 2 listed on Anex I of the Birds Directive (Birds Directive, 2009) (*Anthus campestris*, *Lanius collurio*) and 3 taxa included on the National Red List of Birds Species (MMAP, 2022) (*Carpodacus erythrinus*, *Linaria cannabina*, *Saxicola rubetra*). Best represented groups were *Fringillidae, Motacillidae, Muscicapidae and Sylviidae*, with species that generally thrive in this type of habitats (e.g. *Motacilla alba*, *Motacilla cinerea*, *Sylvia borin*, *Fringilla coelebs*). Considering site size and the surrounding area's landscape, the recorded avian diversity highlights that this mire acts as an effective stepping stone, aiding species regional and long-distance dispersal, thus playing an important role during migrations.

Restoration and awareness actions undertaken

B. humilis along with other wet meadows specialist depends on stable hydrological conditions making its populations highly vulnerable to habitat drainage and anthropogenic alterations (Dihoru & Negrean, 2009).

In this respect, several on-site interventions were implemented to restore and protect the ecological integrity of the Coșna peatland. These included blocking drainage channels to restore the water regime, installing informational panels at site access points for promoting environmental education, and fencing sensitive areas to prevent grazing and allow for natural vegetation regeneration.

Engaging local stakeholders has played a central role in the restoration process. The Institute of Biology Bucharest team facilitated activities aimed at raising awareness about the ecological importance of the site. These initiatives involved the dissemination of educational materials, engagement with local entrepreneurs to highlight the potential of ecotourism, and educational presentations on local flora and fauna delivered in schools. Field trips to the peatland were also organized, offering students hands-on experience in wetland biodiversity monitoring alongside conservation specialists.

A key outcome of the partnership with the Coșna Local Council was initiating the formal procedure to designate the peatland behind the Coșna school as a locally protected area. This step ensures long-term conservation of the site and its valuable habitats and species.

Ex situ* conservation of *B. humilis

B. humilis is currently cultivated in the "Al. Borza" Botanical Garden, Cluj-Napoca, and the "V. Fati" Botanical Garden, Jibou (Hurdu et al., 2022). Additionally, under the PeatRO 2 project, as part of the ex-situ conservation program targeting peatland plant species, seeds were collected to establish collections for ex situ conservation and to support future reintroduction initiatives.

CONCLUSIONS

"Mestecănașul de la Coșna" site in Bukovina stands out as a unique ecological refuge for one of Romania's largest populations of *Betula humilis* Schrank, a glacial relict, together with other species of European conservation interest such as *Ligularia sibirica* and *Lycaena helle*. Although it has suffered from anthropic interference, significantly reducing the overall surface, this peatland still has a high biodiversity value. The conservation of *B. humilis* acts as an umbrella for the other rare and vulnerable species, sharing the same ecological conditions of this very specific habitat, that is already severely reduced at European and national level. The site is also home to a diverse community of amphibians, reptiles, and bird species, several of which are protected under EU legislation. Initial restoration efforts, including hydrological rehabilitation, control of invasive species, local education and awareness initiatives, have contributed to the ecological stabilization of the area. Continuous ecological monitoring, strict protective measures, and adaptive management focused on maintaining hydrological balance and minimizing human impact are essential to ensure the long-term conservation of this ecosystem. The proposed designation of "Mestecănașul de la Coșna" as a Natura 2000 site would not only safeguard *B. humilis*, but also demonstrate a broader commitment to protecting peatland ecosystems within the Eastern Carpathians.

ACKNOWLEDGEMENTS

This research was conducted as part of project RO1567-IBB03/2024 through the Institute of Biology Bucharest, Romanian Academy, and was additionally funded by the EEA Grants under the project "Degraded Mires and Peatlands Restoration in the North-East 1 Region of Romania" (PeatRO2).

REFERENCES

Austin, K.G., Elsen, P.R., Coronado, E.N.H., DeGemmis, A., Gallego-Sala, A.V., Harris, L., Kretser, H.E., Melton, J.R., Murdiyarso, D., Sasmito, S.D. & Swails, E. (2025). Mismatch between global importance of peatlands and the extent of their protection. *Conservation Letters*, *18*(1), p.e13080. Retrieved from https://conbio.onlinelibrary.wiley.com/doi/epdf/10.1111/conl.13080

Anomynus (2015). Planul de management al sitului ROSCI0245 Tinovul de la Romănești. Retrieved from chrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/https://mmediu.ro/app/webroot/uploads/files/2015-11-05_PM_Tinovul_de_la_Romanesti.pdf

Bonn, A., Allott, T., Evans, M., Joosten, H., & Stoneman, R. (2016). Peatland restoration and ecosystem services: an introduction. *Peatland restoration and ecosystem services: Science, policy and practice*, 1-16. Retrieved from https://doi.org/10.1017/CBO9781139177788

Boșcaiu, N., Coldea, G. & Horeanu, C. (1994). Lista Roșie a plantelor vasculare dispărute, periclitate, vulnerabile și rare din Flora României. *Ocrotirea Naturii și a Mediului Înconjurător, 38*, 45–56.

Botez, C. (1977). *Epopeea feroviară românească*, Editura Sport–Turism, București.

Bibby, C. J., Burgess, N. D., & Hill, D. A. (1992). Bird Census Techniques. Cambridge: Cambridge Univ. Press. Retrieved from https://www.ebcc.info/wp-content/uploads/2020/06/gregory-bird-census-and-survey-techniques.pdf

Birds Directive (BD) (2009). Directive 2009/147/EC of the European Parliament and of the Council of 30 November 2009 on the conservation of wild birds (codified version). Official Journal of the European Union, L20, 7–25. Retrieved from https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32009L0147

Chrzanowska A., Jadwiszczak K.A., Kłosowski S., Banaszek A. & Sozinov O.V. (2016) Sexual reproduction efciency and genetic diversity of endangered *Betula humilis* Schrk. populations from edge and sub-central parts of its range. *Folia Geobotanica*, 51: 161–173. Retreive from https://doi.org/10.1007/s12224-016-9244-1

Ciocârlan, V. (2009). *Flora ilustrată a României*. Editura Ceres, București, 1141 p.

Convention on Biological Diversity (CBD) (2022). Kunming-Montreal Global Biodiversity Framework. United Nations Environment Programme (UNEP). Retreive from https://www.unep.org/resources/kunming-montreal-global-biodiversity-framework (Accessed: March 2025)

Craioveanu, C., Sitar, C., & Rákosy, L. (2014). Mobility, behaviour and phenology of the Violet Copper *Lycaena helle* in North-Western Romania:

Implications for conservation. In J. C. Habel, M. Meyer, & T. Schmitt (Eds.), *Jewels in the mist: A biological synopsis on the endangered butterfly Lycaena helle* (pp. 150). Pensoft. Retrieved from https://bit.ly/4iLQhd0

Dihoru, G. & Dihoru, A. (1994). Plante rare, periclitate și endemice în flora României - Lista Roșie. *Acta Horti Botanici Bucurestiensis, 23*, 173–197.

Dihoru, G. & Negrean, G. (2009). *Cartea roșie a plantelor vasculare din România*. Editura Academiei Române. București.

Doniță, N., Popescu, A., Paucă-Comănescu, M., Mihăilescu, S. & Biriș, I.A. (2005). *Habitatele din România*. Editura Tehnică Silvică, București.

European Commission (2020). EU Biodiversity Strategy for 2030: Bringing nature back into our lives. Brussels: European Commission. Retreive from https://environment.ec.europa.eu/strategy/biodiversity-strategy-2030_en

Euro+Med (2006). Euro+Med PlantBase - the information resource for Euro- Mediterranean plant diversity. Retrieved from http://ww2.bgbm.org/EuroPlusMed/. Accessed February, 2025.

Georgescu, C.C. (1952). Betulaceae. In: T. Săvulescu (Ed.), *Flora Republicii Populare Române*, I, (pp. 189-210), Editura Academiei Republicii Populare Române.

Goia, I. & Fărcaș, S. (1997). Mlaștinile de turbă – impact antropic, conservare și reconstrucție ecologică. *Studii și Cercetări, Științe ale Naturii Bistrița*, 3. 283-289.

Habitats Directive (1992). Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora. Official Journal of the European Communities, L206, 7–50. Retrieved from https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A31992L0043

Harenda, K.M., Lamentowicz, M., Samson, M. & Chojnicki, B.H. (2018). The Role of Peatlands and Their Carbon Storage Function in the Context of Climate Change. In: Zielinski, T., Sagan, I., Surosz, W. (eds) Interdisciplinary Approaches for Sustainable Development Goals. GeoPlanet: Earth and Planetary Sciences. Springer, Cham. Retreive from https://doi.org/10.1007/978-3-319-71788-3_12

Hurdu, B.I., Coste, A., Halmagyi, A., Szatmari, P.M., Farkas, A., Pușcaș, M., Turtureanu, P.D., Roșca-Casian, O., Tănase, C., Oprea, A. and Mardari, C., (2022). Ex situ conservation of plant diversity in Romania: A synthesis of threatened and endemic taxa. *Journal for Nature Conservation*, *68*, p.126211. Retrieved from https://ui.adsabs.harvard.edu/link_gateway/2022JNatC..6826211H/doi:10.1016/j.jnc.2022.126211

Ion, M.C., Manu, M., Stănescu, M., Maican, S., Helepciuc, F.E., Moroșanu, A.M., Ștefănuț, M.M., Bîrsan, C.C., Tamas, G., Nicoară, R.G. & Ștefănuț, S., (2023). The rediscovery of *Lycaena helle* (Lepidoptera: Lycaenidae) in Dorna Depression (România), 125 years after its first mention. *Scientific Papers. Series D. Animal Science*, *46*(2): 612-620. Retreive from chrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/https://animalsciencejournal.usamv.ro/pdf/2023/issue_2/Art76.pdf Accessed February, 2025.

Jabłońska, E. (2012). Vegetation with *Betula humilis* in Central Europe. *Phytocoenologia*, 42: 259–277. Retreive from https://doi.org/10.1127/ 0340-269X/2012/0042-0527

Jadwiszczak, K.A., Banaszek, A., Jabłońska, E. & Sozinov, O.V. (2012). Chloroplast DNA variation of *Betula humilis* Schrk. in Poland and Belarus. *Tree Genetics & Genomes*, 8, 1017-1030. Retreive from https://doi.org/10.1007/s11295-012-0482-y

Jadwiszczak, K. A., Banaszek, A., Chrzanowska, A., Kłosowski, S., & Sozinov, O. V. (2014). The admixture zone of *Betula humilis* Schrk. phylogenetic lineages follows the eastern central European suture zone. *Plant Ecology & Diversity*, *8*(3), 323–329. Retreive from https://doi.org/10.1080/17550874.2014.893593

Kaplan, Z. (2017). Flora and phytogeography of the Czech Republic. *Flora and vegetation of the Czech Republic*, 89-163.

Joosten, H. & Clarke, D. (2002). Wise use of mires and peatlands: Background and principles including a framework for decision making; International Mire Conservation Group/International Peat Society Saarijärvi, Finland.

Joosten, H. & Tanneberger, F., & Moen, A. (2017). Mires and peatlands of Europe: Status, distribution and conservation. Schweizerbart Science Publishers.

László, G. (1915). *A tőzeglápok és előfordulásuk Magyarországon* [Peat bogs and their distribution in Hungary]. A.M.K. Földtani Intézet Kiadványai.

Leifeld, J., Menichetti, L. (2018). The underappreciated potential of peatlands in global climate change mitigation strategies. *Nature Communication,* 9, 1071. Retreive from https://doi.org/10.1038/s41467-018-03406-6

Limpens, J., Berendse, F., Blodau, C., Canadell, J. G., Freeman, C., Holden, J., ... & Schaepman-Strub, G. (2008). Peatlands and the carbon cycle: from local processes to global implications–a synthesis. *Biogeosciences*, *5*(5), 1475-1491.

Ministerul Mediului, Apelor și Pădurilor (MMAP). (2022). *Lista roșie națională a speciilor de păsări din România* (Monitorul Oficial al României, Partea I, nr. 798 bis/11.VIII.2022). București. Retreive from https://www.sor.ro/wp-content/uploads/1991/01/Lista-Rosie-Monitorul-Oficial-Partea-I-nr.-798Bis.pdf

Oltean, M., Negrean, G., Popescu, A., Roman, N., Dihoru, G., Sanda, V. & Mihăilescu, S. (1994). Lista roșie a plantelor superioare din România. In: Oltean M. (coord.), *Studii, sinteze, documentații de ecologie. Academia România, Institutul de Biologie București,* 1, 1-52. Retreive from https://www.researchgate.net/publication/318654406_Lista_rosie_a_plantelor_superioare_din_Romania

Oprea, A. (2005). *Lista critică a plantelor vasculare din România*. Ed. Univ. Alexandru Ioan Cuza, Iași.

Peterschilka, F. 1928. Pollenanalyse einiger Hochmoore Neurumäniens 80 (Vorläufi ge Mitteilung). *Ber. Dtsch. Bot. Ges., 46*(3), 190-197.

Pop, E. (1928). *Betula nana* L. şi *Betula humilis* Schrank în România. *Buletinul Grădinii Botanice şi a Muzeului Botanic Cluj*, *7*(1), 1-9.

Pop, E. (1929). Analize de polen în turba Carpaților Orientali (Dorna-Lucina). *Buletinul Grădinii Botanice şi a Muzeului Botanic Cluj, 9*(3-4), 81-209.

Pop, E. (1960). Mlaştinile de turbă din R.P.R. Editura Academiei R.P.R.

Pop, E. (1976). Specii relicte în flora României. In T. Săvulescu (Ed.), *Flora Republicii Populare Române*, XIII, 106-111. Editura Academiei Republicii Populare Române.

Pop, A. M., Hognogi, G. G., & Bătinaş, R. H. (2025). Who's voice counts? Managing conservation and sustainable valorisation of peatlands. *Environmental Sciences Europe*, 37(1), 1-19. Retreive from https://doi.org/10.1186/s12302-025-01087-9

POWO (2025). Plants of the World Online. Facilitated by the Royal Botanic Gardens, Kew. Retreive from https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:295106-1 (accessed on March 2025).

Pricop, E., Filip, P.N. and Negrea, B.M. (2019). Floristic study of a protected wetland from Borsaros-Sâncraieni, Harghita County, Romania. *Advances in Agriculture & Botanics*, 11(2), 97-108. Retreive from https://www.proquest.com/docview/2395873816?pq-origsite=gscholar&fromopenview=true&sourcetype=Scholarly%20Journals

Rákosy, L. (2021). *Lycaena helle*. In D. Murariu & S. Maican (Eds.), *The Red Book of Invertebrates of Romania* (p. 318). Romanian Academy Publishing House, Bucharest.

Rákosy, L. (2024). *A field guide to the butterflies of Romania*. Exeter, UK: Pelagic Publishing.

Sanda, V., Ölerrer, K., & Burescu, P. (2008). *Fitocenozele din România*. Bucharest, RO: Ars Docendi Publishing House

Sârbu I., Ştefan N. & Oprea A. (2013): *Plante vasculare din România. Determinator ilustrat de teren*. Victor B Victor, București.

Shaw, K., Roy, S., Wilson, B. (2014). *Betula humilis*. *The IUCN Red List of Threatened Species*. Retreive from e.T194645A2355398. https://dx.doi.org/10.2305/IUCN.UK.2014-.RLTS.T194645A2355398.en.

Swindles, G. T., Morris, P. J., Mullan, D. J., Payne, R. J., Roland, T. P., Amesbury, M. J., ... & Warner, B. (2019). Widespread drying of European peatlands in recent centuries. *Nature Geoscience*, *12*(11), 922-928. Retreive from https://doi.org/10.1038/s41561-019-0462-z

Szatmari, P.M. & Hurdu, B.I. (2022). Low altitude glacial relicts in the Romanian flora. *Contributii Botanice*, (57), 19-51. Retreive from DOI: 10.24193/Contrib.Bot.57.2

Tanneberger, F., Tegetmeyer, C., Busse, S., Barthelmes, A., Shumka, S., Mariné, A. M., . . . Joosten H. (2017). The peatland map of Europe. *Mires and Peat*, *19*(22), 1–17. Retreive from https://doi.org/10.19189/MaP.2016.OMB.264

Tanneberger, F., Moen, A., Barthelmes, A., Lewis, E., Miles, L., Sirin, A., Tegetmeyer, C., Joosten, H. (2021) Mires in Europe-regional diversity. Mires in Europe - Regional diversity, condition and protection. *Diversity*, *13*(8), 381 Retreive from https://doi.org/10.3390/d13080381

Tantau, I., Reille, M., de Beaulieu, J. L., Farcas, S., Goslar, T., & Paterne, M. (2003). Vegetation history in the Eastern Romanian Carpathians: pollen analysis of two sequences from the Mohoş crater. *Vegetation History and Archaeobotany*, *12*, 113-125.

Tolman, T., & Lewington, R. (2008). *Collins butterfly guide: The most complete field guide to the butterflies of Britain and Europe* (384 pp). Harper Collins Publishers.

UNEP (2022). Global Peatlands Assessment–The State of the World's Peatlands: Evidence for action toward the conservation, restoration, and sustainable management of peatlands. *Main Report. Global Peatlands Initiative*.

Verhoeven, J. T. (2014). Wetlands in Europe: perspectives for restoration of a lost paradise. *Ecological Engineering*, *66*, 6-9. Retreive from https://doi.org/10.1016/j.ecoleng.2013.03.006

Witkowski, Z. J., Król, W., Solarz, W. (2003). *Carpathian List of Endangered Species*. Vienna-Krakow: WWF and Institute of Nature Conservation, Polish Academy of Sciences.

Załuski, T., Pisarek, W., & Kucharczyk, M. (2001). *Betula humilis* Schrank. In: Kaźmierczakowa R., Zarzycki K. (eds.), *Polish Plant Red Data Book*, Polish Academy of Sciences, Kraków.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

ECOLOGY AND PHYTOSOCIOLOGY OF THE SPECIES *CIRSIUM CANDELABRUM* GRISEB. (SECT. CIRSIUM, ASTERACEAE, CYNAREAE) IN THE UPPER BASIN OF THE GILORT RIVER, CARPATHIANS MOUNTAINS

Mariana NICULESCU, Paula IANCU

University of Craiova, 19 Libertăţii Street, Craiova, Romania

Corresponding author email: paula.iancu@edu.ucv.ro

Abstract

According to Flora Europaea, Cirsium candelabrum Griseb. (Asteraceae) includes the territory of the Balkans (Balkan endemic): Bosnia and Herzegovina, Montenegro, Albania, Serbia, Kosovo, Macedonia, Greece, Bulgaria and southwest Romania. Cirsium candelabrum occurs in ruderal habitats along roads, especially when natural vegetation has been removed by road construction or reconstruction. The species is found in intensely sunny and extremely dry areas, newly filled warehouses with construction waste, most often in urban and suburban areas. It is an anthropogenic species with tendencies to spread in natural habitats and agro-ecosystems. From a phytosociological point of view, two plant communities built by this species have been described in Europe: Picrido hieracioidis-Cirsietum candelabri Jasprica, Milović & Pandža 2015 (Croatia) and Cirsietum candelabri Matvejeva ex Čarni, Kostadinovski & Matevski 2001 (North Macedonia and Bosnia) and Herzegovina. In Romania, the species is found sporadically, especially on skeletal, sandy or stony soils, in Hunedoara, Caras-Severin, Mehedinți, Gorj and Vâlcea. From an ecological and phytosociological point of view, the species shows a relatively high variability corroborated with the current eco-pedo-climatic conditions.

Key words*: Cirsium candelabrum, distribution, ecology, Gilort basin, phytosociology.*

INTRODUCTION

The genus *Cirsium* Mill. is one of the largest genera in *Asteraceae*. It contains about 250 species, distributed in Europe, North Africa, Eastern, Central, and South-West Asia, and North and Central America (Davis & Parris, 1975; Petrak, 1979; Yıldız et al., 2009). In Romania, the genus *Cirsium* is represented by 19 taxa, 17 species and 2 subspecies. *Cirsium candelabrum* (Syn. *C. helmeum* Orph.) is a biennial plant that develops a rosette of basal leaves in the first year and a tall, much-branched stem with numerous flower heads in the following year. This plant has a very particular habitus and can be easily distinguished from other *Cirsium* species by the following characteristics: completely glabrous plants, stems very branched from the base to the synflorescence, capitula in groups of 4-12 at the tips of the later (Săvulescu coord, 1964; Werner, 1976). *Cirsium candelabrum* is a Balkan endemic that occurs in ruderal habitats along roads, especially where natural vegetation has been removed by road construction or reconstruction (Borovečki-Voska et al., 2022). The species occurs in intensely sunny and extremely dry areas, freshly filled construction waste dumps, most often in urban and suburban areas. It is an anthropogenic species with tendencies to spread in natural habitats and agro-ecosystems.In the last 25 years, a lot of construction has been done in the upper basin of the Gilort, especially in the locality of Rânca, this tourist resort becoming a cluster of constructions, many of which were started and not completed, some of which ended up in ruins. This has led to the existence of many plots of land with garbage or waste resulting from construction activities in this area. A specific ruderal vegetation has also developed on these plots. The *Cirsium candelabrum* species that we studied in the period 2023-2024 has found a habitat conducive to development, with populations developed numerically, phenotypically, ecologically, physiologically, and cenotically in many areas. The aim of this study is to describe the chorology, ecology and phytosociology of *Cirsium candelabru* Griseb. (*Asteraceae*) species in the upper basin of the Gilort, an area where this species has proven to be very widespread along the roads, mostly in

the areas of recent constructions, on the remains or waste collected from them, on the side of the roads, in sunny, drier places, participating in the floristic composition of the ruderal vegetation in this area.



Figure 1. *Cirsium candelabrum* in the upper basin of the Gilort river (Gorj County)

MATERIALS AND METHODS

Study area. The paper presents the results of the investigations carried out in the ruderal vegetation in the upper basin of the Gilort in the Parâng Mountains, in the Transalpina area, between Novaci and Rânca. From a conservative point of view, the studied territory is an integral part of the ROASC0128 Nordul Gorjului de Est protected area. The upper basin of the Gilort river has significant ecological and economic importance, being an area with high biodiversity and valuable natural resources. It is part of the Jiu River basin, which is characterized by a temperate-continental climate, with variations from north to south and from east to west. This leads to significant variations in temperature and precipitation throughout the year (https://inundatii.ro). The relief is characterized by steeply sloping mountain valleys, with predominantly clayey soils and undeveloped soils (https://www.mmediu.ro). The mountainous area, which includes the upper basin of the Gilort, has lower average annual temperatures and more abundant precipitation compared to the plain areas. Precipitation is more frequent in the form of rain and snow, due to the higher altitude (https://legislatie.just.ro). The relief is characterized by steeply sloping mountain valleys, with predominantly clayey soils and undeveloped soils (https://www.mmediu.ro). The mountainous area, which includes the upper basin of the Gilort river, has lower average annual temperatures and more abundant precipitation compared to the plain areas. Precipitation is more frequent in the form of rain and snow, due to the higher altitude (https://legislatie.just.ro).The upper basin of the Gilort river presents a climatic characteristic specific to the mountainous areas of Romania. The main climatic characteristics of this region are described below. The average annual temperatures are lower compared to the plain areas, due to the high altitude. In general, the average annual temperature in the mountainous areas of Romania varies between 2°C and 6°C, but can be lower depending on the altitude. Precipitation is abundant, with a high annual average, which can exceed 1000 mm. This can fall in the form of rain or snow, depending on the season. The climate is influenced by cold air masses from the north and warm air from the south, which leads to significant variations in temperature and precipitation throughout the year.

Methods. In order to identify this species, we looked into: Romanian Flora, vol. IX (Săvulescu, T. coord., 1964), Flora Europaea, Vol. 4 (Werner, 1976) and Flora d'Italia (Pignatti, 1982). For the phytosociological studies, the following works developed in Romania by: Coldea, 1991 and Sanda et al., 1997, 2001 were used. Also, works developed at international level by: Mucina et al. (2016, 1993), Oberdorfer (1992) and Rodwell et al. (2002) were consulted.

RESULTS AND DISCUSSIONS

Chorology. *Cirsium candelabrum* popularly called *Candelabra thistle* is a biennial species widespread from the columnar to the upper montane levels. In the southern Carpathians of Romania, this species of Balkan origin is found only in the counties of Hunedoara, Caraș-Severin, Mehedinți, Gorj and Vâlcea. Information about the exact distribution of this species is limited, but it is known to be presentin

the lowlands and promontories of the Carpathians, along the Danube.There are also mentions of its presence in the Iron Gates area, where a hybrid form, *Cirsium candelabrum x creticum* (Parascan, 2014), taxa subsequently validated, was also identified. There are studies on the ecology and distribution of some species of the genus *Cirsium* in Romania, such as *Cirsium candelabrum*, which were investigated in the context of the vascular flora of the country (Oprea, 2005). The same author mentions that Grințescu studied the presence of *Cirsium candelabrum* in Romania in 1932. The species is mentioned in the context of the chorology of some meridional species in the Carpathians (https://pnscc.ro).Regarding the present studies, the species was identified and analyzed in Gorj County, in the upper basin of the Gilort River in the Parâng Mountains, at an altitude of between 600-1750 m. Following field studies carried out in the period 2023-2024, it was observed that this species is quite common in this area, in places with stony or even sandy substrate, in littered places, with a lot of construction waste, on the side of paved or unpaved roads, on the side of paths, on degraded and anthropized lands due to construction works, in the immediate vicinity of buildings under construction or of buildings that have recently been put into use (Figures 2, 3).



Figure 2. *Cirsium candelabrum* in Rânca Resort (Gorj County)

The species was found in Novaci, Rânca Resort, along the Transaplina road, in the areas of La Cârlig and Lupii Râncii, Telescaun Păpușa, Belvedere Transalpina, Panorama Parângul Mare, as well as in the stream basins: Dâlbanul, Gilorțel, Romanu, Setea Mare.



Figure 3. *Cirsium candelabrum* in the Coada Râncii area, on the edge of unfinished constructions

Ecology. *Candelabra thistle* is usually found on nutrient-poor, stony, and dry soils in resorts with lower temperatures, specific to the mountainous area of the Romanian Carpathians. The species can also be found in clearings, at the edge of forest habitats or in thickets, especially of blackthorn or blackberry, if the ecological and especially pedological conditions are favourable. In the researched territory, the species was also found in grassy ditches at the contact limit of the meadows of *Agrostis capillaris* and *Festuca rubra*, in the Romanu and Setea Mare streams, tributaries of the Gilort river (Figures 4, 5).



Figure 4. *Cirsium candelabrum* in the Romanu stream basin



Figure 5. *Cirsium candelabrum* in the Setea Mare stream basin

Phytosociological assessment of *Cirsium candelabrum* in plant communities

In Romania, the species is found sporadically, especially on skeletal, sandy or stony soils, in Hunedoara, Caraș-Severin, Mehedinți, Gorj and Vâlcea.

From an ecological and phytosociological point of view, the species shows a relatively high variability corroborated with the current eco-pedo-climatic conditions.

From a phytosociological point of view, two plant communities built by this species have been described in Europe: *Picrido hieracioidis-Cirsietum candelabri* Jasprica, Milović & Pandža 2015 from Croatia and *Cirsietum candelabri* Matvejeva ex Čarni, Kostadinovski & Matevski 2001 from North Macedonia and Bosnia and Herzegovina.

In Romania, there is no study on the coenology of this species.

In the upper basin of the Gilort River, *Candelabra thistle* is found in the floristic structure of ruderal plant communities, most frequently in *Tussilaginetum farfarae* Oberd. 1949 (Syn. *Poo-Tussilaginetum* Tx. 1931) plant community (Table 1).

This plant community is foundon eroded land, broken banks, at the edge of roads and paths, next to streams, stony and sandy-loamy soils, on usually sloping slopes but also in the habitats produced by the degradation of larger areas in construction works alongside the edifying species *Tussilago farfara* meeting *Cirsium candelabrum* but also other ruderal species, some of them invasive: *Erigeron annuus, Conyza canadensis, Picris hieracioides, Chondrilla juncea, Cichorium intybus, Artemisia absinthium, Melilotus officinalis, M. albus, Crepis foetida, Ambrosia artemisiifolia, Lotus corniculatus, Daucus carota, Poa annua*, *Plantago major*, *Ranunculus repens*, *Rumex obtusifolius*, *Equisetum arvense*, etc.

From an ecological point of view, the structure of these phytocoenosis is dominated by mesophilic, micro-mesothermic and euryionic species.

We can mention that in many locations in the studied area, the species *Cirsium candelabrum* was found in populations of over 10 individuals, being accompanied by an assemblage of a few species, many of which are ruderal. Thus, our field studies show that a series of species in the coenotic structure that have a greater constancy and abundance-dominance: *Melilotus albus, Echium vulgare, Galeopsis speciosa, Urtica dioica, Lapsana communis, Cichorium intybus*, *Daucus carota, Echium vulgare,* Senecio ovatus, *Verbascum chaixii, Hypericum perforatum, Conyza canadensis, Scrophularia nodosa, Thymus pulegioides.*

This assemblage of species, from a phytosociological point of view, is similar to that characteristic of the plant community *Cirsietum candelabrum* Matvejeva ex Čarni, Kostadinovski & Matevski 2001 from North Macedonia (Haziri, 2015), Slovenia (Grošelj, 2012; Šilc & Čarni, 2012), Bosnia and Herzegovina.

In order to establish with certainty the presence of this plant community in the studied area, more field studies and at least two more growing seasons are necessary to establish the exact cenotic core and, most importantly, the syndromancy of the phytocoenosis that we consider to be built by *Cirsium candelabrum*.

Table 1 - *Tussilaginetum farfarae* Oberd. 1949 plant community

No. of relevée	1	2	3	4	5	6	7	8	9	10	K	ADm
Altitude m.o.s. (x 10 m)	70	85	110	120	110	75	90	120	90	110		
Exposure	10	5	10	10	7	5	10	15	15	10		
Inclination (in grades)	SE	SV	E	SE	NE	E	V	SE	S	E		
Canopy (%)	70	75	75	80	80	80	60	70	60	80		
Coverage of herbacaeous layer (%)	30	10	20	25	25	20	25	20	10	30		
Char. ass.												
Tussilago farfara	3-4	4	4	4	5	4	4	4	4	5	V	66.25
Artemisietea et Artemisietea												
Arctium lappa	+	+	-	-	+	-	-	-	-	-	II	0.15
Urtica dioica	+	+	-	-	+	+	-	-	+	-	III	0.25
Rumex obtusifolius	-	-	+	+	-	-	+	+	-	+	III	0.25
Cirsium candelabrum	2	1	2	1	2	2	2	2	1	2	V	13.75
Chondrilla juncea	+	+	-	-	-	+	+	-	-	+	III	0.25
Artemisia absinthium	-	-	+	+	-	-	-	-	-	+	II	0.15
Cichorium intybus	+	+	-	-	+	-	+	-	-	+	III	0.25
Crepis foetida	+	-	+	+	+	+	-	+	-	+	IV	0.35
Ambrosia artemisiifolia	-	+	+	+	-	-	+	+	-	-	III	0.25
Chenopodietea								-				
Daucus carota	-	-	+	+	-	-	-	-	-	+	II	0.15
Echium vulgare	+	+	+	-	-	-	-	-	-	-	II	0.15
Capsella bursa-pastoris	+	+	-	-	-	+	-	-	-	-	II	0.15
Plantaginetea majoris												
Lolium perenne	+	+	-	-	-	+	+	-	-	+	III	0.25
Plantago major	+	+	+	-	+	-	+	+	-	+	IV	0.35
Poa annua	1	+	-	+	-	1	+	-	+	+	IV	1.25
Ranunculus repens	1	1	+	-	+	-	+	+	+	+	IV	1.30
Rorippa sylvestris	+	+	-	-	-	+	+	-	-	+	III	0.25
Melilotus officinalis	+	1	1-2	1	+	+	+	1	+	+	V	3.40
Melilotus albus	+	+	+	-	+	-	+	+	-	+	IV	0.35
Cirsium arvense	+	+	-	-	+	+	-	-	-	-	II	0.20
Molinio-Arrhenatheretea												
Dactylis glomerata	+	+	-	-	-	+	-	-	+	-	II	0.20
Taraxacumm officinale	-	+	-	-	+	-	-	-	-	-	I	0.10
Lotus corniculatus	-	+	+	-	-	-	-	-	-	-	I	0.10
Variae Syntaxa												
Deschampsia caespitosa	-	+	+	+	-	-	-	+	+	-	III	0.25
Ajuga reptans	+	+	-	-	-	-	-	-	-	-	I	0.20
Erigeron annuus	1	1	+	-	+	-	+	+	+	+	IV	1.30
Conyza canadensis	1	+	-	+	-	1	+	-	+	+	IV	1.25
Equisetum arvense	1	1	1	+	+	+	1-2	+	+	V	3,40	1
Rumexobtusifolius	+	+	-	+	+	-	-	+	-	-	III	0.25

Place and data of the relevés: 1-10, Upper basin of the Gilort river, 17.V.2023, 23.VI.2023, 18.VII.2024, 10.VIII.2024

CONCLUSIONS

Cirsium candelabrum is a Balkan endemic species, in Romania it is found sporadically, especially in the southwestern part of Romania, preferring oligotrophic soils. Most often it settles on degraded surfaces, with waste resulting from construction sites. Sometimes it finds habitat suitable for development in some anthropized, dry meadows or on the edge of roads and paths, especially paved ones.

From a phytosociological point of view in the upper of the Gilort river, *Candelabra thistle* is found in the floristic structure of ruderal plant communities, most frequently in the *Tussilaginetum farfarae* Oberd. 1949 (Syn. *Poo-Tussilaginetum* Tx. 1931) plant community but can also form the *Cirsietum candebra* Matvejeva ex Čarni, Kostadinovski & Matevski 2001 plant community described in North Macedonia and Bosnia and Herzegovina. The species is of conservation interest and has a pedogenetic role.

Considering the physiognomy, structure and floristic composition of the phytocoenosis studied in the upper basin of the Gilort river, as well as the high frequency and abundance-dominance of this species, it is important to

carry out complex cenotic studies not only in the area but also in other locations in the southwest of the country where the species constantly vegetates.

REFERENCES

Borovečki-Voska, L., Randić, M., Šegota, V., Alegro, A. (2022). New localities of *Cirsium candelabrum* Griseb. (Asteraceae) in Croatia. *Journal of the Croatian Botanical Society, 9*(2): 95-103.

Čarni, A., Kostadinovski, M., Matevski, V. (2001). New ruderal associations of *Artemisietea vulgaris* in the Republic of Macedonia. 75 years of Macedonian Museum of Natural History, 225-235.

Čarni, A. (2012): Conspectus of vegetation syntaxa in Slovenia. *Hacquetia, 11*(1): 113-164.

Coldea, Gh. (1997). *Les associations vegetales de Roumanie*. Ed. Presses Universitaires de Cluj, Cluj-Napoca.

Coldea, G. (1991). Prodrome des associations végétales des Carpates du Sud-Est (Carpates Roumaines). Documents Phytosociologiques, N.S., 13, Camerino, 317-539.

Davis, P.H., Tan, K & Mill, R.R. eds (1988). *Flora of Turkey and the East Aegean Islands*, Edinburgh University Press, Vol. 10 (suppl. 1), pp. 164-165.

Davis, P.H & Parris, S.B. (1975). *Cirsium* Mill. In: Davis PH, *Flora of Turkey and the East Aegean Islands*, Edinburgh University Press, Vol. 5, pp. 370-412.

Greuter, W. (2006). Compositae (pro partemajore). In: Greuter, W., Raab-Straube, E. von (ed.). *Compositae. Euro+MedPlantbase – the information resource for Euro-Mediterranean plant diversity*. – http://www.emplantbase.org/home.html [Accessed April 06, 2024].

Grošelj, P. (2012). *Cirsium candelabrum* Griseb.: Novo naha jliščetu jerodne vrstev Sloveniji. [Newlocality of an alien species in Slovenia]. *Hladnikia*, 29: 51-54.

Haziri, A. (2015). Asteraceae (Compositae) in the Northwest region of Macedonia. *International Journal of Advances in Pharmacy, Biology and Chemistry, 5*(1): 122-125.

Matvejeva, J. (1982). Ruderal nata vegetacijana SR Makedonija. Makedonska akademija na naukite iumetnostite, Oddelenie za biološki I medicinski nauki, Skopje, 1- 70.

Milović, M., Pandža, M., Radečić, K. (2014): New localities of *Cirsium candelabrum* Griseb. in Croatia. In: Jelaska, S.D. (ed.): *Book of Abstracts* of the First Croatian Symposium on Invasive Species with International Participation, Croatian Ecological Society, Zagreb, 39-40.

Milović, M., Pandža, M., Radečić, K. (2015). *Cirsium candelabrum* Griseb. (Asteraceae) in Croatia –the beginning of its invasive spread outside natural range? *Journal of the Croatian Botanical Society, 3*(2): 15-22.

Mucina, L.,Bültmann, H.,Dierßen, K.,Theurillat, J.P., Raus, T., Tichý, L. (2016). Vegetation of Europe: hierarchical floristic classification system of vascular plant, bryophyte, lichen, and algal communities. In: *Applied Vegetation Science, 9*(Suppl. 1), 3–264, Doi: 10.1111/avsc.12257.

Mucina, L., Grabherr, G., Ellmauer, T. & Wallnöfer, B. (eds.) (1993). Die Pflanzengesell schaften Österreichs. Band I-III. G. Fischer, Jena.

Nenad, J., Milović, M., Pandža, M. (2015). Picrido hieracioidis - *Cirsietum candelabri* - a ruderal association new to Croatia, *Journal of the Croatian Botanical Society, Vol. 3*, No. 2, pp. 4-14, 2015.

Oberdorfer, E. (1992). Süddeutsche Pflanzen – gesellschaften, Teil IV: Wälder und Gebüsche 2, Stark berabeilete Auflage Texband, Gustav Fischer Verlang Jena, New York.

Oprea, A. (2005). *Lista critică a plantelor vasculare din România*, Edit. Univ. "Alexandru Ioan Cuza", Iaşi: 668.

Parascan, D. (2014). Profil de magistru: Profesorul Iuliu Morariu. *Bucovina Forestieră, 14*(1): 107-112.

Petrak, F. (1979). *Cirsium* Mill. In: Rechinger, KH, ed. Flora Iranica. Compositae III- Cynareae, 139a: 231-285. Graz: Akademische Drucku Verlagsanstalt.

Petronić, S., Pavlović, D. (2006): *Cirsietum candelabra* ruderal nazajednica područja Pala. In: Knežević, D. (ed.): Zbornikradova Simpozija s međunarodni msudjelovanjem "Una pređenjepoljo privredne proizvodnjena teritoriju Kosova iMetohije", Poljoprivredni fakultet, Lešak, 245- 250.

Pignatti, S. (1982): *Flora d'Italia*. 1-3. Edagricole, Bologna.

Rodwell, J. S., Schaminée, J.H.J., Mucina, L., Pignatti, S., Dring Moss, J.D. (2002). The diversity of European vegetation – An overview of phyto-sociological alliances and their relationships to EUNIS habitats. National Reference Centre for Agriculture, Nature and Fisheries. [report no. EC-LNV 2002(054)], Wageningen.

Sanda, V., Popescu, A. & Stancu, D.I. (2001). *Structura cenotică şi caracterizarea ecologică a fitocenozelor din România*. Pitesti: Edit. Cophis.

Sanda, V., Popescu, A., Barabaş, N. (1997). Cenotaxonomia şi caracterizarea grupărilor vegetale din România. *St. Com., Muz. Şt. Nat. Bacău*, 14: 5-366.

Săvulescu, T. (Ed) (1952-1976). *Flora R.P. Române*. R.S România, 1-XIII. București. Edit. Acad. R.P. Române (R. S. România), București.

Šilc, U., Čarni, A. (2012): Conspectus of vegetation syntaxa in Slovenia. *Hacquetia, 11*(1): 113-164.

Werner, K. (1976): *Cirsium* Miller. In: Tutin, T.G. et al. (eds.): *Flora Europaea, Vol. 4*, Cambridge University Press, Cambridge, 232-240.

Yıldız, B., Dirmenci, T., Arabacı, T. (2009): A newrecord for the flora of Turkey: *Cirsium candelabrum* Griseb. (Cirsium sect. Cirsium, Asteraceae, Cynareae). *Turkish Journal of Botany*, 33: 47-51.

https://pnscc.ro (accessed 03.03.2025)

https://www.mmediu.ro(accessed 03.03.2025)

https://legislatie.just.ro(accessed 03.03.2025)

https://inundatii.ro(accessed 03.03.2025)

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

SOME ASPECTS OF THE MORPHO-ANATOMICAL FEATURES OF THE MEDICINAL PLANT *ARNICA MONTANA* L.

Sorina NIȚU (NĂSTASE)[1, 2], Vasilica LUCHIAN[3], Monica TOD[1], Paul ZEVEDEI[1], Tudor ENE[1], Virgil NIȚU[2], Adrian BLAJ[1]

[1]Research And Development Grasslands Institute, 5 Cucului Street, 500128, Brașov, Romania
[2]University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, 3-5 Calea Mănăştur Street, 400372, Cluj-Napoca, Romania
[3]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăşti Blvd, District 1, Bucharest, Romania

Corresponding author email: vasilica.luchian@horticultura-bucuresti.ro

Abstract

The objective of this work was the morphological and anatomical study of vegetative organs in the species Arnica montana L. it belongs to the Asteraceae family. The genus includes approximately 30 species, herbaceous, perennial, has yellow or orange flowers, reaches a height of approximately 60 cm. It originates from the mountain regions of Europe, North America. It is commonly found in alpine meadows, on wetlands and in forests. Arnica montana L. is an endangered medicinal plant species endemic to Europe. Traditionally used in Romania for its anti-inflammatory and analgesic properties, it stimulates blood circulation, reduces edema, has a healing and antimicrobial effect. After analyzing the morphology and anatomy of arnica leaves, it was found that in both epidermises there are peri tectors and glands, short and long, the mesophyll is bifacial type, differentiated into palisade tissue with 1-2 rows of cells, under upper epidermis and lacunate tissue, with 4-5 rows of cells, with intercellular spaces, below the lower epidermis. The leaf blade is amphistomatic, with stomata in both epidermises.

Key words*: root anatomy, leaf anatomy, endemic, morphology.*

INTRODUCTION

The genus *Arnica* includes 32 species, which are distributed in the boreal zone and mountainous regions of Europe and North America. *Arnica montana* L. is native to Europe and can be found in its natural habitats from the mountainous regions of southern Iberia to southern Scandinavia and in the Carpathian Mountains of Romania (Pljevljakušić et al., 2014). Some species of the *Arnica* genus are medicinal plants widely used in both the pharmaceutical and cosmetic industries. Since 1977, *A. montana* L. has been declared a natural monument. Due to the fact that *Arnica montana* L., a species that was once quite widespread in Romania, has seen a significant decline in its populations in recent years, it has been included in Annex 5 of the Government Emergency Ordinance OUG 57/2007 (Antofie, 2012). Although *Arnica montana* is a protected species under the EU Habitats Directive and the EU regulation on trade in flora and fauna, it is currently listed only on the IUCN Red List as a species of "least concern" at the European level (https://eunis.eea.europa.eu/species/Arnica%20montana).

The inflorescences of *Arnica montana* L. are widely used in various types of phytopharmaceuticals, such as ointments, creams, and gels, due to their anti-inflammatory action, which is used in the local treatment of contusions and sprains (Pljevljakušić et al., 2014). The inflorescences of *Arnica montana* are characterized by antiseptic, anti-inflammatory, antifungal, antioxidant, and antisclerotic properties (Gaspar et al., 2014). They exhibit cardiotonic and cardiotoxic effects (Malarz et al., 1993) anxiety-reducing and analgesic effects (Ahmad et al., 2013), insecticidal effects (Ahmad et al., 2013), and hepatoprotective activity (Marchishin, 1983).

Plant extracts demonstrate antioxidant, antibacterial, anti-inflammatory, antifungal, and immunomodulatory activities, as well as antitumor, choleretic, cholagogue, diuretic, antirheumatic, anticancer, and musculoskeletal pain-relieving effects (Macêdo et al. 2004;

Kriplani et al., 2017; Rotar, 2005; Žitek et al., 2022; Nieto-Trujillo et al., 2021; Schmidt, 2023; Milani et al., 2023; Röhrl et al., 2023; Attiq et al., 2018; Sugier et al., 2020; Parafiniuk et al., 2023; Röhrl et al., 2023; Verre et al., 2024). Gautam et al., 2018, studied *Arnica* for Carpal Tunnel Syndrome, which generally affects women over the age of 30. Epistaxis can often be treated with *Arnica* (May Loo, 2009). Animal studies with microcurrent and *Arnica* gel have proven effective in treating skin lesions through anti-inflammatory effects and by reducing the presence of macrophages in the skin (Zacarias et al., 2023).

As a result of numerous chemical studies on *Arnica* species, approximately seventy sesquiterpene lactones, more than eighty flavonoids, phenolic acids, thymol derivatives, diterpenes, and pyrrolizidines have been identified (Kowalski et al., 2015). The use of *Arnica* before and after surgery to reduce bruising, swelling, pain, and soreness has been documented, with some variations in potency (strength) and dosing frequency (Burke, 2017).

Arnica reduces pain and has an anti-inflammatory effect after surgeries (Iannitti et al., 2016) and reduces bruising following rhinoplasty surgeries (Chaiet & Marcus, 2016). Some studies indicate that the essential oil from the roots (2-4%) and rhizomes (3-6%), which mainly contains thymol and derivatives with bactericidal and fungicidal activity, contributes to these effects (Kohlmünzer, 2003).

MATERIALS AND METHODS

In this study, the morphological and anatomical characteristics of the vegetative organs of *Arnica montana* L. were investigated.

The plants used in the experiment were collected from the Blana Bucegi experimental center, under the coordination of the Research and Development Institute for Pastures in Brașov (ICDP Brașov), during the period of July 5-10, 2024.

After collection, they were transferred and acclimatized in the greenhouse of the Medicinal and Aromatic Plants Laboratory within the same institute.

These steps ensured optimal conditions for plant development prior to conducting the morphological and anatomical research (Figure 1).

The research material consisted of young plants at the 6-8 leaf stage. At this stage, the leaf of *A. montana* is fully developed and represents a stable biological unit that can be effectively analyzed to obtain precise data. The use of fresh material is important, as it allows for the best results in structural analysis, with the cells and tissues being in an active state, unaltered by dehydration processes that may occur during preservation.

The study of leaf anatomy is of great importance in taxonomy, as both the internal and external structures of leaves are often used for plant classification and for understanding their evolution and adaptability. A detailed knowledge of these features significantly contributes to deciphering the phylogenetic relationships between species and deepening our understanding of plant diversity in nature.

The microscopic examination was carried out on cross-sections of leaves treated with chloral hydrate for clarification, facilitating the observations. The study was conducted in the Botany Laboratory - Plant Morphology and Anatomy Department of the Faculty of Horticulture at USAMV of Bucharest, using Leica and Optika binocular microscopes, instruments that allow detailed observation of the internal structures of the leaf. Photographs were taken with a Motorola digital camera, using objectives of different magnifications to capture details at

various levels, which are essential for precise analysis of leaf anatomy.



Figure 1 a, b. - a - Arnica obtained from seeds;
b - Arnica obtained from the mother plant

Research on the morphology of *Arnica montana* L. has been conducted by Sugier et al., 2022, Parafiniuk et al., 2023. Knowledge of anatomical elements is crucial in the field of taxonomy.

Studies related to the anatomy of the species under study, as well as other species from the Asteraceae family, have been conducted by Pljevljakušić, 2012; Kostina & Muravnik, 2019; Milan et al., 2006; and Kromer, et al., 2016.

RESULTS AND DISCUSSIONS

Morphology and Anatomy of the Root and Leaf of *Arnica montana* L.

Morphology and Anatomy of the Root

Transverse sections were made through the fibrous root of *Arnica montana* (Figure 2).



Figure 2. Fragment from the fibrous root of Arnica

In transverse section, the root has a circular contour (Figure 3 a, b).

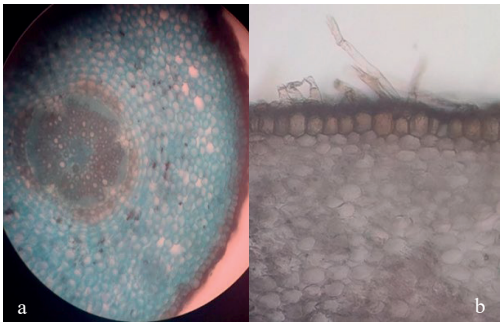


Figure 3 a, b – a - Root - Transverse section - general appearance; b - Rhizodermis with absorbent hairs

The outer surface of the root is covered by rhizodermis, with a single row of cells and absorbent hairs, cortex with parenchymal cells, and intercellular spaces (Figure 3), along with secretory canals, which are rounded or oval in shape. The central cylinder contains the pericycle, xylem, and phloem, while the pith consists of parenchymal cells with thin walls at the center of the section (Figures 4 and 5).

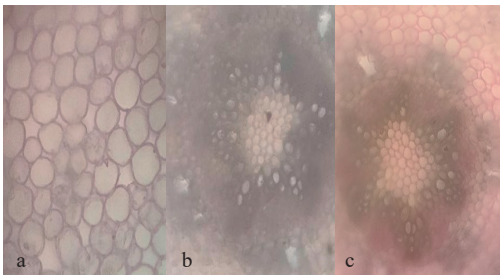


Figure 4 a, b, c – a - Root - Transverse section - cortical parenchyma with intercellular spaces;
b, c - Secretory canals and central cylinder

The structure of the fibrous root from plants obtained from seeds and from cuttings was the same, and thus the sections will not be presented separately.

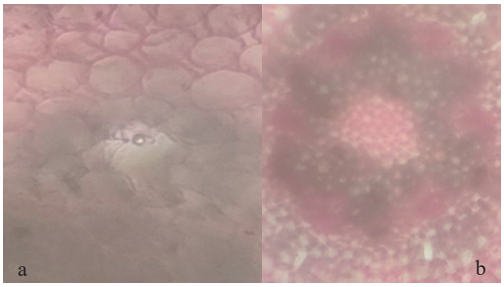


Figure 5 a, b – a - Root - Transverse section - secretory canal with oil droplet; b - Central cylinder with pith at the center

Morphology and Anatomy of the Leaf

Anatomy of the Petiole

The petiole is very short, with the leaf being nearly sessile.

It contains bicolateral vascular bundles embedded in parenchymal tissue, which are surrounded by a unistratified epidermis. Characteristic trichomes are present on the leaf surface (Figure 6 a, b, c).

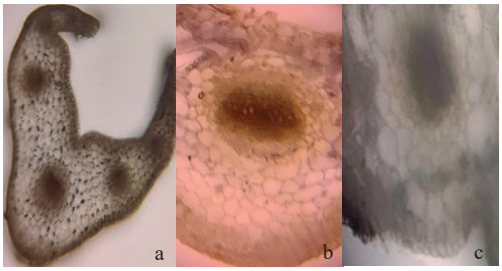


Figure 6 a, b, c – a - petiole overview; b, c - Petiole with the main vascular bundle - detail

Micromorphology and Anatomy of the Leaf

Leaves from both seed-grown and cutting-propagated plants were analyzed. Since no significant structural differences were observed, only the elements that stood out are mentioned. The leaves exhibited long and short multicellular protective trichomes on the margins, as well as glandular trichomes, both sessile and pedicellate, uniseriate and multiseriate, moniliform, of the same types (Figure 7 a,b,c, Figure 8 a,b,c, Figure 9 a,b,c,d, Figure 10 a, b, c, d).

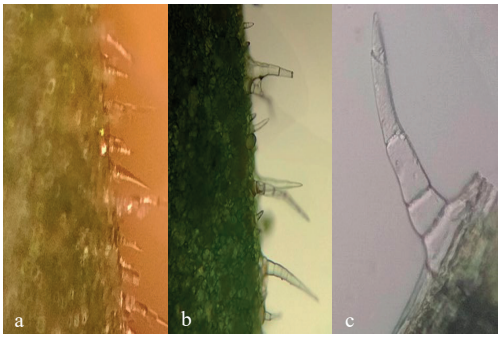


Figure 7 a, b, c – a - Short and long multicellular glandular trichomes on the leaf margin; b - Protective and secretory hairs on the leaf margin; c - Detail of multicellular protective hair (plant obtained from seeds)

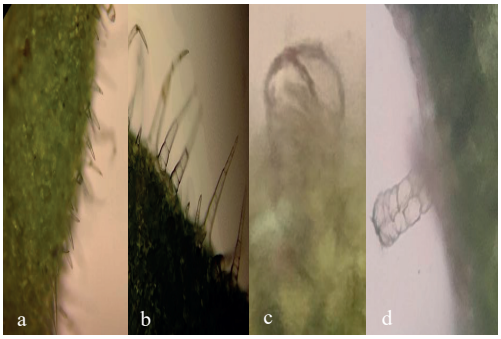


Figure 8 a, b, c, d – a, b – Short and long multicellular protective trichomes on the leaf margin; c – Bicapitate glandular trichome; d – Multiseriate glandular trichome (on the mother plant).

Anatomy of the Lamina

The leaf of *Arnica montana* in transverse section shows the upper epidermis, the lower epidermis, and the mesophyll with vascular bundles (Figure 9 a, b, c, d).

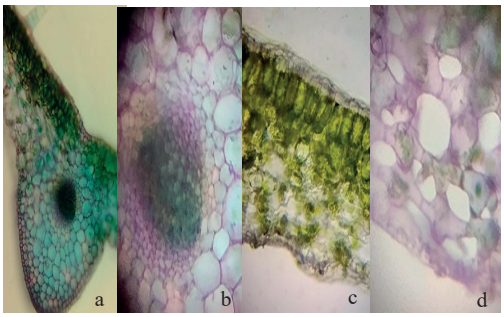


Figure 9 a, b, c, d – a, b - Transverse section of the lamina and main vein - overview and detailed view of the vascular bundle; c - Transverse section of the lamina

The mesophyll is bifacial, differentiated into palisade tissue consisting of 1-2 rows of chloroplast-rich cells located beneath the upper epidermis, and lacunose tissue formed by 4-5 rows of isodiametric cells with intercellular spaces and spherical secretory canals, located beneath the lower epidermis, containing bicolateral vascular bundles. The leaf is amphistomatic, with more stomata in the lower epidermis compared to the upper epidermis. Both epidermises contain protective trichomes, including uniseriate, short and long, slightly curved multicellular trichomes, as well as glandular trichomes - short and globular, as well as long, uniseriate, multiseriate, and bicapitate.

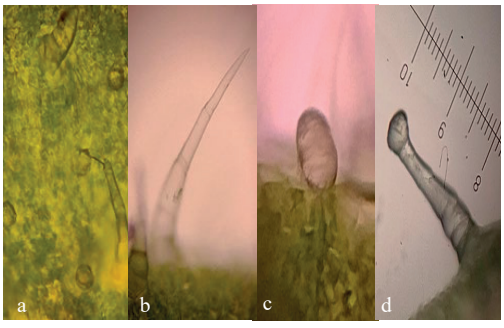


Figure 10 a, b, c, d – a - Upper epidermis with protective and glandular trichomes; b - Protective trichome; c - Sessile glandular trichome; d - Multicellular uniseriate glandular trichome (seed-grown plant)

In leaves of cutting-propagated plants, multi-seriate bicapitate glandular trichomes predominate.

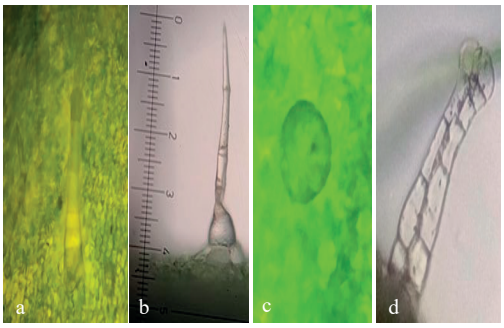


Figure 11 a, b, c, d – a - Upper epidermis with protective and secretory trichomes; b - Protective trichome; c - Glandular trichomes; d - Multiseriate glandular trichome (mother plant)

In the epidermises, there are both long and short multicellular protective trichomes and glandular trichomes, which are sessile, spherical, and pedicellate, uniseriate and multiseriate (Figure 10 a, b, c, d; Figure 11 a, b, c, d, ; Figure 12 a, b, c; Figure 13 a, b, c).

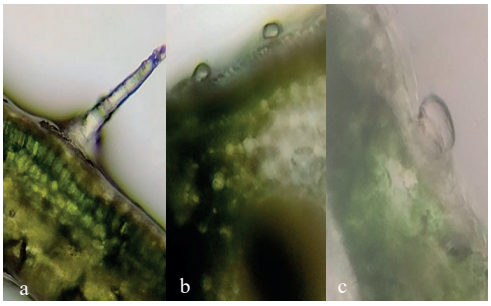


Figure 12 a, b, c – a - Upper epidermis with protective trichomes; b, c - Glandular trichomes, overview and detail (transverse section - mother plant)

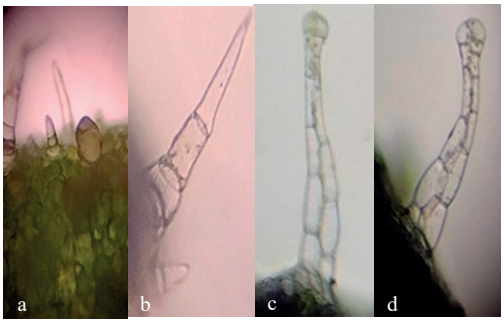


Figure 13 a, b, c, d – a - Lower epidermis with long and short multicellular protective trichomes and glandular trichomes; b - Long multicellular protective trichomes and short trichomes; c, d - Multiseriate glandular trichomes (mother plant)

On the lower epidermis of the leaf, protective trichomes, multicellular and glandular, spherical, and multiseriate, are observed (Figure 13 a, b, c, d).

In species of Asteraceae, trichomes are predominantly biseriate (Appezzato-da-Gloria et al., 2012 and others).

Glandular trichomes accumulate various products of primary and secondary metabolism, which, in addition to being essential elements for species identification, also contribute to the medicinal properties of the plant (Gopfert et al., 2005; Muravnik et al., 2016, 2019).

In the upper epidermis, rare stomata are observed, and the epidermal cells have undulated walls, with no intercellular spaces. The epidermis at the level of the main vein contains polygonal cells and stomata (Figure 14 a, b, c, d).

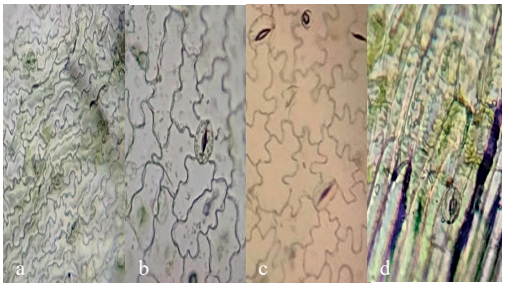


Figure 14 a, b, c, d – a, b - Upper epidermis of seed-grown Arnica; c - Upper epidermis of cutting-propagated Arnica (mother plant); d - Upper epidermis above the main vein (mother plant)

In the lower epidermis, more numerous stomata and glandular trichomes are observed, and the epidermal cells have undulated walls, with no intercellular spaces (Figure 15 a, b, c; Figure 16 a, b, c).

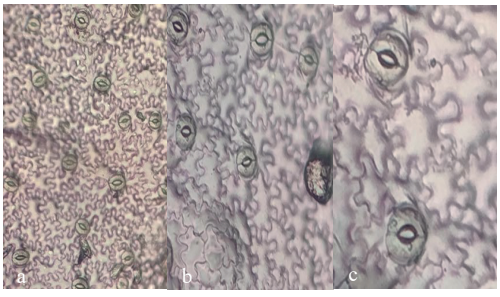


Figure 15 a, b, c – a, b - Lower epidermis of seed-grown Arnica; c - Upper epidermis of the mother plant

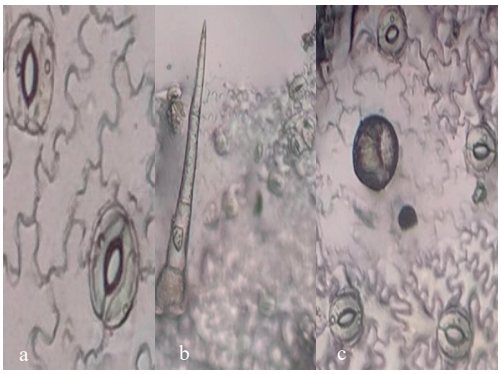


Figure 16 a, b, c – a, b, - Lower epidermis of seed-grown Arnica; c - Lower epidermis of the mother plant

CONCLUSIONS

Morphological and anatomical analyses are crucial for taxonomy, systematic botany, and for verifying the authenticity of semi-processed material obtained from this medicinal plant.

These morphological and anatomical analyses are being carried out for the first time in Romania for this species, and the detailed characteristics are presented in the Results and Discussion.

Both in the root and in the leaves, structures secreting essential oils, used for medicinal purposes, were observed.

The morphological and structural characteristics are similar in plants obtained from seeds and those obtained from cuttings, with the exception that in plants propagated through cuttings, multiseriate glandular trichomes predominated on the leaves.

REFERENCES

Ahmad, M., Saeed, F. A. R. A. H., Mehjabeen Jahan, N. (2013). Neuro-pharmacological and analgesic effects of *Arnica montana* extract. Int J Pharm Pharm Sci, 5(4), 590-3.

Antofie, M.M. (2012). Conservarea biodiversității - considerații politice și legislative, Ed. Universității Lucian Blaga din Sibiu 275 p.

Appezzato-da-Gloria, ´ B., Da Costa, F.B., da Silva, V.C., Gobbo-Neto, L., Rehder, V.L.G., Hayashi, A.H. (2012). Glandular trichomes on aerial and underground organs in *Chrysolaena* species (Vernonieae – Asteraceae): structure, ultrastructure and chemical composition. Flora 207, 878–887. https://doi.org/10.1016/j. flora.2012.10.003.

Attiq A., Jalil J., Husain K., Ahmad W. (2018). Raging the War Against Inflammation with Natural Products. Front. Pharmacol.; 9:976. doi: 10.3389/fphar.2018.00976. [DOI] [PMC free article] [PubMed] [Google Scholar]

Burke Lennihan (2017). Homeopathy for Pain Management, Alternative and Complementary Therapies, Vol. 23, No.5 https://doi.org/10.1089/act.2017.29129.ble

Camelia Sava Sand (2015). *Arnica montana* L. As A Medicinal Crop Species. Scientific Papers. Series "Management, Economic Engineering in Agriculture and rural development", Vol. 15 ISSUE 4, PRINT ISSN 2284-7995, 303-308.

Chaiet S.R., Marcus B.C. (2016). Perioperative *Arnica montana* for reduction of ecchymosis in rhinoplasty surgery. Ann Plast Surg.; 76(5): 477-82. [PubMed ID: 25954844]. https://doi.org/10.1097/SAP.0000000000000312.

David Rakel Editor(s) (2018). Integrative Medicine (Fourth Edition), Elsevier, Pages 697-706.e2,

European Nature Information System *Arnica montana* L. [(accessed on 25 September 2024)]. Available online: https://eunis.eea.europa.eu/species/Arnica%20montana.

Gaspar, A., Cracinescu, O., Trif, M., Moisei, M., Moldovan, L. (2014). Antioxidant and anti-inflammatory properties of active compounds from *Arnica montana* L. Rom. Biotechnol. Lett. 19, 9353–9365.

Gautam J. Desai, Dennis J. Dowling, Joshua W. Harbaugh, Chapter 70 - Carpal Tunnel Syndrome.

Gopfert, ¨ J.C., Heil, N., Conrad, J., Spring, O. (2005). Cytological development and sesquiterpene lactone secretion in capitate glandular trichomes of sunflower. Plant Biol. 7, 148–155. https://doi.org/10.1055/s-2005-837575.

https://doi.org/10.1016/j.jep.2023.117064.

Iannitti T, Morales-Medina JC, Bellavite P, Rottigni V, Palmieri B. (2016). Effectiveness and safety of *Arnica montana* in post-surgical setting, pain and inflammation. Am J Ther.; 23(1): 184-97. [PubMed ID: 25171757]. https://doi.org/10.1097/MJT.0000000000000036.

ISBN 9780323358682, https://doi.org/10.1016/B978-0-323-35868-2.00070-0.

Justine Verre, Marie Boisson, Anne Paumier, Sandra Tribolo, Naoual Boujedaini (2024). Anti-inflammatory effects of *Arnica montana* (mother tincture and homeopathic dilutions) in various cell models, Journal of Ethnopharmacology,Volume 318, Part B, ,117064,ISSN 0378-8741,

Kohlmünzer, S. (2003). Farmakognozja. 5th. *Warszawa: Lekarskie PZWL*.

Kostina, O.V., Muravnik, L.E. (2019). Comparative characteristics of the glandular trichomes in four species of *Arnica (Asteraceae*): localization, morphology, ultrastructure and histochemical analysis. In: Timonin, A.C., Sokoloff, D.D. (Eds.), Plant Anatomy: Traditions and Perspectives. MAKC Пресс, Moscow, pp. 134–138.

Kowalski, R., Sugier, D., Sugier, P., Kołodziej, B. (2015). Evaluation of the chemical composition of essential oils with respect to the maturity of flower heads of *Arnica montana* L. and *Arnica chamissonis* Less.

cultivated for industry. Ind. Crop. Prod. 76, 857–865. https://doi.org/10.1016/j.indcrop.2015.07.029

Kriplani P., Guarve K., Baghael U.S. (2017). *Arnica montana* L. - a plant of healing: review. J Pharm Pharmacol.; 69(8):925-945. doi:10.1111/jphp.12724

Krystyna Kromer, Agnieszka Kreitschitz, Thomas Kleinteich, Stanislav N. Gorb, Antoni Szumny (2016). Oil Secretory System in Vegetative Organs of Three *Arnica taxa*: Essential Oil Synthesis, Distribution and Accumulation, Plant and Cell Physiology, Volume 57, Issue 5, May 2016, Pages 1020–1037, https://doi.org/10.1093/pcp/pcw040

Kromer, K., Kreitschitz, A., Kleinteich, T., Gorb, SN și Szumny, A. (2016). Sistemul secretor de ulei în organele vegetative a trei taxoni de Arnica: sinteza, distribuția și acumularea uleiurilor esențiale. *Plant and Cell Physiology*, *57* (5), 1020-1037.

Macêdo S.B.; Ferreira, L.R.; Perazzo, F.F.; Tavares Carvalho, J.C. (2004). Anti-inflammatory activity of *Arnica montana* 6cH. Preclinical study in animals. Homeopathy, 93, 84–87. [Google Scholar] [CrossRef]

Malarz, J., Stojakowska, A., Dohnal, B., & Kisiel, W. (1993). Helenalin acetate in *in vitro* propagated plants of *Arnica montana*. Planta medica, 59(01), 51-53.

Marchishin, S. M. (1983). Efficacy of the phenol compounds of Arnica in toxic lesion of the liver. Farmakologiia i Toksikologiia, 46(2), 102-106.

May Loo,CHAPTER 35 - Epistaxis,Editor(s): May Loo,Integrative Medicine for Children, W.B. Saunders,

Milan, P., Hayashi, A.H., Appezzato-da-Gloria, ´ B. (2006). Comparative leaf morphology and anatomy of three Asteraceae species. Braz. Arch. Biol. Technol. 49, 135–144. https://doi.org/10.1590/S1516-89132006000100016

Milani, F.; Bottoni, M.; Bardelli, L.; Colombo, L.; Colombo, P.S.; Bruschi, P.; Giuliani, C.; Fico, G. (2023). Remnants from the Past: From an 18th Century Manuscript to 21st Century Ethnobotany in Valle Imagna (Bergamo, Italy). Plants, 12, 2748. https:// doi.org/10.3390/ plants12142748

Muravnik, L.E., V. Kostina, O.V., Zaporozhets, N.L. (2022). Structure and functions of the glandular trichomes in three *Arnica species* (Asteraceae), depending on their location on leaves and flowers, Flora, Volume 290, 2022, 152047, ISSN 0367-2530, https://doi.org/10.1016/j.flora.2022.152047.

Muravnik, L.E., Kostina, O.V., Mosina, A.A. (2019). Glandular trichomes of the leaves in three *Doronicum* species (Senecioneae, Asteraceae): morphology, histochemistry and ultrastructure. Protoplasma, 256, 789–803. https://doi.org/10.1007/s00709-018- 01342-2.

Muravnik, L.E., Kostina, O.V., Shavarda, A.L. (2016). Glandular trichomes of *Tussilago farfara* (Senecioneae, Asteraceae). Planta 244, 737–752. https://doi.org/10.1007/ s00425-016-2539-x.

Nieto-Trujillo, A.; Cruz-Sosa, F.; Luria-Pérez, R.; Gutiérrez-Rebolledo, G.A.; Román-Guerrero, A.; Burrola-Aguilar, C.; Zepeda-Gómez, C.; Estrada-Zúñiga, M.E. (2021). *Arnica montana* cell culture establishment, and assessment of its cytotoxic, antibacterial, α-amylase inhibitor, and antioxidant in vitro bioactivities. Plants, 10, 2300. [Google Scholar] [CrossRef]

Parafiniuk, A., Kromer, K., Fleszar, M. G., Kreitschitz, A., Wiśniewski, J., & Gamian, A. (2023). Localization of sesquiterpene lactones biosynthesis in flowers of Arnica taxa. *Molecules*, *28*(11), 4379.

Pljevljakusˇic´, D., Rancˇic´, D., Ristic´, M., Vujisic´, L., Radanovic´, D. and Dajic´- Stevanovic´, Z. (2012) Rhizome and root yield of the cultivated *Arnica montana* L., chemical composition and histochemical localization of essential oil. Ind. Crops Prod. 39: 177–189

Pljevljakušić D. (2012). Rhizome and root yield of the cultivated *Arnica montana* L., chemical composition and histochemical localization of essential oil, Industrial Crops and Products 39 (2012), 177–189

Pljevljakušić D., Teodora Janković, Slavica Jelačić, Miroslav Novaković, Nebojša Menković, Damir Beatović, Zora Dajić-Stevanović (2014). Morphological and chemical characterization of Arnica montana L. under different cultivation models, Industrial Crops and Products, Volume 52,

Raghibi A., Salar A., Askari H., Keykha R. (2018). Investigating the Effect of *Arnica* Ointment and Distraction on the Pain Caused by Fistula Needle Insertion in Hemodialysis Patients: A Clinical Trial. Med Surg Nurs J.; 7(2): 85338. https://doi.org/10.5812/msnj.85338.

Röhrl J., Piqué-Borràs M.R., Jaklin M., Werner M., Werz O., Josef H., Hölz H., Ammendola A., Künstle G. (2023). Anti-Inflammatory Activities of *Arnica montana* Planta Tota versus Flower Extracts: Analytical, In Vitro and In Vivo Mouse Paw Oedema Model Studies. Plants; 12(6):1348. https://doi.org/10.3390/plants12061348

Röhrl J., Piqué-Borràs M.R., Jaklin M., Werner M., Werz O., Josef H., Hölz H., Ammendola A., Künstle G. (2023). Anti-Inflammatory Activities of *Arnica montana* Planta Tota versus Flower Extracts: Analytical, In Vitro and In Vivo Mouse Paw Oedema Model Studies. Plants (Basel). Mar 16; 12(6):1348. doi: 10.3390/plants12061348. PMID: 36987036; PMCID: PMC10053944.

Rotar, I., (2005). Conservarea plantelor medicinale in Europa de Est: *Arnica montana* – Romania, grant 007.01.

Schmidt TJ. (2023). *Arnica montana* L.: Doesn't Origin Matter? Plants; 12(20): 3532. https://doi.org/10.3390/plants12203532

Sugier P., Rysiak A., Sugier D., Winiarczyk K., Wołkowycki D., Kołos A. (2022). Differentiation and Propagation Potential of *Arnica montana* L. Achenes as a Consequence of the Morphological Diversity of Flowers and the Position of Flower Heads on the Plant. Plants; 11(24):3424. https://doi.org/10.3390/plants11243424

Sugier, P., Jakubowicz-Gil, J., Sugier, D., Kowalski, R., Gawlik-Dziki, U., Kołodziej, B., et al. (2020). Chemical Characteristics and Anticancer Activity of Essential Oil from *Arnica montana* L. Rhizomes and Roots. Molecules 25, 1284. doi:10.3390/molecules25061284

Sugier, P.; Jakubowicz-Gil, J.; Sugier, D.; Kowalski, R.; Gawlik-Dziki, U.; Kołodziej, B.; Dziki, D. (2020). Chemical characteristics and anticancer activity of essential oil from *Arnica montana* L. rhizomes and roots. Molecules, 25, 1284. [Google Scholar] [CrossRef] [PubMed] [Green Version]

Zacarias C.A., de Mendonça Florenziano R.F., de Andrade T.A.M., de Aro A.A., do Amaral M.E.C., Dos Santos G.M.T., Esquisatto M.A.M. (2023). *Arnica montana* L. associated with microcurrent accelerates the dermis reorganisation of skin lesions. Int J Exp Pathol. Apr; 104(2):81-95. doi: 10.1111/iep.12469. Epub 2023 Feb 8. PMID: 36752313; PMCID: PMC10009304.

Žitek, T.; Postružnik, V.; Knez, Ž.; Golle, A.; Dariš, B.; Marevci, M.K. (2022). *Arnica montana* L. supercritical extraction optimization for antibiotic and anticancer activity. Front. Bioeng. Biotechnol., 10, 897185. [Google Scholar] [CrossRef]

(https://www.sciencedirect.com/science/article/pii/B9780323358682000700) [(accessed on 17 February 2025)].

(https://www.sciencedirect.com/science/article/pii/B9781416022992100350) [(accessed on 17 February 2025)].

(https://www.sciencedirect.com/science/article/pii/S0367253022000445) [(accessed on 17 February 2025)].

(https://www.sciencedirect.com/science/article/pii/S0378874123009327) [(accessed on 17 February 2025)].

(https://www.sciencedirect.com/science/article/pii/S0926669013005876) 2009, Pages 333-334, ISBN 9781416022992, [(accessed on 17 February 2025)].

https://doi.org/10.1016/B978-141602299-2.10035-0. 2014, Pages 233-244, ISSN 0926-6690, [(accessed on 17 February 2025)].

https://doi.org/10.1016/j.indcrop.2013.10.035. [(accessed on 17 February 2025)].

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

METHODS OF COMBATING AND CONTROL REGARDING FALSE INDIGO-BUSH IN CLOSE CORRELATION WITH INVASIVE CAPACITY IN THE FORESTRY PLANT COMMUNITIES IN THE JIU-DANUBE CONFLUENCE ZONE

Silvestru Ilie NUȚĂ, Mariana NICULESCU, Călin SĂLCEANU, Paula IANCU, Marin SOARE

University of Craiova, Faculty of Agronomy, 19 Libertăţii Street, Craiova, Romania

Corresponding author email: mniculescum@yahoo.com

Abstract

The false indigo-bush – Amorpha fruticosa L. is an invasive shrub, native to south-western North America. It was acclimatized in Europe as an ornamental shrub, its invasive-aggressive nature being noted over time. Areas of this species have greatly expanded in recent years across the country. The current eco-pedo-climatic conditions as well as the geographical position allow the installation of invasive species within these meadow phytocoenoses, some of them having a very high abundance-dominance. Among the species with high abundance-dominance, we studied the species Amorpha fruticosa which has an increase aggressive character both in the Jiu meadow, but especially in the Danube meadow. Although both 2022 and especially 2023 were dry years, the false indigo-bush had a massive development, most populations having a high number of individuals/m^2 within phytocoenoses. This species influences the successional dynamics and the floristic composition of the forest plant communities, occupying increasingly more and more space. Each tested method (mechanical and chemical) has its advantages and challenges and often a combination of strategies is necessary for sustainable management of the species.

Key words*: Amorpha fruticosa, combating methods, forest habitat, invasive species, Jiu-Danube Confluence.*

INTRODUCTION

Amorpha fruticosa L. is also known as shrubby amorpha or indigo-bushand is an invasive species that can have significant negative effects on biological diversity. This plant, originally from North America, was introduced to many regions, including Europe, where it quickly adapted and began to spread. Horticultural strategies emphasize promoting plants with a visually appealing appearance, but these kinds of plants often possess characteristics that predispose them to becoming invasive (Raicu et al., 2024). In Romania it was introduced for ornamental uses and to stabilize river banks. The plant spreads rapidly through flood waters, becoming a major problem for local pastures and hayfields. *Amorpha fruticosa* occupies large territories from protected areas of Mureș Floodplain Natural Park (Suciuet al., 2018)and other un-vegetated or sparsely vegetated shores, water-fringing reed beds and tall helophytes (Anastasiu et al., 2007). The species has incidence in western Romania, being spread along all the waterways (Mureş River, Danube River, Crişul Alb, Crişul Negru, Timiş, Bega, Bârzava) and their affluents, along the terrestrial transport ways, but it started to be found in grasslands too (Sărățeanu, 2010).

Negative effects on biodiversity relate to competition with native species, with *Amorpha fruticosa* tending to compete with native plants for resources such as light, water and nutrients. This can lead to a decrease in the number of native species, thus affecting the diversity of the ecosystem (Grabić et al., 2022). Another effect refers to the modification of the habitat, this invasive species can alter the structure of the habitats by forming monodominance where the false indigo bush becomes dominant, thus limiting the space and resources for other species. In the long term, *Amorpha fruticosa* invasion can destabilize local ecosystems, affecting essential ecological processes such as pollination and seed dispersal.

To control this invasive species, projects have been initiated in various areas of Romania, but the works are complex and expensive, requiring significant financial resources.

The purpose of this research was to evaluate the control methods efficiency generated by the expansion of the invasive species *Amorpha fruticosa* in the area at the confluence of the Jiu River with the Danube.

MATERIALS AND METHODS

The study was made in 2022-2023 in the territory at the confluence of Jiu River and Danube (Figure 1). The Jiu-Danube confluence (43°59′38″N - 23°53′48″E) is a protected area, located in southern Romania, on the administrative territory of Dolj county. The area was declared a Special Avifaunistic Protection Area by Government Decision no. 1284 of October 24, 2007 (regarding the declaration of areas of special avifaunistic protection, as an integral part of the European ecological network: Natura 2000 in Romania) (Hotărâre nr. 1284 din 24 octombrie 2007) and starting from February 2013 the site is protected as a wetland of international importance by the Ramsar Convention (Ramsar.org). The confluence of the Jiu with the Danube is a landscape of flowing waters, ponds and canals, bordered by meadow forests, with areas of pasture and arable land.



Figure 1. Study area-Jiu-Danube Confluence (source: https://www.google.ro/maps)

There are also forests with centuries-old examples of oaks, ash trees, elms, etc.
The analysis of the climatic elements that characterize the territory of the site is specific to the geographical space in the southwest of the country. The air temperature increases slightly from north to south: -2.5^{0}C in Târgu Jiu, respectively -1.8^{0}C in Calafat (in January) and 22.8-23.2^{0}C in July, but also successive increasing with temperature increases above average usual.

Atmospheric precipitation falls in liquid form during most of the year. In winter, there are on average 20 days with snow in the southern part of the site and about 30 days in the north. The multiannual averages of atmospheric precipitation are recorded around 540-550 mm. The great non-periodic variability of precipitation and the frequent advections of warm air cause the occurrence of relatively frequent dryness and drought phenomena in the plains (www.coridoruljiului.ro/PM_ROSCI0045.pdf). The identification of taxa was based on Romanian Flora (Săvulescu T. (Ed), 1952-1976) and Flora Europaea (Tutin et al., 1978). In order to establish the type of plant community and the level of invasiveness of the *Amorpha fruticosa* species within each plant community, sample markets were established, for each plant community 10 phytosociological surveys were carried out. In order to identify and establish the degree of invasiveness, specialized works developed by Pyšek et al. (2004); Pyšek et al., (2005); Pyšek et al. (2012) and Sîrbu & Oprea (2011), as well as the database The European Network on Invasive Alien Species (NOBANIS) (https://www.nobanis.org) were consulted. For the phytocoenotic research in the study area was used the method of phyto-sociologic research of the Central European School elaborated by Braun-Blanquet (1932). The plant communities were identified and distinguished according to the characteristic, edifying, dominant and differential species. For the identification of the plant communities and the coenotaxonomic classification, synthesis works of Coldea (1991), Sanda et al. (1997, 2001), as well as Oberdorfer (1992), Mucina et al. (1993, 2016), Rodwell (2002) were used. In the studied territory, 10 relevés were made within the phytocoenoses edified by Salix alba and *Salix fragilis*. A total of 30 sample plots of 400 m^2 each were considered in our studies, for the 4 plant communities in whose floristic composition *Amorpha fruticosa* populations were analyzed.

Control methods for the species *Amorpha fruticosa*

To restore forest phytocenoses, both from the forestry, bio-conservative and economic point of view, two methods were used: mechanical and chemical control and the economic calculation

regarding the costs of control.The mechanical and chemical control of the invasive species Amorphafruticosa was applied to 50 individuals, within the plant community that is an integral part of the 92A0 habitat in one location, within the administrative radius of the Ostroveni commune.

The mechanical method

Once *Amorpha fruticosa* has established itself in an area, it can form dense colonies, shading out other plants and competing with native species for resources. This ability to rapidly invade new territories complicates mechanical control efforts because constant intervention is needed to prevent spread. This method of mechanical control was achieved by cutting the branches with a chainsaw at a height of 10 cm above the ground, but also by pulling out the shoots and stems of the invasive species *Amorpha fruticosa*.

The chemical method

Chemical control can be effective, but its use must be carefully managed to minimize impact on native species and the environment. It is essential to use herbicides that are selective and do not affect the local flora.The chemical method was also applied in the test surfaces in order to highlight which of the methods is more effective. The chemical method was carried out by injecting the stem of the *Amorpha fruticosa* shrub with a systemic herbicide based on glyphosate, at a height of 10 cm from the ground. The following equipment was used for trunk injection:

- drill with a diameter of 0.6 cm and a length of 10 cm, to make holes in the stem of the shrub (Figure2);



Figure 2. Preparation of *Amorpha fruticosa* stems for herbicide application (photo: Călin Sălceanu)

- a syringe used to apply the herbicide directly into the hole in the stem.

The stem was perforated, to a depth of 4-6 cm, then a quantity of approx. 40 ml of herbicide was inserted directly, avoiding the leakage of the herbicide, in order to prevent contamination of the surrounding environment (Figures 3, 4). The diameter of the stems injected with herbicide did not exceed 3-5 cm, in the case of mature specimens.

The most effective absorption of the herbicide is in autumn, when nutrients from the leaves are transported to the root. 2 l of herbicide were used to inject 50 *Amorpha fruticosa* shrubs, of which 40 were completely dried.



Figure 3. Application of the chemical method for controlling the *Amorpha fruticosa* species in the Jiu-Danube confluent area (photo: Călin Sălceanu)



Figure 4. Herbicide injection of the stem of *Amorpha fruticosa* (photo: Călin Sălceanu)

RESULTS AND DISCUSSIONS

Amorpha fruticosa already covers significant areas (inside and outside protected habitats) in

Romania and the species has the tendency to naturally continuous spreading into new territories. To similar conclusion reached Ciuvăț et al. (2016) and they recommended the opportunity to sustainably control its spread by valuing its biopontential medicinal, food and industrial.

As results of the studies carried out in the Danube meadow, in the area of the confluence of the Jiu with the Danube, *Amorpha fruticosa* invades forest habitats over very large areas. This excessive development of the species causes a very large disturbance of the structure and floristic physiognomy of the poplar and willow plant communities in this area.

Amorpha fruticosa settles in the form of continuous strips along the Danube, with widths between 2 and 30 m, sometimes difficult to penetrate from one side to the other. The extremely developed populations of this species, especially in poplar groves, have a negative impact on the phytocoenoses, acting from the level of the grassy layer to that of the trees, resulting in a decrease in the value of biodiversity and the productivity of these forests. The decrease in the productivity of the poplar groves is automatically reflected in the decrease in the economic efficiency of the forestry in the area.

Phytosociological studies

From a phytosociological point of view, the studies indicate high dominance-abundance of the *Amorpha fruticosa* species in the following forest plant communities: *Salici-Populetum* Meijer-Drees 1936; *Salicetum albae* Issler 1924 (Syn. *Salicetum albae-fragilis* R. Tüxen 1937) and *Salicetum fragilis* Passarge 1957 (Figure 5). The invasive species *Amorpha fruticosa* has a strong negative impact on these types of forests both structurally and functionally, but also economically.

From conservative point of view, the identified plant communities edify Natura 2000 habitats of maximum importance: 91E0* - Alluvial forests with *Alnus glutinosa* and *Fraxinus excelsior* (*Alno-Padion, Alnion incanae, Salicion albae*) (according to the Palearctic Classification it corresponds to 44.3 - Middle European stream ash-alder woods, 44.2 - Boreo-alpine riparian galleries, 44.13 - Middle European white willow forests habitat types) and 92A0 *Salix alba* and *Populus alba* galleries (according to the Palearctic Classification it corresponds to: 44.141, 44.162 and 44.6 forests habitat types).

White poplar and white willow forests included in the plant community - *Salici-Populetum* Meijer-Drees 1936 occupies very large areas (hundreds of km) in the studied area, having a particular importance from an eco-pedological, bio-conservative, but also economic point of view for the entire area of the confluence of the Jiu River with the Danube, as for all hydrographic basins.

These types of white poplar forests, pure or mixed with white willow, generally develop on more developed alluvial soils. Along with the edifying species *Populus alba* and *Salix alba* are also found in the tree layers: *Fraxinus angustifolia, F. pallisiae, Ulmus laevis, U. minor*. A number of species have a high constancy in the structure of phytocenoses: *Vitis vinifera* subsp. *sylvestris, Cornus sanguinea, Humulus lupulus, Galium rubioides, G. palustre, A. tenuifolius, Urtica dioica, Calystegia sepium, Solanum dulcamara, Rubus caesius*. Often, the invasive species *Amorpha fruticosa* has a very high dominant abundance, and is accompanied by other invasive species: *Ailanthus altissima, Erigeron annuus, Oenothera glazioviana, Sicyos angulatus, Ecballium elaterium, Symphyotrichum lanceolatum, Oxalis stricta, Bidens frondosa*. In most of the analyzed phytocoenoses, the invasive species *Amorpha fruticosa* occupies large areas, has an excessive development, suffocating the species in the grassy and shrub state, white poplar and white willow saplings, especially preventing the natural regeneration of ancestral types of forest habitats.

Amorpha fruticosa presents a high degree of interpenetration, often forming an impenetrable network, and the root system is extremely developed, reaching great depths and extending over large surfaces in the horizontal plane. In order for the state of conservation, the structure and functions of this type of forest to have appropriate values, controlling the species is extremely necessary in the Jiu and Danube Valleys.

Another plant community identified and analyzed in the Jiu-Danube confluence area is *Salicetum albae* Issler 1924 (Syn. *Salicetum albae-fragilis* R. Tüxen 1937).



Figure 5. *Amorpha fruticosa* in the plant communities of the studied area (photo: M. Niculescu)

In this area, the phytocoenoses of this plant community have been invaded by several invasive species, but the supremacy is held by *Amorpha fruticosa* and in some phytocoenoses it is in competition with another invasive species *Sicyos angulatus* that also produces structural, functional, bioconservative and economic disturbances in these types of forests.

The edifying species are: *Salix alba* and *Salix fragilis.* In the floristic composition of the phytocoenoses of this plant community, they are also found scattered: *Salix purpurea, Populus canadensis, P. nigra* and *Fraxinus angustifolia.* The grassy layer largely represented by: *Mentha longifolia*, *Agrostis stolonifera*, *Glechoma hederacea*, *Ranunculus repens*, *Urtica dioica*, *Equisetum maximum, Aegopodium podagraria, Stachys palustris, Humulus lupulus, Polygonum hidropiper, Ranunculus ficaria, Stellaria nemorum, Agrostis stolonifera, Eclipta prostrate, Bidens tripartita, Vitis vinifera* ssp. *sylvestris, etc*. is often destroyed or taken over by populations of false indigo-bush.

And within these phytocoenoses, in the floristic structure, in addition to the species characteristic of the plant community, the following invasive species were identified along with *Amorpha fruticosa*: *Ailanthus altissima, Oenothera glazioviana, Bidens frondosus*, *Erigeron annuus, Cyperus difformis*, *Conyza canadensis, Morus alba, Echinocystis lobata, Robinia psudoaccacia, Abutilon theophrasti, Sicyos alngulatus, Reynoutria × bohemica*, *Galinso gaquadriradiata, Symphyotrichum lanceolatum.*
In the researched area, but on smaller areas, the plant community is also found *Salicetum albae-fragilis* Issler 1926 em. Soó 1957.
The phytocoenoses of this association are found in the form of narrow strips, on one side and the other of the Jiu River, close to its confluence with the Danube.
The shrub layer also consists of species like: *Sambucus nigra*, *Corylus avellana*, *Clematis vitalba*, *Hedera helix*, *Viburnum opulus*, *Salix triandra*, *Euonymus europaeus*, *Ligustrum vulgare***,** *Hedera helix*, *Cornus sanguinea, Clematis vitalba.*
In the grassy layer, the following species are found with high constancy: *Aegopodium podagraria*, *Carex remota, C. sylvatica, Equisetum arvense, Lycopus europaeus, Rumex sanguineus, Urtica dioica, Polygonum hidropiper, Petasites albus, Ranunculus ficaria, Stellaria nemorum, Agrostis stolonifera, Bidens tripartita, Brachypodium sylvaticum, Impatiens noli-tangere, Cardamine impatiens, Lysimachia nummularia, Humulus lupulus, Glechoma hederacea,* etc.
In the analyzed phytocoenoses, *Amorpha fruticosa* has a fairly high abundance-dominance, but lower than a number of other invasive species (allochthonous): *Sicyos angulatus, Ailanthus altissima, Oxalis stricta, Phytolacca americana, Ambrosia artemisiilfolia, Erigeron annuus, Gleditsia triacanthos*, *Echinocystis lobata, Falopia dumetorum.*
Following the studies, it was found that these phytocenoses also have a high degree of anthropization.
The three communities of woody plants analyzed have an inadequate state of conservation, the greatest anthropic pressure being exerted by the exuberant development of the *Amorpha fruticosa* species, which modified the floristic structure of the phytocoenoses with a direct impact on the entire natural capital in the studied area and not only that, but also on the vitality and state of health of the wood species from the composition of the forest plots in close correlation with their economic value.

The economic efficiency regarding the control of invasive species *Amorpha fruticosa* within the plant communities in the Jiu-Danube Confluence area

The results of this study show that the species analyzed can be controlled, with all affected individuals dying. Mechanical control alone is not very effective in the long term. Chemical control may be more effective, but requires repeated applications and compliance with rest periods for environmental protection.

Efficiency (%) = no. of shrubs removed/no. of treated shrubs x 100.
40:50 x 100 = 80%.

Costs:
The cost of one liter of Roundup herbicide is 80 Ron x 2 liters = 160 Ron.

The mechanical costs consisted of sawing with a chainsaw, where 2 liters of gasoline were consumed, and the equipment needed to collect the logs (tractor, trailer), to transport the wood and the effort of the staff responsible for transporting the wood resulting from cutting the logs from the height of 10 cm above the ground, generated much higher costs than the chemical control of trees *Amorpha fruticosa*.
Estimation of the costs for controlling the *Amorpha fruticosa* species, on an area of 50 shrubs:
- **Chemical method** – the rate 25 Ron/hour/worker x 8 hours = 200 Ron, plus 2liters of herbicide 80 Ron x 2 liters = 160 Ron, in total 360 Ron to control an area of 500 m^2 of *Amorpha fruticosa*;
- **Mechanical method** – rate 25 Ron/hour/worker x 8 hours = 200 Ron x 2 workers = 400 Ron, total labor cost (400 Ron) plus equipment (300 Ron) = 700 Ron.

Estimated total for mechanical control = 700 Ron;

Estimated total for chemical control = 400 Ron.

As a result of the calculations, mechanical methods are more expensive and require considerable human effort, so they are not always practical for large areas, while for chemical methods, although cheaper,

Amorpha fruticosa is a significant threat to local biodiversity and control measures are needed to prevent its spread and protect native species. The woody plant communities in most of the river basins in Romania present the invasive species in the floristic structure of the phytocoenoses of this specie. Regarding these plant communities, studies were carried out on the state of conservation, anthropogenic impact, threats, pressure on these priority habitats. These studies can be considered the basis of observations and future studies regarding the evolution of habitats, the syndamics of phytocoenoses, floristic composition, physiognomy of phytocoenoses, the range and degree of expansion of priority habitats.

This species has a high constancy within these phytocoenoses, and the dominant abundance varies depending on the eco-pedo-climatic conditions, but most of the time it has a percentage value of over 35%. As for the phytocoenoses analyzed in the Jiu-Danube confluence area, they are characterized by a very high degree of anthropization, the excessive development of the invasive species *Amorpha fruticosa* being one of the disruptive factors that act both from a silvo-biological, silvo-technical, conservative point of view and economic. Thus, it is very important to define and apply methods to control the species to restore these plant communities, especially in the meadow forests of the large hydrographic basins and especially in the Danube basin.

CONCLUSIONS

Amorpha fruticosa reproduces quickly and has a high capacity to adapt to various environmental conditions. This means that even after cutting or uprooting, the plant can quickly regenerate from the roots or remaining shoots, making mechanical methods insufficient to completely eradicate the species. Mechanical control often requires significant resources, both in terms of manpower and the equipment required. Also, plucking efforts must be performed repeatedly, which can become expensive and ineffective in the long run.

Chemical control is considered one of the most effective methods for managing *Amorpha fruticosa*. This is necessary because mechanical methods such as pulling or cutting are not effective enough to eradicate the plant once it has established itself in an area. The use of specific herbicides can help reduce the population of this invasive species and prevent its spread to other habitats.

Chemical control of *Amorpha fruticosa* is a crucial component in managing this invasive plant, but must be done responsibly to protect local ecosystems.

Due to the invasive character of *Amorpha fruticosa* and the difficulties encountered in its control by mechanical methods, it is recommended to use integrated strategies that include chemical control or other more effective methods to manage this invasive species.

REFERENCES

Anastasiu, P., Negrean, G., Bașnou, C., Sârbu, C., Oprea, A. (2007). A preliminary study on the neophytess of wetlands in Romania, in Rabitsch, W., F. Essl & F. Klingenstein (Eds.): Biological Invasions from Ecology to Conservation. *Neobiota*, 7: 181-192.

Braun-Blanquet J. (1932). Plant sociology. In *The Study of Plant Communities* (Transl. G. D. Fullerand H. S. Conrad); McGraw-Hill: New York, NY, USA. p. 539.

Ciuvăț, A.L., Vasile, D., Dinu, C., Apostol, E., Apostol, B., Petriţan, A.M. (2016). Valorisation possibilities of invasive indigobush (*Amorphafruticosa* L.) in Romania. *Revista de Silvicultură şi Cinegetică*, Nr. 39, p. 96-99.

Coldea, G.(1991). Prodrome des associations végétales des Carpates du Sud-Est (Carpates Roumaines). Documents Phytosociologiques, N.S., 13, Camerino, 317-539.

Grabić, J., Ljevnaić-Mašić, B., Zhan, A., Benka, P., Heilmeier, H. (2022). A review on invasive false indigo bush (*Amorpha fruticosa* L.): Nuisance plant with multiple benefits. *Ecol Evol*., Sep 14; 12(9):e9290. doi: 10.1002/ece3.9290. PMID: 36177113; PMCID: PMC9475134.

Mucina, L., Grabherr, G., Ellmauer, T. &Wallnöfer, B. (eds.) (1993). Die Pflanzen gesell schaften Österreichs. Band I-III. *G.* Fischer, Jena.

Mucina, L., Bültmann, H., Dierßen, K., Theurillat, J.P., Raus, T., Tichý, L. (2016). Vegetation of Europe: hierarchical floristic classification system of vascular plant, bryophyte, lichen, and algal communities. In: *Applied Vegetation Science*, 19 (Suppl. 1), 3–264, Doi: 10.1111/avsc.12257.

Oberdorfer, E. (1992). SüddeutschePflanzen – gesellschaften, Teil IV: Wälder und Gebüsche 2, Stark berabeileteAuflageTexband, Gustav Fischer Verlang Jena, New York.

Pyšek, P., Richardson, D.M., Rejmánek, M., Webster, G., Williamson, M., Kirschner, J. (2004b). Alien plants in checklists and floras: towards better communication between taxonomists and ecologists. *Taxon*, 53:131–143.

Pyšek, P., Jarošík, V., Chytrý, M., Kropáč, Z., Tichý, L., Wild, J. (2005). Alien plants in temperate weed communities: prehistoric and recent invaders occupy different habitats. *Ecology*, 86:772–785.

Pyšek, P., Jarošík, V., Hulme, P.E., Pergl, J., Hejda, M., Schaffner, U., Vilŕ, M. (2012). A global assessment of invasive plant impacts on resident species, communities and ecosystems: the interaction of impact measures, invading species' traits and environment. *Global Ecology and Biogeography*, 18: 1725–1737.

Raicu, M., Camen-Comănescu P., Urziceanu, M., Anastasiu, P., Toma, F. (2024). The role of ornamental horticulture in plantinvasion: a case study in Romania. *Scientific Papers. Series B, Horticulture*. Vol. LXVIII, No. 2, p. 717-726.

Rodwell, J.S., Schaminée, J.H.J., Mucina, L.,Pignatti, S., Dring, Moss J.D. (2002). The diversity of European vegetation – An overview of phyto-sociological alliances and their relationships to EUNIS habitats. National Reference Centre for Agriculture, Nature and Fisheries [report no. EC-LNV 2002(054)], Wageningen.

Sîrbu, C., Oprea, A. (2011). Plante adventive in flora României, Ed. Ion Ionescu de la Brad, Iasi, pp. 733.

Sanda V., Popescu A., Barabaș N.(1997). Cenotaxonomia și caracterizarea grupărilorvegetale din România. *St. Com., Muz. Șt. Nat. Ba*cău, 14: 5-366.

Sanda V., Popescu, A. & Stancu, D.I. (2001*). Structura cenotica si caracterizarea ecologica a fitocenozelor din România*. Pitesti: Edit. Cophis.

Sărățeanu, V. (2010). Assessing the influence of *Amorpha fruticosa* L. invasive shrub species on some grassland vegetation types from Western Romania. *Research Journal of Agricultural Science*, 42(1), p. 536-540.

Săvulescu T. (Ed)(1952-1976). *Flora R.P. Române* - R.S. România, 1-XIII. București. Edit. Acad. R.P. Române (R.S. România), București.

Suciu, C., Pancuru, E.B., Marin, D., Petronam, C., Petronam, I. (2018). The impact of alien species of plants on Mures floodplain Natural Park fauna. *Lucrări Științifice Management Agricol*, Seria 1, Vol. 20, No. 3, p. 162-167.

Tutin T.G., Heywood V.H., Burges N.A., Moore D.M., Valentine D.H., Walters S.M., Webb D.A.(1978). *Flora Europaea*, Vol. 2, pàg. 299.

https://apmbv.anpm.ro/-/specii-invazive (accesed04.02.2025).

https://www.nobanis.org - The European Network on Invasive Alien Species (NOBANIS) (accessed 05 February 2025).

www.coridoruljiului.ro/PM_ROSCI0045.pdf.

Hotărâre nr. 1284 din 24 octombrie 2007, publicate în Monitorul Oficial al României, nr. 739 din 31 octombrie 2007.

Ramsar.org - The Ramsar Convention on Wetlands - Romania names four new Ramsar Sites.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

MODELLING THE DISTRIBUTION OF THE RARE, ENDEMIC, TERTIARY RELICT AND VULNERABLE SPECIES *SYRINGA JOSIKAEA* JACQ. FROM APUSENI MOUNTAINS (WESTERN ROMANIAN CARPATHIANS)

Marilena ONETE[1], Florian Paul BODESCU[2], Roxana NICOARĂ[1], Minodora MANU[1], Simona MIHAILESCU[1]

[1]Institute of Biology Bucharest of Romanian Academy, 296 Splaiul Independenței, District 6, Bucharest, Romania
[2]Multidimension SRL, 7 Ciprian Porumbescu Street, Săveni, Ialomița County, Romania

Corresponding author email: florianbodescu@gmail.com

Abstract

The spontaneous species Syringa josikaea Jacq. (Transylvanian lilac, wind wood) is a very important species in Romanian flora (rare, endemic, tertiary relict, and vulnerable), being under Romanian and European special protection. Small populations with a low number of individuals are distributed only in western Romanian Carpathians (Apuseni Mountains) and Ukrainian Carpathians (Latorica River watershed). We developed, in Maxent, a distribution model based on the following parameters: ecological (climatic, geomorphologic, soil distribution), biological (interactions among species, characteristic plant species communities), anthropic (land use, biodiversity conservation), and cultural (local and traditional practices). The development of such a model leads to: the assessment of the impact of climate, and to analyze projections that may affect the distribution of the species in the future, the conservation of the characteristic habitats by developing management strategies thus ensuring the sustainability of the species. This model is also a direct support for the EU-specific reporting of Romania in the context of Article 17 of the Habitats Directive. Due to its beauty, the Transylvanian lilac is cultivated in the gardens and urban parks of the world.

Key words*: Syringa josikaea, distribution, modelling, Maxent, Western Romanian Carpathians.*

INTRODUCTION

When scientists recorded the influence of the environmental variables on the development and distribution of plant species (Crawley, 1997; Hageer et al., 2017), they used qualitative estimations but also accurate numerical techniques (Rosenzweig, 1995; Rockwood, 2006) and developed models that have strong predictive capabilities for describing species distribution in their natural range (Guisan & Thuiller, 2005; Reese et al., 2005; Elith & Leathwick, 2009).

Following species inventory from an area and recording the spatial characteristics or/and environmental factors from that area, for estimating their complex relationship, it has been developed species distribution models (SDMs) (Elith et al., 2011; Wisz et al., 2013).

Species distribution models for plants and animals (called also bioclimatic or environmental niche modelling) progressed hugely for usage in: forecasting anthropogenic effects on patterns of biodiversity at different spatial scales (Guisan & Thuiller, 2005; Reese et al., 2005), providing estimates of the spatial distributions of rare species with limited occurrence for their conservation management (Hernandez et al., 2006; Sharma et al., 2018) or with very small populations (Liu et al., 2024) estimating species responses to climate change (Austin, 2007; Hosseini et al., 2024), estimating the potential for species populations to occur in areas not previously surveyed (Hernandez et al., 2008; Rhoden et al., 2017; Ma & Sun, 2018; Bao et al., 2022). In the recent years many methods are available in ecology, conservation biology, biogeography (Franklin, 2013), being distinctive throughout the data used (Elith et al., 2011). Some database might contain accurate data but other data are limited in presence-absent and occurrence of the species (Merow et al., 2013). But not all the time a good data set is

available for developing a good predictive model for most of the taxa (Wisz et al., 2008).

Following recording environmental factors together with the presence-only occurrence of a species, many ecological applications use Maxent (maximum entropy) model for predicting: the potential suitable habitat of species (historic, current and future distribution trends) (Dai et al., 2022), the suitable habitat for a species new populations (even those species having specialized habitat requirements and narrow geographical ranges) (Williams et al., 2009), and also for transferring it in another climatic conditions or geographic areas (Hernandez et al., 2008), the influence of climate change on the potential distribution of medicinal plant (Yang et al., 2013; Khanum et al, 2013; Yi et al., 2016; Tang et al., 2021), of invasive plant species (Qin et al., 2014), relict plants species (Rotllan-Puig & Traveset, 2016), extremely endangered plant species (Qin et al., 2017), colonisations and extinctions of plant species (Venne & Currie, 2021), global potential distribution of some plant species (Zhang et al., 2021), suitable habitat for rehabilitation of different type of forest at landscape-scale (Tesfamariam et al., 2022).

Maxent model can use small datasets and run complex models; for presence-only modelling methods are necessary only data about species distribution together with topographic, climatic, edaphic, biogeographic data as predictor variables (Phillips & Dudík, 2008). Maxent program implement the maximum-entropy method and is a machine-learning algorithm capable both to model the spatial distribution of species and predict their presence in a geographic area relying on presence-only occurrence data of the species in the locations without considering the locations of documented absences (Lissovsky & Dudov, 2021).

In the situation that scientists need more ecological information about species habitats (vegetation presence, net primary productivity, and photosynthetic efficiency, anthropogenic impact, etc.). The data can be excerpts from the multispectral satellite images and aerial high-resolution orthophotos, to provide Normalized Difference Vegetation Index (NDVI) NDVI (Dunea et al., 2014; Boc et al., 2020), Green Normalized Difference Vegetation Index (GNDVI) (Dunea et al., 2021), etc.

Genus Syringa L. (lilac) together with Jasminum and Forsythia are beautiful ornamental shrubs belonging to Oleaceae Family (including also Fraxinus, Ligustrum, Olea genera) (Ciocârlan, 2009). The Romanian Flora comprise two *Syringa* species: *vulgaris* L. and *josikaea* Jacq. f. ex. Rchb., both shrubs and very ornamental but only *S. josikaea* is rare, Carpathians endemic, tertiary relict in Romanian Flora (Boşcaiu & Onete, 2009; Sârbu et al., 2013).

As ornamental plant, some comparative studies have been developed on *S. josikaea* about quantitative anatomy of epidermal cells in smaller leaf side considered adaptive mechanism for reducing its asymmetry (Polonskiy & Polyakova, 2015). Individual of *S. josikaea* introduced in urban arctic climate develop polyvariance of the floral organogenesis (based on morpho-physiological analysis of the reproductive buds) (Vasilevskaya & Morozova, 2020) and high morphological variability and large proportion of sterile pollen due to pollution (Vasilevskaya & Morozova, 2024). *S. josikaea* is used as an ornamental shrub in different cities (Gizhitskaya & Lyakh, 2020). For expanding the genetic diversity of the species, have been developed studies on the hybridization of lilac genotypes with different origins (taxonomic and genetic); the crosses combinations ended up by good fruits sets in the presence of pollinators (Pavlenkova, 2024).

Based on chemical substances (iridoids) content mainly in *S. josikaea* but also *Fraxinus excelsior* and *S. vulgaris*, Damtoft et al. (1995) suggest that the taxonomy of the Oleaceae Family should be discussed, the Family should be considered member of Gentianales Order rather than Lamiales Order.

Apart of being very important for horticulture (USDA, 2025), Lendvay et al. (2013) developed genetic studies, mainly characterizing and optimizing microsatellite markers for using them on *S. josikaea.* The authors studied later (2015) the historical biogeography and intraspecific variation of the species using nuclear ribosomal DNA sequences, multiple DNA sequences and microsatellites. The scientists learned that during Early Pleistocene, *S. josikaea* disrupted from its Asian relatives or colonized from one glacial refugia, and have a very low sequence variation and complete identity of cpDNA among all samples (implying

a bottleneck), Also, in *S. josikaea* populations from Ukrainian and Romanian Carpathians indicate no geographic differentiation based on the presence on four different ribotypes and weak differentiation based on microsatellite variation.

MATERIALS AND METHODS

Syringa josikaea Jacq. f. ex Rchb. is an extant (resident) species in Romania and Ukraine, an endemic Carpathian (Witkowski et al., 2003; Ciocârlan, 2009; Sârbu et al., 2013; Macalik et al., 2013) (Figure 1), subendemic Romanian (Oltean et al., 1994) and relict (Boșcaiu & Onete, 2009; Dihoru & Negrean, 2009) species from Oleaceae Family, vulnerable (Witkowski et al., 2003) and rare (Ciocârlan, 2009; Sârbu et al., 2013). Boșcaiu et al. (1994) consider this species as critically endangered and in risk of extinction, Dihoru & Negrean (2009) consider it as low risk of danger (Least Concern - LC according to IUCN) and is listed as Endangered under IUCN criteria (IUCN, 2018) (Figure 1).



Figure 1. Distribution of *S. josikaea* in Europe (After IUCN, 2018) (left) and Romanian alpine bioregion Art17 (2012 report – right up and 2018 report – right down)

S. josikaea has 2186 as Natura 2000 code, is distributed in alpine biogeographic region (ALP) from Romania and is present in Annex II (Animal and plant species of community interest whose conservation requires the designation of special areas of conservation) of Habitat Directive (H.D., 1992).

Data collection started with desk investigation of reports, scientific articles and management plans, followed by field trips.

Romania must report the conservation status of the plants and habitats of European interest, thus, during two monitoring periods: 2011-2015 in the framework of P.O.S. project (2011) and 2019-2023 in the farmwork of P.O.I.M. (2023) project (Mihăilescu et al., 2024). The field assessment and monitoring of the conservation status of Romanian species of community interest started in 2011 and continued in 2019, during two reporting periods (six years each) toward the European Commission. Based on our data and knowledge, the Annex 1 from M.O. 3351 (2023) was developed and published.

For generating the distribution maps, we used the standard methods: grid ETRS89 of 10 x 10 Km plot and 1 x 1 km grid, projective ETRS LAEA 5210. The necessary data used for generating the maps have been collected during one year or one period for monitoring the species because ethe mapping the distribution of a species might necessitate a few years of field identification and assessment. (Figure1- right up and down).

The location of the individuals has been marked with GPS Garmin eTrex Summit. The data regarding biotic and abiotic factors influencing the distribution of the species have been collected from the field in filed data sheets and used for modelling with Maxent version 3.4.4 software.

The method relied on complete study or a solid statistical estimation, using good data regarding environmental variables like geomorphological (elevation, exposition, slope, plan and profile curvature), climatic (temperature and precipitation), biological (interactions among species, characteristic plant species communities), anthropic (land use, biodiversity conservation), and cultural (local and traditional practices) from the entire distribution range of the species for calibrating and evaluating the predictive performances.

We assessed the conservation status of *S. josikaea* in its distribution area according with the methods established in the methodological guides (Mihăilescu et al., 2015a; 2015b; M.O. 3351, 2023). From the field, data have been collected on field data forms comprising: location, population parameters, habitats characteristics, pressures and threats. Romanian reporting format toward European Commission has individuals/population density as reporting unit for the species belonging of the group "Vascular plant species from the water's edge or wet areas, places periodically flooded, water banks (more or less steep)" (M.O. 3351, 2023) in which *S. josikaea* belongs to.

The direct (presence/absence, population size, age/size classes) and indirect (niche availability, hydrology, water quality, vegetation structure, impact factors, negative indicators such as: shadow, competition, fragmentation, extreme drought, alien species) attributes are mandatory to be evaluated during the optimal flowering season (May-June). The monitoring plots have been set on transect along river banks where *S. josikaea* was recorded, according with the management needs, length and width of the vegetation distribution and habitat discontinuities. The frequency of the monitoring activities depends both on the distribution impact or reporting cycles for European Commission (6 years). If the direct and indirect attributes are not met in the field, it means that there are unfavourable conditions for existence. The population size was reported as an interval (minim and maxim) or a single value (measured or estimated) when it is known only one single value obtained throughout expert opinion.

The monitoring method used in the filed investigations for detecting the population size and the range of the species habitat (two very important populational parameters) have been realized accordingly with the growth strategies of the species. In areas where the individuals' growth was lax, counting the individual stems have been possible. Where the stems of the individuals' growth were dens (due to vigorous clonal reproduction), we measured the projection area of their crowns on the soil.

Monitoring protocols may differ in relation to the size of the population of each species, the integrating habitat and their biological and ecological characteristics. Direct and indirect attributes are mandatory. If these attributes do not meet, it means that there are unfavourable conditions of existence.

Field trips realized in 2011 (POS, 2011) on Valea Iadului (Stâna de Vale Locality, Bihor County) have been made for 5 x 5 m plots installation for monitoring *S. josikaea*. We installed 3 permanent monitoring plots on Valea Iadului.

Our paper focused on using the Maxent model to estimate the distribution areal for *S. josikaea* and taking into account the high confidence of estimates for respectively value equal with 1. For estimating the population size, we used the medium value of density for 0.1 ha 5 individuals multiplied with normalized of hotspot analysis index.

RESULTS AND DISCUSSIONS

The rare, endemic, tertiary relict and vulnerable species *S. josikaea* is distributed in eastern Carpathian Mountains, in 17 known localities (2 uncertain) from Latorica, Rika, Uzh and Stryi Rivers in the Ukrainian Carpathians and 7 known localities (6 uncertain) from Crişul Negru, Valea Iadului (Bihor County), Valea Sebeşului, Someşul Cald (Cluj County), Arieş (Alba County) Rivers in Romanian Carpathians (Apuseni Mountains) (Lendvay et al., 2013; 2015; Macalik et al., 2013). A proper conservation strategy might consist in maintaining connectivity among sites and understanding the species' reproductive strategy (clonal propagation in detriment of sexual reproduction). The population might consist in a few clones only thus might enhance the resilience of the population (Lendvay et al., 2015) or determine the rapid deterioration of the individuals due to low genetic variability (Crawley, 1997; Rockwood, 2006).

The distribution of the studied species on small areas is strongly related with river valleys: alongside river margins (on the shore) (Figure 2), adjacent areas (rocky shores, cliffs) (Figure 3) and in hilly areas in rivers floodplains.



Figure 2: *S. josikaea* individuals distributed on Valea Iadului river shore

The species is integrated in *Alno incanae-Syringetum josikaeae* (Borza 1965) Rațiu et al. 1984 Association (Romanian habitat R4413 South-East Carpathian thickets with *Syringa josikaea*) (Doniță et al., 2005).



Figure 3: *S. josikaea* individuals distributed on adjacent Valea Iadului river margins (cliffs)

This plant association and Romanian habitat are integrated in Natura 2000 habitat 40A0* Subcontinental peri-Pannonic scrub (Gafta & Mountford, 2008; Goriup, 2008). The habitat is distributed on narrow mountainous valleys, on humid districtsambosols with 5.8-7.5 soil reaction (pH), temperature 7.0-5.5^{0}C and 800-900 mm rainfall (Doniță et al., 2005). In Apuseni Mountains, the distribution localities are situated at 500-1100 m attitude, on different expositions according with the river direction.

In Bihor County, Stâna de Vale Locality, we installed in 2011, monitoring plots in habitats with 100 ha surface area in which the distribution of *S. josikaea* is on about 50 m^2. The locations of the plots are different, according to the distribution of *S. josikaea* in the region (Table 1).

The surrounding vegetation structure consists in trees layer in one plot (Valea Iadului 3 and 4, alongside river shore close to the forest), highest shrubs layer in two plots (Valea Iadului 1 and 2, vegetating on rocky shores, cliffs, at 3-4 m above the 108J road) and one richest species site (Leșu touristic locality, hilly areas in rivers floodplains).

The Natura 2000 protected areas in which the species might be distributed are: ROSCI0002 Apuseni, ROSCI0062 Defileul Crișului Repede - Pădurea Craiului, ROSCI0121 Muntele Vulcan, ROSCI0262 Valea Iadei (S.F., 2011). Following the field trips in these protected areas, the presence of *S. josikaea* was not confirmed in ROSCI0062 and ROSCI0121.

The Maxent model reveal the total potential distribution area of *S. josikaea* is about 177.31 ha with estimated population size of circa 90,464 individuals (Table 2).

Table 1. Surrounding vegetation structure and species richness in plots comprising *S. josikaea*

Sit's name	Valea Iadului 1	Valea Iadului 2	Valea Iadului 3	Valea Iadului 4	Leșu
Total vegetation coverage (%)	100	100	100	100	100
Vegetation layers					
- max. height of the trees layer (m)	-	-	7	-	-
- max. coverage of the trees layer (%)	-	-	30	-	-
- max. height of the shrubs layer (m)	3	4	2.7	3	3
- max. coverage of the shrubs layer (%)	50	70	50	50	50
- max. height of the herbaceous layer (cm)	100	30	100	100	100
- max. coverage of the herbaceous layer (%)	100	30	100	100	100
Species richness (number)	7	3	10	6	16
Alno incanae-Syringetum josikaeae Association					
Characteristic species					
Alnus incana			+		
Syringa josikaea	+	+	+	+	+
Other species					
Anemone ranunculoides					+
Campanula serrata					+
Clematis alpina	+				+
Fagus sylvatica			+		+
Geum rivale			+		
Luzula sp.	+				+
Melica uniflora				+	+
Myosotis sylvatica	+	+			+
Petasites albus		+	+		
Polygonum bistorta				+	
Polygonatum verticillatum				+	+
Rosa pendulina					+
Rubus idaeus			+		
Salix capraea					+
Salix sp.	+		+	+	+
Sambucus nigra			+		
Sorbus aucuparia	+				+
Spiraea sp.					+
Trolius europaea				+	
Urtica dioica			+		
Valeriana montana	+		+		+
Veratrum album					+

Table 2. Estimates surfaces for areal distribution for *S. josikaea* in alpine bioregion Romania

Category	Number	Minimum size (ha)	Maximum size (ha)	Surface (ha)	Estimated number of individuals
Cold spot 99% confidence	3,159	0.00633	0.3022	30.136	21,698
Cold spot 95% confidence	2,141	0.00635	0.1398	21.243	14,020
Cold spot 90% confidence	1,014	0.00635	0.4341	10.283	6,170
Not significant	3,197	0.00634	0.2569	36.338	19,623
Hot spot 90% confidence	75	0.00681	0.1087	1.1374	546

Hot spot 95% confidence	234	0.00681	0.3228	4.3586	1,831
Hot spot 99% confidence	3,788	0.00637	1.4581	73.821	26,576
Total	**13,608**	**0.00633**	**1.4581**	**177.31**	**90,464**

The development of such a model leads to: the assessment of the impact of climate, and to analyse projections that may affect the distribution of the species in the future, the conservation of the characteristic habitats by developing management strategies thus ensuring the sustainability of the species. This model is also a direct support for the EU-specific reporting of Romania in the context of Article 17 of the Habitats Directive (Figure 4).

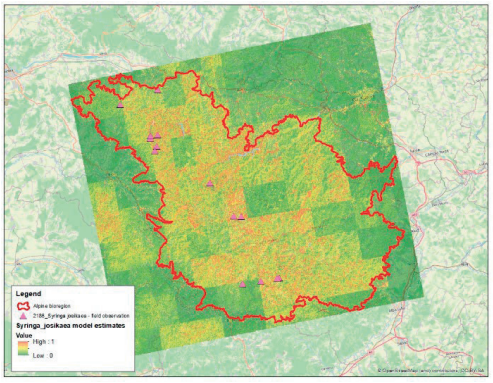


Figure 4. The Maxent model estimates for *S. josikaea* distribution

Conservation measure for central (Hotspot - red) and marginal (Coldspot - blue) spots relay on maintain the current range of the populations and/or the current areal of habitats for the species. On the interval confidence of both cold spot >90% (confidence blue spots) and Hot spot >90% (confidence red spots) (Figure 5), the dominant pressure and threats are clear-cutting (removal of all trees), the destruction of the species habitats (*i.e.* hydrotechnical engineering of mountainous water courses) and the collecting of the species from the wild and move it in the peoples' gardens.

S. josikaea is a threatened species of the Carpathians mostly growing as extremely small populations at distant sites, our analysis reveals the estimated areal distribution is in small surfaces (max. 1.4 ha) cover total 177 ha in 5,856 km^2.

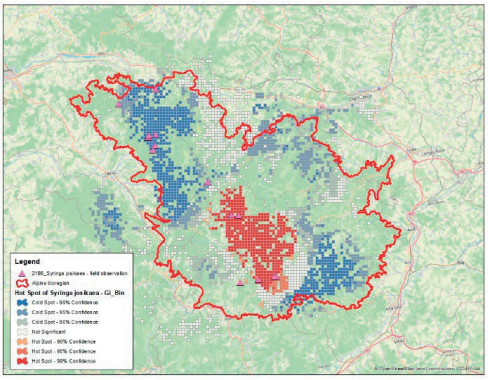


Figure 5. Hot spot analysis for *S. josikaea* distribution areal

In this respect we observe that the reported surface on 2,400 km^2 is overestimated base on chorological methods of reporting tool. The estimated population size near to 90,000 individuals need to be validated before next report to European Commission. The hot spot analysis of *S. josikaea* areal estimates shows the clustering of metapopulation for *S. josikaea* distribution plots, central areas (hot spot >90% confidence) and marginal areas (cold spot >90% confidence), to validate the specific appropriate conservation measures to achieve the conservation objectives especially for Natura 2000 sites.

In June, the monitoring of the population of *S. josikaea* from downstream the dam Beliș toward Valea Neagră confluence reported 300 individuals in 2020 and 2021-2022 distributed on about 16 ha, indicating that this population is relatively stable, with a favourable conservation status. It was identified a new location on Someșul Cald valley with 6 individuals. The main threats affecting the species distribution and populations density are: collecting individuals from the wild and the proliferation of exotic/invasive species. In ROSCI0002 Apuseni, *S. josikaea* has a favourable conservation status and due to low impact of the threats, the perspectives are good, determining the conservation of this species in the wild (APNA, 2022).

Our field trips in 2022 (P.O.I.M., 2023) at the beginning of June on Valea Iadului, upstream of Lești lake (Lești touristic locality), showed difficulties to find the species individuals in the wild but we found about 500 individuals

forming a green fence of a private property that developed till the edge of the river water course (Figure 6).



Figure 6. Private fence formed by *S. josikaea* individuals

In the *Habitats Directive* (92/43/EEC), species monitoring belonging to Annex II and Annex IV is required in both Article 11 and Article 17 preceding the reporting of the species conservation status at every six years toward the European Commission. Measures taken pursuant to the *Habitats Directive* shall be designed to maintain or restore, at favourable conservation status for species of wild flora of Community interest.

In the last 12 years, all Member States, including Romania, has reported two times to the Commission: in 2013 (for the evaluation period 2007-2012) and 2019 (for the evaluation period 2013-2018), based on every 6 years of the assessed conservation status for habitats of community interest. For the next report (deadline 31 July 2025), was approved in 2023 the reporting format on species types (DG Environment, 2023).

For the period 2013-2018 the population size measured in number of individuals was estimated between 10 and 240.

Under the Habitats Directive - Article 17 reporting process these assessments have been carried out in EU25 for the period 2001-2006, in EU 27 for the period 2007-2012 and in EU28 for the period 2013-2018 (EIONET, 2019).

During 2007-2019, the conservation status was ”unfavourable - inadequate” (U1), due to increasing trends in the intensity of different impacts upon the species habitat. In the period 2020-2024, after implemented the conservation measures included in the Management Plan of the Apuseni Natural Park (O.M., 2024), this overall assessment was changed as ”favourable” (FV) (Table 3).

Table 3. Overall assessment of the conservation status on each reporting period

Species	2013	2019	2025
	ALP	ALP	ALP
2186 *Syringa josikaea* Jacq.	U1	U1	FV

List of pressures and threats and conservation measures was updated in 2024 for the period 2019-2024 (EIONET, 2025). During 2007-2019, main conservation measures according with EU code: CB01 - Prevent conversion of (semi-) natural habitats into forests and of (semi-) natural forests into intensive forest plantation.

CONCLUSIONS

The spontaneous species *Syringa josikaea* Jacq. (Transylvanian lilac, Hungarian lilac, wind wood) is a very important species in Romanian flora (rare, endemic, tertiary relict and vulnerable), being under Romanian and European special protection. The species populations distributed in western Romanian Carpathians (Apuseni Mountains) are basically small (low number of the individuals), genetically homogenous, distributed at distant sites. The distribution model developed using Maxent highlighted the anthropogenic and climate impacts that can affect the population density and distribution. The analysis of the projection of the main threats and pressures that may affect the distribution of the species in the future, lead the actions regarding the conservation of the characteristic habitats by developing management strategies thus ensuring the sustainability of the species. The hot spot analysis of *S. josikaea* areal estimates help to validate the specific appropriate conservation measure to achieve the conservation objectives especially for Natura 2000 sites.

The populations are relatively stable, with a favourable conservation status and protected in areas under conservation regulation. Maintaining the favourable conservation status still depends on the continued application of conservation measures, both for the species and its habitat.

Due to its beauty, the Transylvanian lilac is cultivated in the gardens and urban parks of the world.

ACKNOWLEDGEMENTS

The data have been obtained in the framework of the Sectorial Operational Programme ENVIRONMENT 2007-2013 (POS no. 130537/10.01.2011) and the Large Infrastructure Operational Programme 2014-2020 (POIM no. 238/11.03.2019) projects financed by the Ministry of Investments and European Projects. The paper was written in the framework of projects RO1567-IBB01, RO1567-IBB03 and RO1567-IBB04 from Institute of Biology Bucharest of Romanian Academy financed by Romanian Academy.

REFERENCES

APNA (2022). RNP Romsilva-Administration of Natural Park Apuseni, Activity report for natural protected areas Natural Park Apuseni, ROSPA0081 Munții Apuseni-Vlădeasa, ROSCI0002 Apuseni, ROSCI0016 Buteasa and the 55 natural protected areas that make the subject of the contract no. 132/18.11.2014

Austin, M. (2007). Species distribution models and ecological theory: a critical assessment and some possible new approaches. *Ecological modelling*, *200*(1-2), 1-19.

Bao, R., Li, X., Zheng, J. (2022). Feature tuning improves Maxent predictions of the potential distribution of Pedicularis longiflora Rudolph and its variant. *PeerJ*, *10*, e13337.

Boc, V. I., Mihalache, B., Tudor, T. I., Zeca, L. (2020). Vegetation habitats mapping in Văcăreşti Natural Park. *Scientific Papers. Series B, Horticulture*, *LXIV*(1): 549-553

Borza, A. (1925). *The flora of peasant Romanian gardens: plants of adornment, medicine, charms and beliefs*. Institutul de Arte Grafice Ardealul.

Boşcaiu, N., Coldea, G., Horeanu, C. (1994). The red list of extinct, threatened, vulnerable and rare vascular plant species form Romanian Flora. *Ocrotirea Naturii şi a Mediului Înconjurător, 38*(1) 45-56.

Boşcaiu, N., Onete, M. (2009). A tertiary relict in Romanian Flora: *Syringa josikaea* Jacq., Argesis. *Studii şi Comunicări Seria Ştiinţele Naturii*, Piteşti, XVII: 43-46.

Ciocârlan, V. (2009). *Ilustrated flora of Romania. Pteridophyta et Spermatophyta*, Editura Ceres, Bucureşti. 1141 p.

Crawley, M.J. (1997). *Plant Ecology*, Blackwell Science, 717 p.

Dai, X., Wu, W., Ji, L., Tian, S., Yang, B., Guan, B., Wu, D. (2022). Maxent model-based prediction of potential distributions of *Parnassia wightiana* (Celastraceae) in China. *Biodiversity Data Journal* 10: e81073.

Damtoft, S., Franzyk, H., Jensen, S.R. (1995). Biosynthesis of iridoids in Syringa and Fraxinus: Secoiridoid precursors. *Phytochemistry*, *40*(3), 773-784.

DG Environment (2023). *Reporting under Article 17 of the Habitats Directive: Guidelines on concepts and definitions – Article 17 of Directive 92/43/EEC, Reporting period 2019-2024*. Brussels, https://cdr.eionet.europa.eu/help/habitats_art17

Dihoru, G., Negrean, G. (2009). *The Red Book of vascular plants from Romania*, Editura Academiei Române, Bucureşti, 630 p.

Doniţă, N., Paucă-Comănescu, M., Popescu, A., Mihăilescu, S., Biriş, I. A. (2005). *Habitatele din România*. Bucureşti. Editura Tehnică Silvică.

Dunea, D., Bretcan, P., Purcoi, L., Tanislav, D., Serban, G., Neagoe, A., Iordache, V., Iordache, Ş. (2021). Effects of riparian vegetation on evapotranspiration processes and water quality of small plain streams. *Ecohydrology & Hydrobiology, 21*(4), 629-640.

Dunea, D., Dincă, N., Iordache, Ş. (2014). Spatio-temporal characterization of natural grasslands from Bucegi Natural Park using remote sensing resources. *Scientific Papers - Series A, Agronomy*, 57, 180-185.

EIONET (2019). Article 17 web tool - the Article 17 web tool on biogeographical assessments of conservation status of species and habitats under Article 17 of the Habitats Directive, https://nature-art17.eionet.europa.eu/article17/species/report/?period=3&group=Vascular+plants&country=RO&region=

EIONET (2025). *Reference portal for reporting under Article 17 of the Habitats Directive*, https://cdr.eionet.europa.eu/help/habitats_art17 (accessed January 2025).

Elith, J., Leathwick, J. R. (2009). Species distribution models: ecological explanation and prediction across space and time. *Annual review of ecology, evolution, and systematics*, *40*(1), 677-697.

Elith, J., Phillips, S. J., Hastie, T., Dudík, M., Chee, Y. E., Yates, C. J. (2011). A statistical explanation of Maxent for ecologists. *Diversity and distributions*, *17*(1), 43-57.

Franklin, J. (2013). Species distribution models in conservation biogeography: developments and challenges. *Diversity and distributions*, *19*(10), 1217-1223.

Gafta, D., Mountford, O.J. (Coord.) (2008). *Manual of interpreting Natura 2000 from Romania*, Editura Risoprint.

Gizhitskaya, S.A., Lyakh, E.M. (2020). Potential for using ornamental bushes for greening the city of Novosibirsk: in case of the genus Syringa L. In *BIO Web of Conferences* (Vol. 24, p. 00024). EDP Sciences.

Goriup, P. (Coord.) (2008). Natura 2000 in Romania. Species fact sheets. EU Phare Project on Implementation of Natura 2000 Network in Romania EU Phare EuropeAid/12/12160/D/SV/RO.

Guisan, A., Thuiller, W. (2005). Predicting species distribution: offering more than simple habitat models. *Ecology letters*, 8(9), 993-1009.

H.D. (1992) Habitat Directive, Council Directive 92/43/EEC on the conservation of natural habitats and of wild fauna and flora, *Official Journal of the European Union*, 206(7), 50. https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:31992L0043

Hageer, Y., Esperón-Rodríguez, M., Baumgartner, J. B., Beaumont, L. J. (2017). Climate, soil or both? Which variables are better predictors of the distributions of Australian shrub species?. *PeerJ*, *5*, e3446.

Hernandez, P.A., Franke, I., Herzog, S.K., Pacheco, V., Paniagua, L., Quintana, H.L., Soto, A., Swenson, J.J., Tovar, C., Valqui ,T.H., J. Vargas, Young, B.E. (2008). Predicting species distributions in poorly-studied landscapes. *Biodiversity and conservation*, *17*, 1353-1366.

Hernandez, P.A., Graham, C.H., Master, L.L., Albert, D.L. (2006). The effect of sample size and species characteristics on performance of different species distribution modeling methods. *Ecography*, *29*(5), 773-785.

Höhn, M., Lendvay, B. (2018). *Syringa josikaea*. *The IUCN Red List of Threatened Species*, 2018: e.T162267A99428926. https://dx.doi.org/10.2305/IUCN.UK.2018-1.RLTS.T162267A99428926.en.

Hosseini, N., Ghorbanpour, M., Mostafavi, H. (2024). Habitat potential modelling and the effect of climate change on the current and future distribution of three Thymus species in Iran using Maxent. *Scientific Reports*, *14*(1), 3641.

IUCN (2018). *Syringa josikaea*. *The IUCN Red List of Threatened Species. Version 2024-2*, www.iucnredlist.org/species/162267/99428926.

Khanum, R., Mumtaz, A. S., Kumar, S. (2013). Predicting impacts of climate change on medicinal asclepiads of Pakistan using Maxent modeling. *Acta Oecologica*, *49*, 23-31.

Lendvay, B., Kadereit, J. W., Westberg, E., Cornejo, C., Pedryc, A., Höhn, M. (2015). Phylogeography of *Syringa josikaea* (Oleaceae): Early Pleistocene divergence from East Asian relatives and survival in small populations in the Carpathians. *Biological Journal of the Linnean Society*, *119*(3), 689-703.

Lendvay, B., Pedryc, A., Höhn, M. (2013). Characterization of Nuclear Microsatellite Markers for the Narrow Endemic *Syringa josikaea* Jacq. fil. ex Rchb. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, *41*(1), 301-305.

Lissovsky, A.A., Dudov, S.V. (2021). Species-distribution modeling: advantages and limitations of its application. 2. Maxent. *Biology Bulletin Reviews*, *11*(3), 265-275.

Liu, Y., Cai, L., Sun, W. (2024). Current conservation status and potential distribution under climate change of *Michelia lacei*, a Plant Species with Extremely Small Populations in Yunnan, China. *Oryx*, *58*(5), 631-640.

M.O. (2023). Ministry Order no. 3351 from 28 December 2023 for the approval of the "Guide on protocols and unitary methodologies for monitoring the conservation status of species of Community interest", Annex 1. Guide with protocol and methodology for monitoring the conservation status of plant species of community interest, https://legislatie.just.ro/public/Detalii Document/ 278140

Ma, B., Sun, J. (2018). Predicting the distribution of Stipa purpurea across the Tibetan Plateau via the Maxent model. *BMC ecology*, *18*, 1-12.

Macalik, K., Tamás, R., Kolcsár, L.P., Keresztes, L. (2013). Present status of the *Syringa josikaea* Jacq. ex Rchb., an endemic species which contributes to the diversity of the Flora of the Carpathians. *Studia Universitatis Babeş-Bolyai. Series Biologia*, *58*(2), 31-40.

Merow, C., Smith, M.J., & Silander Jr, J.A. (2013). A practical guide to Maxent for modeling species' distributions: what it does, and why inputs and settings matter. *Ecography*, *36*(10), 1058-1069.

Mihăilescu, S., Onete, M., Bodescu, F.-P., Gheorghe, I.F., Strat, D., Sahlean, T, Nicoară, G-R., Mihai, L.-S., Manu, M., Mountford, J.O., (2024). Approaches to the assessment of some habitats of community importance in Romania, *Scientific Papers. Series A. Agronomy*, LXVII (2): 529-539

Mihăilescu, S., Anastasiu, P., Popescu, A., Alexiu, A.F., Negrean, G.A., Bodescu, F., Manole (Aiftimie), A., Ion, R.G., Goia, I.G., Holobiuc, I., Vicol, I., Neblea, M.A., Dobrescu, C., Mogîldea, D.E., Sanda, V., Biță-Nicolae, C.D., Comănescu, P. (2015a). *Monitoring Guide for community interest plant species from Romania*, Editura Dobrogea.

Mihăilescu, S., Strat, D., Cristea, I., Honciuc, V. (2015b). *Synthetic report regarding conservation status of community interest plants and habitats from Romania*, Editura Dobrogea.

O.M. (2024). Official Monitor I Part no. 1071Bis, Annex at Order of Environment, Water, and Forests Ministry no. 2.238/2024 regarding the approval of integrative management Plan of Apuseni Natural Park and integrated protected areas, https://lege5.ro/MonitorOficial/mongq3danzygiyq/monitorul-oficial-partea-i-nr-1171-25-11-2024

Oltean, M., Negrean, G., Popescu, A., Roman, N., Dihoru, G., Sanda, V., Mihăilescu, S., (1994). *The red list of superior plants from Romania*, Institute of Biology, Romanian Academy.

P.O.I.M. (2023). Completing the level of knowledge of biodiversity by implementing the system for monitoring the conservation status of species and habitats of Community interest in Romania and reporting on the basis of Article 17 of Habitats Directive 92/43/EEC", funded by the Large Infrastructure Operational Programme, 2014-2020.

P.O.S. (2011). *Monitoring the conservation status of species and habitats from Romania under article 17 of Habitat Directive*, Contract no. 130537/10.01.2011, https://www.ibiol.ro/posmediu/index.htm.

Pavlenkova, G.A. (2024) Fruit setting as a result of hybridization of Syringa representatives in the conditions of the Orel region, Agrarian Scientific Journal. 12 :57–65.

Phillips, S.J., Dudík, M. (2008). Modeling of species distributions with Maxent: new extensions and a comprehensive evaluation. *Ecography*, *31*(2), 161-175.

Polonskiy, V.I., Polyakova, I.S. (2015). Increased size of epidermal cells in *Syringa josikaea* Jacq. smaller leaf side as an adaptive mechanism for reducing its asymmetry. *Russian Journal of Developmental Biology*, *46*, 356-361.

Qin, A., Liu, B., Guo, Q., Bussmann, R.W., Ma, F., Jian, Z., Xu, G., Pei, S. (2017). Maxent modeling for predicting impacts of climate change on the potential distribution of *Thuja sutchuenensis* Franch., an extremely endangered conifer from southwestern China. *Global Ecology and Conservation*, *10*, 139-146.

Qin, Z., DiTommaso, A., Wu, R.S., Huang, H.Y. (2014). Potential distribution of two *Ambrosia* species in C hina under projected climate change. *Weed Research*, *54*(5), 520-531.

Reese, G.C., Wilson, K.R., Hoeting, J.A., Flather, C.H. (2005). Factors affecting species distribution predictions: a simulation modeling experiment. *Ecological Applications*, *15*(2), 554-564.

Rhoden, C.M., Peterman, W.E., Taylor, C.A. (2017). Maxent-directed field surveys identify new populations of narrowly endemic habitat specialists. *PeerJ*, *5*, e3632.

Rockwood, L.L., (2006). *Introduction to population ecology*, Blackwell Science, 339 p.

Rosenzweig, M. L. (1995). *Species diversity in space and time*. Cambridge University Press, 436 p.

Rotllan-Puig, X., Traveset, A. (2016). Declining relict plants: climate effect or seed dispersal disruption? A landscape-scale approach. *Basic and Applied Ecology*, *17*(1), 81-91.

S.F. (2011). Natura 2000 Standard Form https://www.mmediu.ro/app/webroot/uploads/files/Formulare_standard_SCI.pdf

Sârbu, I., Ștefan, N., Oprea, A. (2013). *Vascular plants from Romania. Illustrated field identification manual*. Editura Victor B Victor. 1320 p.

Sharma, S., Arunachalam, K., Bhavsar, D., Kala, R. (2018). Modeling habitat suitability of *Perilla frutescens* with Maxent in Uttarakhand - A conservation approach. *Journal of Applied Research on Medicinal and Aromatic Plants*, *10*, 99-105.

Tang, X., Yuan, Y., Wang, L., Chen, S., Liu, X., Zhang, J. (2021). Identifying prioritized planting areas for medicinal plant *Thesium chinense* Turcz. under climate change in China. *Ecological Informatics*, *66*, 101459.

Tesfamariam, B. G., Gessesse, B., Melgani, F. (2022). Maxent-based modeling of suitable habitat for rehabilitation of Podocarpus forest at landscape-scale. *Environmental Systems Research*, *11*(1), 4.

USDA (2025). *Syringa josikaea* Jacq. f. ex Rchb., U.S. Department of Agriculture, Natural Resources Conservation Service, https://plants.sc.egov.usda.gov/plant-profile/SYJO4

Vasilevskaya, N., Morozova, D. (2020). Monitoring the growth and development of *Syringa josikaea* Jacq. fil. in the conditions of the euro-arctic region (on the example of the city of Murmansk). Principles of the Ecology, 9, 2(36). DOI: 10.15393/j1.art.2020.10342

Vasilevskaya, N., Morozova, D. (2024). Morphological variability and fertility of *Syringa josikaea* pollen under industrial pollution of the Arctic city. In *BIO Web of Conferences* (Vol. 130, p. 01008). EDP Sciences.

Vasilevskaya, N.V., Morozova, D.A. (2018). Polyvariance of Floral Organogenesis of *Syringa josikaea* Jacq. (Oleaceae) in the Conditions of the Arctic Urbanized Territory. *Ecology and Sustainable Development*, *1*(1), 31-39.

Venne, S., Currie, D. J. (2021). Can habitat suitability estimated from Maxent predict colonizations and extinctions? *Diversity and Distributions*, *27*(5), 873-886.

Williams, J. N., Seo, C., Thorne, J., Nelson, J. K., Erwin, S., O'Brien, J. M., Schwartz, M. W. (2009). Using species distribution models to predict new occurrences for rare plants. *Diversity and distributions*, *15*(4), 565-576.

Wisz, M.S., Hijmans, R.J., Li, J., Peterson, A.T., Graham, C.H., Guisan, A., NCEAS Predicting Species Distributions Working Group. (2008). Effects of sample size on the performance of species distribution models. *Diversity and distributions*, *14*(5), 763-773.

Wisz, M. S., Pottier, J., Kissling, W.D., Pellissier, L., Lenoir, J., Damgaard, C.F., Dormann, C.F., Forchhammer, M.C., Grytnes, J.-A., Guisan, A., Heikkinen, R.K., Høye, T.T., Kühn, I., Luoto, M., Maiorano, L., Nilsson, M.-C., Normand, S, Öckinger, E., Schmidt, N.M., Termansen, M., Timmermann, A., Wardle, D.A., Aastrup, P., Svenning, J.-C., Svenning, J. C. (2013). The role of biotic interactions in shaping distributions and realised assemblages of species: implications for species distribution modelling. *Biological reviews*, *88*(1), 15-30.

Witkowski, Z. J., Król, W., Solarz, W. (Eds.) (2003). *Carpathian list of endangered species, WWF and Institute of Nature Conservation*, Polish Academy of Science, Europress, Kraków, Poland.

Yang, X.Q., Kushwaha, S.P., Saran, S., Xu, J., Roy, P.S. (2013). Maxent modeling for predicting the potential distribution of medicinal plant, *Justicia adhatoda* L. in Lesser Himalayan foothills. *Ecological engineering*, *51*, 83-87.

Yi, Y.J., Cheng, X., Yang, Z.F., Zhang, S.H. (2016). Maxent modeling for predicting the potential distribution of endangered medicinal plant (*H. riparia* Lour) in Yunnan, China. *Ecological Engineering*, *92*, 260-269.

Zhang, Y., Tang, J., Ren, G., Zhao, K., Wang, X. (2021). Global potential distribution prediction of *Xanthium italicum* based on Maxent model. *Scientific reports*, *11*(1), 16545.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

ANTIOXIDANT CAPACITY AND SEASONAL MINERAL COMPOSITION VARIATION IN *ARONIA MELANOCARPA* FRUITS

Laura POP (CARPESCU)[1], Rui COSTA[2], Adrian ASĂNICĂ[1], Stelica CRISTEA[1], Corina PREDESCU[1], Dana TĂPĂLOAGĂ[1], Liliana TUDOREANU[1, 3]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăşti Blvd, District 1, Bucharest, Romania
[2]Research Centre for Natural Resources, Environment and Society (CERNAS), Polytechnic University of Coimbra, Bencanta, 3045-601, Coimbra, Portugal
[3]Interdisciplinary Laboratory for Heavy Metals Accumulation in the Food Chain and Modelling, Department of Animal Production and Public Health, Faculty of Veterinary Medicine Bucharest, University of Agronomic Sciences and Veterinary Medicine of Bucharest, 105 Splaiul Independenței, District 5, Bucharest, Romania

Corresponding author email: laurapop_22@yahoo.com

Abstract

Aronia melanocarpa fruits have been linked to a variety of health benefits due to their nutritional, antioxidant, antiviral, and anti-inflammatory properties. The main objective of this study was to identify the optimal harvest time for maximum nutritional antioxidant potential and nutritional mineral concentrations. Ten berry samples of 'Nero Eggert' Aronia melanocarpa variety were harvested twice per week over 5 weeks (August-September). Aronia berries harvested in early August contain high concentrations of dietary macronutrients: Ca (807.17 mg/kg), K (3,070.15 mg/kg), Mg (201.94 mg/kg), Na (116.77 mg/kg), dietary ultratrace elements: B (168.65 mg/kg) and Sr (1.14 mg/kg) and total flavonoid content (TFC - 1,722.87 mg CE/100 g). Berries harvested in early September contain high total polyphenol concentrations (TPC - 2,358.65 mg GAE/100 g), total antioxidant capacity (TAC - 41,58 mmol TE/ 100 g), total anthocyanin content (TA - 357.17 mg CGE/100 g) and ferric reducing antioxidant power (FRAP - 69.33 mmol Fe(II)/100 g), high concentrations of dietary micronutrients: Cr (0.39 mg/kg), Cu (1.53 mg/kg), Fe (110 mg/kg), Mn (9.2 mg/kg), Zn (108.32 mg/kg), which make them recommended for functional and personalized foods with high polyphenol content.

Key words*: chokeberry, nutritional value, minerals, polyphenols.*

INTRODUCTION

Due to its rich chemical composition, which varies based on a number of parameters including soil composition, fertilization, climate, berry ripeness, harvesting technique, and storage conditions, *Aronia melanocarpa* has attracted a lot of interest in the scientific world and food industry (Tolić et al., 2017). *Aronia melanocarpa* is a 2-3 m shrub (Jurendić and Ščetar, 2021) and one of four recognized species of the genus *Aronia* Medik, in the *Rosaceae* family, also known as black chokeberry (Mahoney et al., 2019).

Aronia berries, also known as chokeberries, are one of the greatest sources of polyphenols and anthocyanins in the plant kingdom, making them an ideal raw material for the development of functional food. Their popularity stems from both their nutritional content and health-promoting properties (Denev et al., 2018). Research has shown that these compounds can have a meaningful beneficial impact on health through their antioxidative, anti-inflammatory, antiviral, anticancer, antiatherosclerosis, hypotensive, antiplatelet, and antidiabetic properties (Borowska and Brzóska, 2016). Being remarkably rich in anthocyanins, flavanols, proanthocyanins, and phenolic acids (King and Bolling, 2020), chokeberries' overall polyphenol content ranges from 1,022 mg/100 g fresh weight (fw) to 1,795 mg/100 g fw, with anthocyanins accounting for 284-686 mg/100 g fw (Denev et al., 2018). A study conducted by Xie et al. (2016) showed that after the consumption of 500 mg aronia extract, their

polyphenols were absorbed and extensively metabolized. Consuming the same quantity also has a beneficial impact on low-density lipoprotein cholesterol (LDL-C); a study on former smokers showed an 8% reduction in LDL-C after 12 weeks compared to the placebo group (Xie et al., 2017).

Aronia melanocarpa is also known for its complex mineral content in fruits, containing calcium, potassium, iron, molybdenum, manganese, phosphorus and iodine (Djuric et al., 2015). Very few information is published on aronia fruits and products (juice, filter bags for infusion and pomace) mineral content (Cindrić et al., 2017; Jurendić & Ščetar, 2021; King & Bolling, 2020; Kulling & Rawel, 2008; Sidor et al., 2019; Pieszka et al., 2015; Pavlovic et al., 2015).

The aim of this study was to determine how the harvest date impacts the antioxidant capacity of *Aronia melanocarpa* berries and their mineral composition.

MATERIALS AND METHODS

Ten samples of *Aronia melanocarpa* variety 'Nero Eggert' fruits were harvested twice per week during August and beginning of September in 2022 from plants located in the experimental orchard of the Integrated Fruit Growing Laboratory of the Faculty of Horticulture, University of Agronomic Sciences and Veterinary Medicine of Bucharest. All samples were frozen at -32°C until time of analysis. All data presented in this paper are for wet weight samples (fresh fruits).

Antioxidant capacity

The following parameters were measured:

- the total polyphenol content (TPC) - was measured using a modified Folin-Ciocalteu method and the results were expressed as milligrams of gallic acid equivalents/100 g fruit (mg GAE/100 g) (Predescu et al., 2016);
- the total flavonoid content (TFC) - was evaluated by aluminium chloride method and the results were expressed as milligrams catechin equivalent/100 g (mg CE/100 g) (Predescu et al., 2016);
- the total anthocyanin content (TA) - was determined by pH differential method and the results were expressed as mg cyanidin-3-glucoside equivalents/100 g fruit (mg CGE/100 g) (Panico et al., 2009);
- the total antioxidant capacity (TAC) - was evaluated using 1,1-diphenyl-2-picrylhydrazyl (DPPH•) and the results were expressed as mmol Trolox equivalents/100 g fruit (mmol TE/100 g) (Predescu et al., 2016);
- a spectrophotometric method was used to estimate the ferric reducing antioxidant power (FRAP) and the results were expressed as mmol Fe(II) equivalents/100 g fruit (Predescu et al., 2016).

Mineral concentratio

Aronia fruits were ground and 0.90 g to a maximum of 1.00 g of fruits were mixed with 8 ml of nitric acid 65% Suprapur Merck.

The samples were digested using a Speedwave Berghov microwave digestion oven for digestion (Table 1). After cooling, the samples were diluted with ultrapure water to the 50 ml flasks (sample solution).

Table1. Microwave digestion oven operating parameters

Parameter	Stage 1	Stage 2	Stage 3
Power (%)	50	50	40
Temperature °C	145	180	100
Execution time (min)	5	10	10

The samples solutions were analysed using an Inductively Coupled Plasma - Optical Emission Spectrometer (ICP-OES) (Table 2).

Table 2. ICP-OES operating conditions

Parameter	Value
Frequency	27.12 MHz
Rf Power	1.5 kw
Argon Gas flow	14 L/min
Nebulizer Type	ciclonic
Stabilization Time	3-5 s
Number of Probes for each Measurement	3

The following micro and macro elements have been determined: Al 396.1 nm; B 208.9 nm, Ba 233.5 nm; Ca 393.9 nm; Cr 267.7 nm; Cu 224.7 nm; Fe 259.9 nm; K 769.8 nm; Mg 280.2 nm; Mn 257.6 nm; Na 589.5 nm; Ni 221.6 nm; Sr 421.5 nm; Zn 213.8 nm.

Statistical analysis

Statistical analysis was performed using MedCalc® Statistical Software version 22.006 (MedCalc Software Ltd, Ostend, Belgium; https://www.medcalc.org; 2024). Data normality was tested using the Shapiro-Wilk test and

Levene's test was used for testing equality of error variances. The post-hoc analysis was performed using Tukey-Kramer test in case of a significant one-way ANOVA analysis. When data was not normally distributed, the Kruskal-Wallis test was applied.

RESULTS AND DISCUSSIONS

Antioxidant capacity

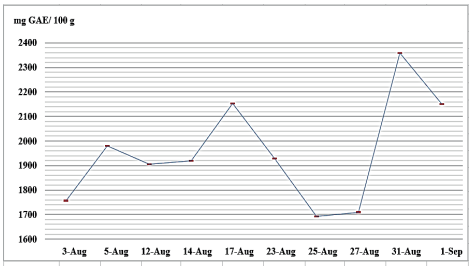
The TPC, TFC, TA, TAC and FRAP for *Aronia melanocarpa* fruits during harvest are presented in Figures 1 to 5.

Total polyphenol content (TPC)

The variation of environment temperature influences the biosynthesis of plant tissues polyphenols (secondary metabolites) the process depending on the gene's activity responsible for phenolic compounds synthesis as a response to the increase in the accumulation of reactive oxygen species (ROS) within plant cells (Zagoskina et al., 2023). This explains the increase of TPC concentrations in aronia berries for the harvests from 17 and 31 August. On the other hand, as the fruits ripen, TPC concentration may decreases (Dobros et al., 2024) explaining the decrease observed for 23-27 August.

Several authors have reported values between 1,022 mg GAE /100 g and 1,795 mg GAE/100 g (Denev et al., 2018) and up to 4,393.50 mg/100 g at their peak ripening stage (Yang et al., 2019). Engin & Mert (2020) reported a steady increase during August and mid-September followed by a decrease until the end of September for an aronia cultivar in Turkey.

For the data in Figure 1, data followed by the same letter are not significantly different for α= 0.05 (Shapiro-Wilk test P=0.7586, Levene's test P=0.418, one-way ANOVA P<0.001).



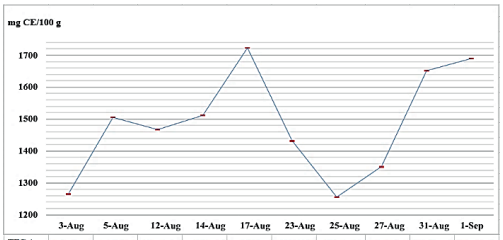
	3-Aug	5-Aug	12-Aug	14-Aug	17-Aug	23-Aug	25-Aug	27-Aug	31-Aug	1-Sep
TPC (mg GAE/ 100 g)	1,756.3 g	1,980.7 c	1,905.1 f	1,920.5 e	2,153.5 b	1,929.2 d	1,692.8 i	1,709.9 h	2,358.6 a	2,151.5 b
SD (%)	2.5	0.7	0.7	1.0	1.2	1.2	0.5	1.7	1.3	1.1

Figure 1. Variation of TPC (mg GAE/100 g) in *Aronia melanocarpa* fruit samples during harvest

Total flavonoid content (TFC)

Light is an important environmental factor in regulating the flavonoid accumulation in fruits, high exposure to sunlight regulating the flavonol biosynthetic gene MdFLS and several anthocyanin biosynthetic genes, including MdCHS, MdCHI, MdF3H, MdDFR1, leucoanthocyanidin dioxygenase (MdLDOX) and MdUFGT (Zoratti et al., 2014). Weather conditions and fruit positioning can explain the high TFC concentrations from August 17 and September 1st harvests. The oxidation of flavonoids to their corresponding semiquinones and quinones by oxidases such as polyphenol oxidases (PPO) and peroxidases (POD) during ripening (Pourcel et al., 2007) can explain the decrease in TFC measured on August 25th harvest. Other research also reported that the variation of TFC is linked with the weather conditions, showing significant changes from one year to the other (Tolić et al., 2017).

In Figure 2, data followed by the same letter are not significantly different for α=0.05 (Shapiro -Wilk test P=0.7536, Levene's test P=0.471, one-way ANOVA P<0.001).



	3-Aug	5-Aug	12-Aug	14-Aug	17-Aug	23-Aug	25-Aug	27-Aug	31-Aug	1-Sep
TFC (mg CE/100 g)	1,264.5 i	1,505.3 e	1,466.9 f	1,511.4 d	1,722.8 a	1,431.4 g	1,256.0 j	1,350.8 h	1,651.0 c	1,688.9 b
SD (%)	1.8	0.6	0.6	0.8	1.0	0.9	0.4	1.4	0.9	0.9

Figure 2. Variation of TFC (mg CE/100 g) in *Aronia melanocarpa* fruit samples during harvest

Total anthocyanins (TA)

Anthocyanins are antioxidants with several health benefits, including anticancer, antiatherogenic, and anti-inflammatory properties, which have been extensively studied for their health advantages over the last two decades. Anthocyanins in foods are key quality indicators that influence customer preferences, and their stability in food is altered by various factors, including pH, solvents, temperature, oxygen, enzymes, and other compounds (Teneva et al., 2022).

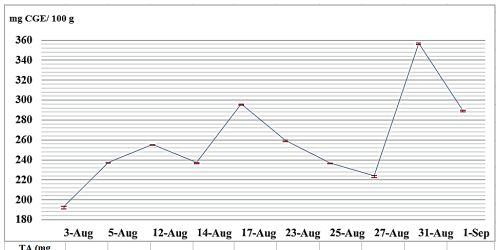
TA had the highest value at the end of August, 45.91% more than at the start of the harvest

(Figure 3). Anthocyanin synthesis is initiated and regulated by the plant hormone abscisic acid (ABA) and variation is influenced by different external factors like light exposure, temperature, nutritional factors (Zoratti et al., 2014).

There are different factors that determine anthocyanin degradation: chemical degradation due to increase of pH in the vacuole microenvironment, external temperature and light exposure; enzymatic degradation by polyphenol oxidase (PPO) and peroxidase (Zhao et al., 2021), explaining the lowest concentrations in August 27th and Septembers 1st harvests.

Engin & Mert (2020) reported values between 558.3 mg CGE/100 g and 843.9 mg CGE/100 g and that anthocyanin content in aronia berries in Turkey increased during ripening; however, it decreased due to overripening.

In Figure 3, data followed by the same letter are not significantly different for α=0.05 (Shapiro-Wilk test P=0.5019, Levene's test P=0.549, one-way ANOVA P<0.001).



TA (mg CGE/ 100 g)	193.2 i	237.2 f	255.2 e	237.3 f	295.9 b	259.6 d	236.7 g	224.0 h	357.1 a	289.5 c
SD (%)	0.3	0.1	0.1	0.1	0.2	0.2	0.1	0.2	0.2	0.2

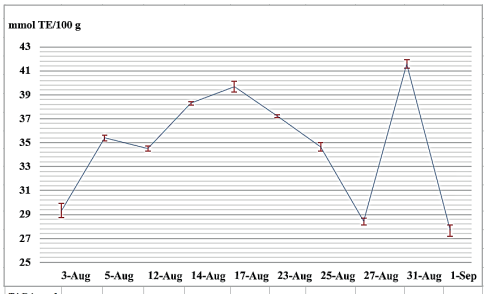
Figure 3. Variation of TA (mg CGE/100 g) in *Aronia melanocarpa* fruit samples during harvest

Total antioxidant capacity (TAC)

In purple colour fruits, the main responsible compounds for a high TAC are anthocyanins (Valero and Serrano, 2013). During harvest, TAC variation (Figure 4) had a similar pattern as the variation of TA (Figure 3), the highest TAC being recorded on August 31st. Degradation of anthocyanins can determine the decrease of TAC, explaining the low values recorded on August 27th and September 1st.

Other studies show a similar pattern; Engin & Mert (2020) reported an 8% increase for an aronia cultivar in Turkey in the first four harvest dates and a gradual decrease between dates four and six of harvest. Bolling et al. (2015) reported a decrease of the antioxidant capacity in the first half of august for an aronia cultivar in USA, followed by a steady increase until mid-September.

In Figure 4, data followed by the same letter are not significantly different for α= 0.05 (Shapiro-Wilk test P=0.7617, Levene's test for equality of error variances P=0.341, one-way ANOVA P<0.001).

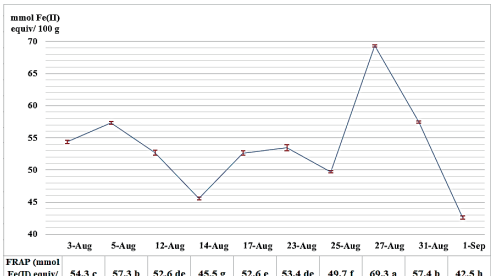


TAC (mmol TE/ 100 g)	29.3 f	35.4 e	34.5 e	38.2 c	39.6 b	37.2 d	34.6 e	28.4 f	41.5 a	27.6 f
SD (%)	0.6	0.2	0.2	0.1	0.5	0.1	0.3	0.3	0.3	0.5

Figure 4. Variation of TAC (mmol TE/100 g) in *Aronia melanocarpa* fruit samples during harvest

The ferric reducing antioxidant power (FRAP)

FRAP was used to measure antioxidant activity of fruits during ripening. The reducing power is primarily associated with the presence of reducing agents, which exhibit antioxidant activity by interrupting free radical chain reactions through the donation of a hydrogen atom (Tolić et al., 2015). Higher total phenolic content (TPC) often correlates with increased FRAP values (Subbiah et al., 2020). FRAP varies from one harvest to the other (Figure 5), with a low peak at the beginning of September and the highest value on 27-Aug harvest. Other studies reported values between 13.50 mmol Fe(II) equivalent/100 g to 68.60 mmol Fe(II) equivalent/100 g (Tolić et al., 2015).



FRAP (mmol Fe(II) equiv/ 100 g)	54.3 c	57.3 b	52.6 de	45.5 g	52.6 e	53.4 de	49.7 f	69.3 a	57.4 b	42.5 h
SD (%)	0.3	0.2	0.4	0.2	0.3	0.5	0.1	0.2	0.2	0.3

Figure 5. Variation of FRAP (mmol Fe(II) equiv/100 g) in *Aronia melanocarpa* fruit samples during harvest

In Figure 5, data followed by the same letter are not significantly different for α= 0.05 (Shapiro-Wilk test P=0.7155, Levene's test P=0.238, one-way ANOVA P<0.001).

Mineral concentrations

The total mineral concentrations during harvest are presented in Figures 6 to 19.

Dietary macro nutrients

Calcium (Ca) accumulation in fruits is highly dependent on water delivery. Also, Ca concentration in fruits depends on plant species, on cell wall interactions in the apoplasm (Hocking et al., 2016). The highest concentrations of Ca have been recorded on August 5th and August 27th (Figure 6). Other authors reported values of 119 mg/kg and 552 mg/kg (Šnebergrová et al., 2014), 601 mg/kg and 1167 mg/kg (Pavlovic et al., 2015), 1,212 mg/kg (Cindrić et al., 2017) and 3,220 mg/kg (Lancrajan, 2012). These large differences in values might be due to the different harvest dates, rainfall regime and varieties/cultivars of Aronia. According to the Romanian Norm from December 13, 2000, emitted by the Romanian Ministry of Health, the daily recommended doze for adults is 1,000 mg Ca/day (Norm 13/12/2000 - Romanian Legislative Portal, 2025).
In Figure 6, data followed by the same letter are not significantly different for α= 0.05 (Shapiro-Wilk test P=0.7105, Levene's test P=0.384, one-way ANOVA P<0.001).

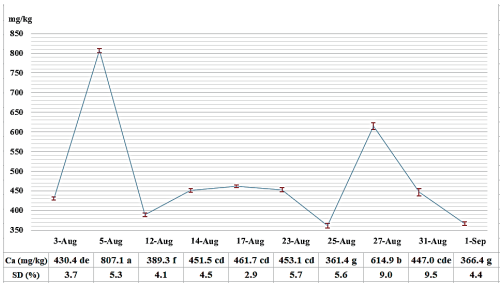


Figure 6. Variation of Ca concentrations (mg/kg) in *Aronia melanocarpa* fruit samples during harvest

Potassium (K) is an essential nutrient that influences the majority of biochemical and physiological processes involved in plant development and metabolism. It also helps plants survive a variety of biotic and abiotic stressors (Wang et al., 2013). However low-K fruits for patients with chronic kidney disease are of major importance (Cui et al., 2024).
During harvest, K in aronia berries had the highest concentrations in the first half of August and the lowest concentration was found on 17 august (Figure 7). Other studies have shown concentrations between 2,707-4,977 mg/kg (Pavlovic et al., 2015), 6,790 mg/kg (Cindrić et al., 2017), and 2,180 mg/kg (Lancrajan, 2012).
According to the Romanian Norm from December 13, 2000, emitted by the Romanian Ministry of Health, the recommended daily dose of K for healthy adults is 3.50 g/day (Norm 13/12/2000 - Romanian Legislative Portal, 2025).
In Figure 7, data followed by the same letter are not significantly different for α= 0.05 (Shapiro-Wilk test P=0.9779, Levene's test for equality of error variances P=0.096, one-way ANOVA P<0.001).

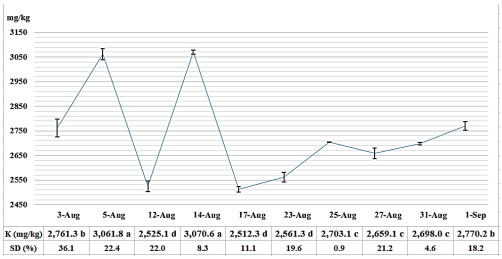
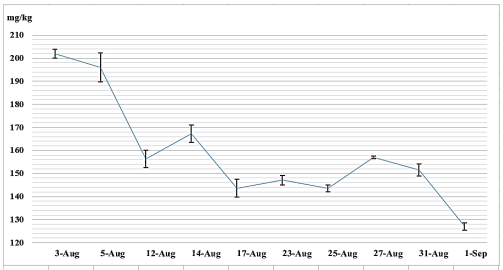


Figure 7. Variation of K concentrations (mg/kg) in *Aronia melanocarpa* fruit samples during harvest

Magnesium (Mg) is well known for its various effects on the human body and is important for nerve transmission and neuromuscular conduction (Kirkland et al., 2018). According to the Romanian Norm from December 13, 2000, emitted by the Romanian Ministry of Health, the recommended daily doze for healthy adults is 350 mg Mg /day (Norm 13/12/2000 - Romanian Legislative Portal, 2025).
During harvest, Mg in aronia berries shows a high peak at the beginning of the harvest (Figure 8) and it gradually decreases until the end of harvest. Other studies have shown concentrations between 164-578 mg/kg (Pavlovic et al., 2015), 669 mg/kg (Cindrić et al., 2017), and 168 mg/kg (Lancrajan, 2012).
In Figure 8, (Shapiro-Wilk test P=0.9269, Levene's test P=0.045, one-way ANOVA

P<0.001) data followed by the same letter are not significantly different for α=0.05.

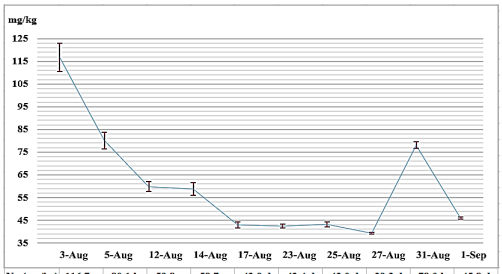


Mg (mg/kg)	201.9 a	196.0 a	156.3 cdef	167.2 b	143.6 efg	147.0 defg	143.5 efg	157.0 cde	151.5 cdefg	127.0 h
SD (%)	2.0	6.4	3.8	3.8	3.9	2.0	1.4	0.6	2.6	1.5

Figure 8. Variation of Mg concentrations (mg/kg) in *Aronia melanocarpa* fruit samples during harvest

Both World Health Organization (*Salt Intake*, 2024) and Romanian Norm from December 13, 2000, emitted by the Romanian Ministry of Health, recommend an intake of maximum 2 g of sodium (Na) per day (Norm 13/12/2000 - Romanian Legislative Portal, 2025). During harvest, Na in aronia berries shows a fluctuation in values, with the lowest amount being recorded on August 27th harvest, and the highest on August 3rd (Figure 9). Other studies reported concentrations of Na between 12.5-16.8 mg/kg (Pavlovic et al., 2015), 4.27 mg/kg (Cindrić et al., 2017) and 26 mg/kg (Lancrajan, 2012).

In Figure 9, data followed by the same letter are not significantly different for α= 0.05 (Shapiro-Wilk test P=0.522, Levene's test P=0.004, one-way ANOVA P<0.001).

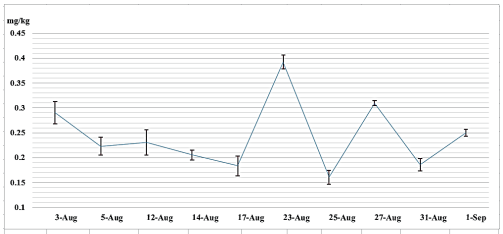


Na (mg/kg)	116.7 a	80.1 b	59.8 c	58.7 c	42.9 d	42.4 d	43.0 d	39.3 d	78.0 b	45.8 d
SD (%)	6.2	3.6	2.3	2.8	1.3	0.8	1.1	0.3	1.4	0.5

Figure 9. Variation of Na concentrations (mg/kg) in *Aronia melanocarpa* fruit samples during harvest

Dietary micro nutrients

Chromium (Cr), an important mineral, appears to play a favorable role in the control of insulin activity and its effects on glucose, protein, and lipid metabolism (Havel, 2004). In the presence of Cr in its physiologically active form, significantly lower quantities of insulin are required in the human body (Anderson, 2003). The National Institute of Health in USA recommends the daily intake of Cr of 25 micrograms/day for adult females and 35 micrograms/day for adult males (*Dietary Reference Intakes (DRIs): Recommended Dietary Allowances and Adequate Intakes, Elements, Food and Nutrition Board, National Academies,* 2025). Cr in fruits can vary due to soil condition and sampling location, depending on the location where the fruits are growing on the plant; also pH of soil and humidity will influence the Cr uptake by plants (Banks et al., 2006). During harvest, the lowest amount of Cr was recorded on August 25th harvest, and the highest on August 23rd (Figure 10). Other studies have shown concentrations between 0.49-0.53 mg/kg (Pavlovic et al., 2015) and 0.029 mg/kg (Cindrić et al., 2017).



Cr (mg/kg)	0.290 bcd	0.223 defghi	0.230 defgh	0.205 defghij	0.183 fghij	0.392 a	0.160 ghij	0.309 bc	0.185 efghij	0.249 cdefg
SD (%)	0.022	0.018	0.025	0.010	0.020	0.014	0.014	0.005	0.012	0.007

Figure 10. Variation of Cr concentrations (mg/kg) in *Aronia melanocarpa* fruit samples during harvest.

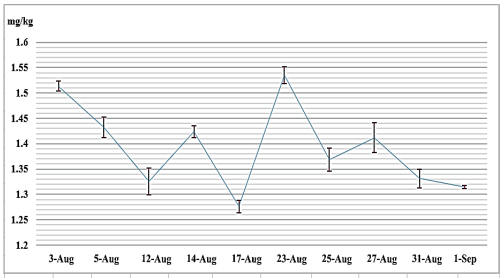
In Figure 10, data followed by the same letter are not significantly different for α=0.05 (Shapiro-Wilk test P=0.8437, Levene's test P=0.380, one-way ANOVA P<0.001).

Copper (Cu) is an essential mineral that occurs naturally in some foods; it is accessible as a dietary supplement and is a cofactor for various enzymes (known as cuproenzymes) in energy production, iron metabolism, neuropeptide activation, connective tissue formation, and neurotransmitter synthesis (Ross et al., 2012). After iron, Cu has gathered a lot of interest in cell death, particularly in causing tumor cell death, inducing autophagy or apoptosis in tumor cells via a variety of mechanisms of action (activation of stress pathways, cell cycle arrest, inhibition of angiogenesis, cuproptosis,

and paraptosis), all of which are promising in cancer therapy and have emerged as new hotspots in research (Ji et al., 2023). According to the Romanian Norm from December 13, 2000, emitted by the Romanian Ministry of Health, the recommended daily doze for adults is 3 mg Cu/day (Norm 13/12/2000 - Romanian Legislative Portal, 2025).

During harvest, Cu in aronia berries shows a concentration fluctuation, with the lowest level being recorded on August 3rd and 5th harvests, and the highest on August 23rd (Figure 11). Other studies have shown concentrations between 0.82-2.11 mg/kg (Djuric et al., 2015; Pavlovic et al., 2015).

In Figure 11, data followed by the same letter are not significantly different for α=0.05. (Shapiro-Wilk test P=0.9805, Levene's test P=0.119, one-way ANOVA P<0.001).



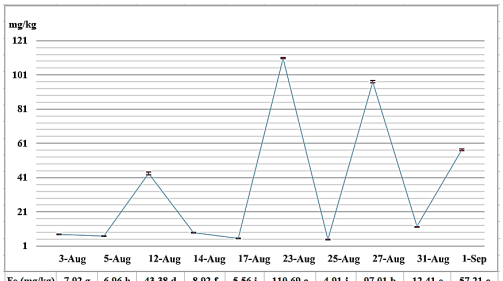
Cu (mg/kg)	1.535 a	1.513 a	1.432 bc	1.423 bc	1.411 bcd	1.368 cdef	1.331 def	1.325defg	1.314 defg	1.276 fg
SD (%)	0.010	0.020	0.027	0.012	0.013	0.016	0.023	0.030	0.018	0.003

Figure 11. Variation of Cu concentrations (mg/kg) in *Aronia melanocarpa* fruit samples during harvest

Iron (Fe) is a mineral found naturally in many foods and is accessible as a nutritional supplement. Fe is an important component of hemoglobin, an erythrocyte (red blood cell) protein that transports oxygen from the lungs to tissues (Ross et al., 2012). According to the Romanian Norm from December 13, 2000, emitted by the Romanian Ministry of Health, the recommended daily doze for adults is 18 mg Fe /day (Norm 13/12/2000 - Romanian Legislative Portal, 2025).

During harvest, Fe in aronia berries shows a great and inconsistent fluctuation in values, ranging from 5.56 mg/kg on August 17th harvest to 110.69 mg/kg on August 23rd harvest (Figure 12). Other studies have shown concentrations between 9.4-14.2 mg/kg (Pavlovic et al., 2015), 1.32 mg/kg (Cindrić et al., 2017) and 9.3 mg/kg (Lancrajan, 2012).

In Figure 12, data followed by the same letter are not significantly different for α=0.05 (Shapiro-Wilk test P=0.0242 - reject normality), Levene's test for equality of error variances P<0.001, Kruskal-Wallis test P= 0.000753).



Fe (mg/kg)	7.92 g	6.96 h	43.38 d	8.92 f	5.56 i	110.69 a	4.91 j	97.01 b	12.41 e	57.21 c
SD (%)	0.05	0.06	0.81	0.12	0.02	0.35	0.07	0.68	0.10	0.41

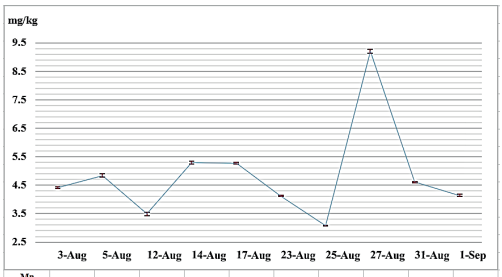
Figure 12. Variation of Fe concentrations (mg/kg) in *Aronia melanocarpa* fruit samples during harvest

Manganese (Mn) is a necessary trace element that can be obtained as a dietary supplement or found naturally in a variety of foods (Ross et al., 2012). Mn is necessary for healthy cellular homeostasis, growth, and development. High Mn exposure results in clinical illness with extrapyramidal symptoms that mimic idiopathic Parkinson's disease (Bowman et al., 2011). There is no evidence in research to support health risks associated with excessive manganese consumption (Finley et al., 2003).

According to the Romanian Norm from December 13, 2000, emitted by the Romanian Ministry of Health, the recommended daily doze for adults is 3 mg Mn /day (Norm 13/12/2000 - Romanian Legislative Portal, 2025). According to the USA National Institute of Health – Office of Dietary Supplements, the adequate intake of Mn is 2.3 mg for adult males and 1.8 mg for adult females (*Dietary Reference Intakes (DRIs): Recommended Dietary Allowances and Adequate Intakes, Elements, Food and Nutrition Board, National Academies,* 2025).

During harvest, Mn in aronia berries shows a fluctuation in values, with the lowest amount being recorded on August 12 harvest, 3.48 mg/kg the highest on August 27th, 9.20 mg/kg (Figure 13). Other studies reported concentrations between 5.49-17.89 mg/kg (Pavlovic et al., 2015) and 0.82 mg/kg (Cindrić et al., 2017).

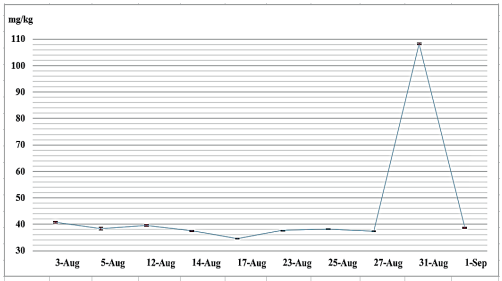
In Figure 13, data followed by the same letter are not significantly different for α=0.05 (Shapiro-Wilk test P=0.9805, Levene's test for equality of error variances P=0.036, one-way ANOVA P<0.001).



	3-Aug	5-Aug	12-Aug	14-Aug	17-Aug	23-Aug	25-Aug	27-Aug	31-Aug	1-Sep
Mn (mg/kg)	4.41 e	4.84 c	3.48 g	5.29 b	5.27 b	4.12 f	3.07 h	9.20 a	4.60 d	4.14 f
SD (%)	0.04	0.05	0.06	0.05	0.03	0.02	0.01	0.08	0.03	0.04

Figure 13. Variation of Mn concentrations (mg/kg) in *Aronia melanocarpa* fruit samples during harvest

Zinc (Zn) in aronia berries exhibits minimal fluctuations in values during August, with the lowest amount reported on August 17th, and a surge in concentration on August 31st (Figure 14). Other studies report values ranging from 4.09-8.40 mg/kg (Pavlovic et al., 2015) and 0.55 mg/kg (Cindrić et al., 2017). According to the Romanian Norm from December 13, 2000, emitted by the Romanian Ministry of Health, the recommended daily doze for adults is 15 mg Zn /day (Norm 13/12/2000 - Romanian Legislative Portal, 2025). According to the USA Office of Dietary Supplements, the adequate intake of Zn is 8 mg for adult males and 11 mg for adult females (*Dietary Reference Intakes (DRIs): Recommended Dietary Allowances and Adequate Intakes, Elements, Food and Nutrition Board, National Academies,* 2025).



	3-Aug	5-Aug	12-Aug	14-Aug	17-Aug	23-Aug	25-Aug	27-Aug	31-Aug	1-Sep
Zn (mg/kg)	40.7 b	38.4 de	39.6 c	37.5 fg	34.5 h	37.6 efg	38.2 def	37.4 fg	108.3 a	38.7 de
SD (%)	0.3	0.5	0.2	0.2	0.1	0.1	0.1	0.1	0.3	0.2

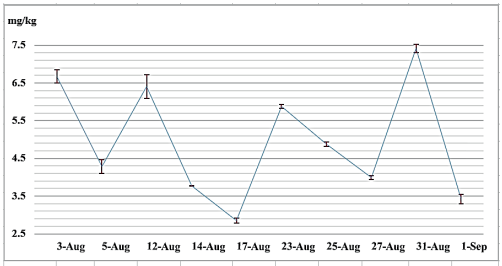
Figure 14. Variation of Zn concentrations (mg/kg) in *Aronia melanocarpa* fruit samples during harvest

In Figure 14, data followed by the same letter are not significantly different for α=0.05 (Shapiro-Wilk test P=0.6960, Levene's test for equality of error variances P=0.060, one-way ANOVA P<0.001).

Dietary ultra trace elements

Aluminium (Al) concentrations show a great variation throughout harvest, as shown in Figure 15, the lower value being 2.85 mg/kg and the highest 7.41 mg/kg. Other research have shown between 2.88-4.40 mg/kg (Pavlovic et al., 2015), or 158 mg/kg (Cindrić et al., 2017). According to the European Food Safety Authority (EFSA), a tolerable intake of 1 mg Al/kg body weight/week is acceptable (Authority, E. F. S. 2008). Al can be found in fruits either due to intake from the geologic environment throughout growth, or from any potential contamination from Al articles that come into touch with food and additives, as well as veterinary medications, fertilizers, and the air (Stahl et al., 2011).

In Figure 15, data followed by the same letter are not significantly different for α= 0.05 (Shapiro-Wilk test P=0.2729, Levene's test for equality of error variances P=0.002, one-way ANOVA P<0.001).



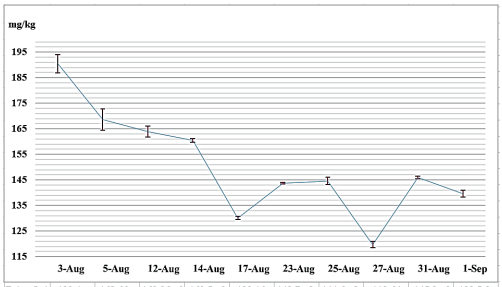
	3-Aug	5-Aug	12-Aug	14-Aug	17-Aug	23-Aug	25-Aug	27-Aug	31-Aug	1-Sep
Al (mg/kg)	6.67 b	4.28 e	6.40 b	3.77 efg	2.85 h	5.87 c	4.88 d	3.99 ef	7.41 a	3.42 fg
SD (%)	0.17	0.18	0.31	0.00	0.07	0.05	0.06	0.06	0.11	0.12

Figure 15. Variation of Al concentrations (mg/kg) in *Aronia melanocarpa* fruit samples during harvest

During harvest, boron (B) in aronia berries showed a gradual decrease from 190 mg/kg at the beginning of August to 119.69 mg/kg at the end of August (Figure 16). Other studies show concentrations around ten times lower, 2.88-14.22 mg/kg (Pavlovic et al., 2015). B is a trace element found naturally in many foods and drinking water (Kuru et al., 2019). It is a structural component of plant cell walls and is necessary for plant growth, pollination, and

seed production (Nielsen and Eckhert, 2020). Some clinical investigations in humans suggest that B may be useful for lowering the symptoms of osteoarthritis, probably by preventing inflammation (Miljkovic et al., 2009). B is a critical nutrient for plant growth, development, production, and quality. It serves numerous important functions in plants, including cell wall synthesis and structural integration. During pollen tube growth and germination, B increases the likelihood of fruit setting and seed production, resulting in higher crop productivity (Shireen et al., 2018).

In Figure 16, data followed by the same letter are not significantly different for α=0.05 (Shapiro-Wilk test P=0.0544, Levene's test for equality of error variances P=0.012, one-way ANOVA P<0.001).

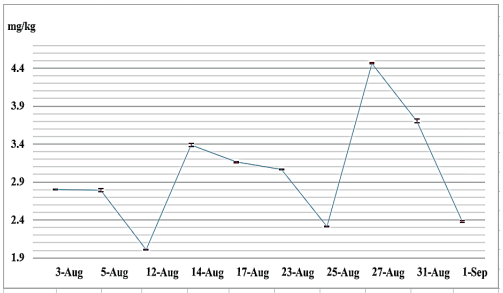


	3-Aug	5-Aug	12-Aug	14-Aug	17-Aug	23-Aug	25-Aug	27-Aug	31-Aug	1-Sep
B (mg/kg)	190.4 a	168.6 bc	163.9 bcd	160.5 cd	130.1 h	143.7 efg	144.6 efg	119.6 i	145.9 ef	139.5 fg
SD (%)	3.5	4.3	2.1	0.7	0.6	0.3	1.4	1.2	0.5	1.3

Figure 16. Variation of B concentrations (mg/kg) in *Aronia melanocarpa* fruit samples during harvest

Barium (Ba) in aronia berries showed a fluctuation in values during harvest, with the lowest amount being recorded on August 12 harvest, 2.01 mg/kg the highest on August 27th, 4.46 mg/kg (Figure 17). Other studies show concentrations between 1.48-6.66 mg/kg (Pavlovic et al., 2015). The European Commission for Public Health and Risk Assessment determined a tolerable daily intake of 0.02 mg Ba/kg bw/day (SCHER, 2012). Eating or drinking significant amounts of Ba compounds that dissolve in water or the stomach can cause comprising kidney diseases, neurological, cardiovascular, mental, and metabolic disorders (Peana et al., 2021).

In Figure 17, data followed by the same letter are not significantly different for α= 0.05 (Shapiro-Wilk test P=0.0205 (reject normality), Levene's test for equality of error variances P=0.002, Kruskal-Wallis test P=0.000753).



	3-Aug	5-Aug	12-Aug	14-Aug	17-Aug	23-Aug	25-Aug	27-Aug	31-Aug	1-Sep
Ba (mg/kg)	2.799 f	2.793 f	2.010 i	3.385 c	3.163 d	3.065 e	2.314 h	4.466 a	3.705 b	2.380 g
SD (%)	0.003	0.024	0.004	0.021	0.007	0.006	0.006	0.007	0.023	0.009

Figure 17. Variation of Ba concentrations (mg/kg) in *Aronia melanocarpa* fruit samples during harvest

Nickel (Ni) is a transitional element that is widely diffused in the environment, including air, water, and soil.

This could be the result of both natural and human action (Genchi et al., 2020).

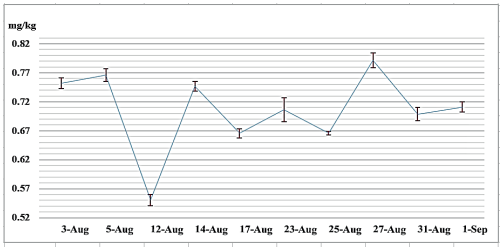
Ni has been shown to disrupt the photosynthetic activity of higher plants; it can significantly degrade soil fertility and induce chronic diseases in humans (Begum et al., 2022).

Even though Ni is a beneficial element for human health, helping microorganisms colonize the gut, exposure to it also represents a potential danger for the human body, such as allergies, nickel-induced carcinogenesis, or infectious disease based on bacteria that rely on nickel-based enzymes to colonize the host (Zambelli and Ciurli, 2013).

According to EFSA, the tolerable daily intake of Ni should not exceed 13 µg/kg body weight, around 0.78 mg/day for the average 60 kg adult (Dieter et al., 2020).

During harvest, Ni in aronia berries shows a fluctuation in values, with the lowest amount being recorded on August 12 harvest, and the highest on August 27 (Figure 18). Other studies have shown concentrations between 0.143-0.741 mg/kg (Pavlovic et al., 2015) and 0.38 mg/kg (Cindrić et al., 2017).

In Figure 18, data followed by the same letter are not significantly different for α=0.05 (Shapiro-Wilk test P=0.2299, Levene's test for equality of error variances P=0.160, one-way ANOVA P<0.001).

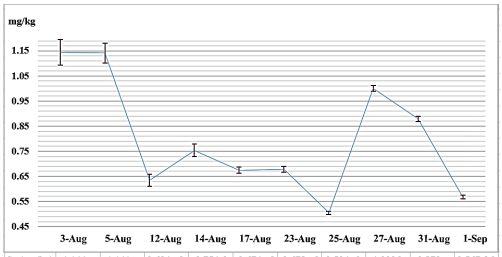


Ni (mg/kg)	0.752 bc	0.766 abc	0.550 f	0.746 bc	0.665 e	0.706 d	0.666 e	0.791 ab	0.698 d	0.711 d
SD (%)	0.009	0.011	0.010	0.009	0.008	0.021	0.003	0.013	0.011	0.008

Figure 18. Variation of Ni concentrations (mg/kg) in *Aronia melanocarpa* fruit samples during harvest

Strontium (Sr) is a common element in the environment, found in all living organisms (Höllriegl 2019). Sr is very important for human health, participating in various physiological functions and biochemical effects, having a protective effect on bone diseases, dental caries, and cardiovascular diseases, but also maintaining reproductive health and having great prospects in the development of health products and natural medicines (Huang et al., 2023). During harvest, Sr in aronia berries shows a fluctuation in values, with the lowest amount being recorded on August 12th harvest, and the highest on August 3rd (Figure 19).

In Figure 19, data followed by the same letter are not significantly different for α=0.05, (Shapiro-Wilk test P=0.9805, Levene's test P=0.014, one-way ANOVA P<0.001).



Sr (mg/kg)	1.144 a	1.141 a	0.634 efg	0.754 d	0.674 ef	0.678 ef	0.504 gh	1.000 b	0.879 c	0.567 fgh
SD (%)	0.052	0.040	0.024	0.024	0.013	0.011	0.006	0.012	0.010	0.007

Figure 19. Variation of Sr concentrations (mg/kg) in *Aronia melanocarpa* fruit samples during harvest

CONCLUSIONS

Aronia melanocarpa fruits may be used in the development of personalised foods as an important source of polyphenols and macro and micro minerals.

Due to the high content of polyphenols, spike of anthocyanins, and decrease of flavonoids, the optimum harvest dates were determined to be August 17th and August 31st.

The spike in K on August 5th and August 14th harvests correlated with the decrease in Ca after August 14th can be associated with the ripening of the berries.

The most valuable harvests from the mineral point of view were the August 23rd harvest for high amounts of Cu, Fe, and Cr, August 31st for zinc, and August 27th for Mn.

The harvest with the maximum mineral concentrations was during the first two weeks of August, and the one with the maximum polyphenol concentrations was in the last weeks of August and beginning of September

Overall, the findings of this study demonstrated that the time of harvest will be important for the use of aronia berry in the development of antioxidant-rich and mineral-rich solid and liquid personalised food products.

ACKNOWLEDGEMENTS

This article was developed within the PhD programme of the Doctoral School of Engineering and Management of Vegetal and Animal Resources, of the University of Agronomic Sciences and Veterinary Medicine of Bucharest, domain Horticulture.

REFERENCES

Anderson, R. A. (2003). Chromium and insulin resistance. *Nutrition research reviews*, 16(2), 267-275.

Authority, E. F. S. (2008). Safety of aluminium from dietary intake-scientific opinion of the panel on food additives, flavourings, processing aids and food contact materials (AFC). *EFSA Journal*, 6(7), 754.

Banks, M. K., Schwab, A. P., & Henderson, C. (2006). Leaching and reduction of chromium in soil as affected by soil organic content and plants. *Chemosphere*, 62(2), 255-264.

Begum, W., Rai, S., Banerjee, S., Bhattacharjee, S., Mondal, M. H., Bhattarai, A., & Saha, B. (2022). A comprehensive review on the sources, essentiality and toxicological profile of nickel. *RSC advances*, 12(15), 9139-9153.

Bolling, B. W., Taheri, R., Pei, R., Kranz, S., Yu, M., Durocher, S. N., & Brand, M. H. (2015). Harvest date affects aronia juice polyphenols, sugars, and antioxidant activity, but not anthocyanin stability. *Food Chemistry*, 187, 189-196.

Borowska, S., & Brzóska, M. M. (2016). Chokeberries (*Aronia melanocarpa*) and their products as a possible means for the prevention and treatment of noncommunicable diseases and unfavorable health effects due to exposure to xenobiotics. *Comprehensive Reviews in Food Science and food safety*, 15(6), 982-1017.

Bowman, A. B., Kwakye, G. F., Hernández, E. H., & Aschner, M. (2011). Role of manganese in neurodegenerative diseases. *Journal of trace elements in medicine and biology*, 25(4), 191-203.

Cindrić, I.J., Zeiner, M., Mihajlov-Konanov, D., & Stingeder, G. (2017). Inorganic macro-and micronutrients in "Superberries" black chokeberries (*Aronia melanocarpa*) and related teas. *International journal of environmental research and public health*, 14(5), 539.

Cui, J., Zhang, Y., Zhang, H., Jin, H., He, L., Wang, H., Lu, P., Miao, C., Yu, J. & Ding, X. (2024). Low-Potassium Fruits and Vegetables: Research Progress and Prospects. *Plants*, 13(14), 1893.

Denev, P., Kratchanova, M., Petrova, I., Klisurova, D., Georgiev, Y., Ognyanov, M., & Yanakieva, I. (2018). Black chokeberry (Aronia melanocarpa (Michx.) Elliot) fruits and functional drinks differ significantly in their chemical composition and antioxidant activity. *Journal of Chemistry*, 2018(1), 9574587.

Denev, P. N., Kratchanov, C. G., Ciz, M., Lojek, A., & Kratchanova, M. G. (2012). Bioavailability and antioxidant activity of black chokeberry (*Aronia melanocarpa*) polyphenols: in vitro and in vivo evidences and possible mechanisms of action: a review. *Comprehensive Reviews in Food Science and Food Safety*, 11(5), 471-489.

Dietary Reference Intakes (DRIs): Recommended Dietary Allowances and Adequate Intakes, Elements, Food and Nutrition Board, National Academies NCBI Bookshelf (n.d.) *Retrieved from https://www.ncbi.nlm.nih.gov/books/NBK545442/table/appJ_tab3/?report=objectonly*

Dieter, S., Margherita, B., Laurent, B., Kevin, C. J., Bettina, G. K., Christer, H., & Elsa, N. (2020). Update of the risk assessment of nickel in food and drinking water. *EFSA Journal*, 18(11).

Djuric, M., Brkovic, D. U. Š. K. O., Milosevic, D., Pavlovic, M., & Curcic, S. (2015). Chemical characterisation of the fruit of black chokeberry grown on different types of soil. *REV CHIM (Bucharest)*, 66, 178-781.

Dobros, N., Zielińska, A., Siudem, P., Zawada, K. D., & Paradowska, K. (2024). Profile of Bioactive Components and Antioxidant Activity of Aronia melanocarpa Fruits at Various Stages of Their Growth, Using Chemometric Methods. *Antioxidants*, 13(4), 462.

Engin, S. P., & Mert, C. (2020). The effects of harvesting time on the physicochemical components of aronia berry. *Turkish Journal of Agriculture and Forestry*, 44(4), 361-370.

Finley, J. W., Penland, J. G., Davis, C. D., & Pettit, R. E. (2003). Dietary manganese intake and type of lipid do not affect clinical or neuropsychological measures in healthy young women. *The Journal of nutrition*, 133(9), 2849-2856.

Genchi, G., Carocci, A., Lauria, G., Sinicropi, M. S., & Catalano, A. (2020). Nickel: Human health and environmental toxicology. *International journal of environmental research and public health*, 17(3), 679.

Havel, P. J. (2004). A scientific review: the role of chromium in insulin resistance. *The Science of Diabetes Self-Management and Care*, (3 SUPPL.), 2-14.

Hocking, B., Tyerman, S. D., Burton, R. A., & Gilliham, M. (2016). Fruit calcium: transport and physiology. *Frontiers in plant science*, 7, 569.

Höllriegl, V. (2019). Other environmental health issues: Strontium in the environment and possible human health effects. *Encyclopedia of Environmental Health (Second Edition). Elsevier*, 797-802.

Huang, X. L., Gao, Y. A., Zhang, Y. D., Wang, J. Q., & Zheng, N. (2023). Research progress on strontium function in foods. *Shipin Kexue/Food Science,* 44(15), 397-406

Ji, P., Wang, P., Chen, H., Xu, Y., Ge, J., Tian, Z., & Yan, Z. (2023). Potential of copper and copper compounds for anticancer applications. *Pharmaceuticals*, 16(2), 234.

Jurendić, T., & Ščetar, M. (2021). *Aronia melanocarpa* products and by-products for health and nutrition: A review. *Antioxidants*, 10(7), 1052.

King, E. S., & Bolling, B. W. (2020). Composition, polyphenol bioavailability, and health benefits of aronia berry: A review. *Journal of Food Bioactives*, 11.

Kirkland, A. E., Sarlo, G. L., & Holton, K. F. (2018). The role of magnesium in neurological disorders. *Nutrients*, 10(6), 730.

Kulling, S. E., & Rawel, H. M. (2008). Chokeberry (*Aronia melanocarpa*) - A review on the characteristic components and potential health effects. *Planta medica*, 74(13), 1625-1634.

Kuru, R., Yilmaz, S., Balan, G., Tuzuner, B. A., Tasli, P. N., Akyuz, S., & Sahin, F. (2019). Boron-rich diet may regulate blood lipid profile and prevent obesity: A non-drug and self-controlled clinical trial. *Journal of Trace Elements in Medicine and Biology*, 54, 191-198.

Lancrajan, I. (2012). *Aronia melanocarpa*, a potential therapeutic agent. *Studia Universitatis "Vasile Goldis" Arad. Seria Stiintele Vietii (Life Sciences Series)*, 22(3), 389.

Mahoney, J. D., Hau, T. M., Connolly, B. A., & Brand, M. H. (2019). Sexual and apomictic seed reproduction in *Aronia* species with different ploidy levels. *HortScience*, 54(4), 642-646.

Miljkovic, D., Scorei, R. I., Cimpoiașu, V. M., & Scorei, I. D. (2009). Calcium fructoborate: plant-based dietary boron for human nutrition. *Journal of dietary supplements*, 6(3), 211-226.

Nielsen, F. H., & Eckhert, C. D. (2020). Boron. *Advances in Nutrition*, 11(2), 461-462.

NORMA 13/12/2000 - Portal Legislativ(n.d.) *Retrieved from https://legislatie.just.ro/public/DetaliiDocument/26542.*

Panico, A. M., Garufi, F., Nitto, S., Di Mauro, R., Longhitano, R. C., Magrì, G., Catalfo, A., Serrentino,

M. E., & De Guidi, G. (2009). Antioxidant activity and phenolic content of strawberry genotypes from *Fragaria* x *ananassa*. *Pharmaceutical Biology*, 47(3), 203-208.

Pavlović, A. N., Brcanović, J. M., Veljković, J. N., Mitić, S. S., Tošić, S. B., Kaličanin, B. M., Kostic, D. A., Eorcrossed D Signevic, M. S., & Velimirovic, D. S. (2015). Characterization of commercially available products of aronia according to their metal content. *Fruits*, 70(6), 385-393.

Peana, M., Medici, S., Dadar, M., Zoroddu, M. A., Pelucelli, A., Chasapis, C. T., & Bjørklund, G. (2021). Environmental barium: potential exposure and health-hazards. *Archives of toxicology*, 95(8), 2605-2612.

Pieszka, M., Gogol, P., Pietras, M., & Pieszka, M. (2015). Valuable components of dried pomaces of chokeberry, black currant, strawberry, apple and carrot as a source of natural antioxidants and nutraceuticals in the animal diet. *Annals of Animal Science*, 15(2), 475.

Pourcel, L., Routaboul, J. M., Cheynier, V., Lepiniec, L., & Debeaujon, I. (2007). Flavonoid oxidation in plants: from biochemical properties to physiological functions. *Trends in plant science*, 12(1), 29-36.

Predescu, N. C., Papuc, C., Nicorescu, V., Gajaila, I. U. L. I. A. N. A., Goran, G. V., Petcu, C. D., & Stefan, G. E. O. R. G. E. T. A. (2016). The influence of solid-to-solvent ratio and extraction method on total phenolic content, flavonoid content and antioxidant properties of some ethanolic plant extracts. *Rev. Chim*, 67(10), 1922-1927.

Ross, A. C., Caballero, B., Cousins, R. J., & Tucker, K. L. (2012). *Modern nutrition in health and disease: Eleventh Edition.* Vol. 40. Wolters Kluwer Health Adis (ESP)

Salt Intake (n.d.) Retrieved from https://www.who.int/data/gho/indicator-metadata-registry/imr-details/3082

SCHER (Scientific Committee on Health and Environmental Risks). (2012). Assessment of the tolerable daily intake of barium. *European Commission*, 13-13.

Shireen, F., Nawaz, M. A., Chen, C., Zhang, Q., Zheng, Z., Sohail, H., Sun, J., Cao, H., Huang, Y., & Bie, Z. (2018). Boron: functions and approaches to enhance its availability in plants for sustainable agriculture. *International journal of molecular sciences*, 19(7), 1856.

Sidor, A., Drożdżyńska, A., & Gramza-Michałowska, A. (2019). Black chokeberry (*Aronia melanocarpa*) and its products as potential health-promoting factors-An overview. *Trends in Food Science & Technology*, 89, 45-60.

Šnebergrová, J., Čížková, H., Neradová, E., Kapci, B., Rajchl, A., & Voldřich, M. (2014). Variability of characteristic components of aronia. *Czech Journal of Food Sciences* 32(1), 25-30.

Stahl, T., Taschan, H., & Brunn, H. (2011). Aluminium content of selected foods and food products. *Environmental Sciences Europe*, 23, 1-11.

Subbiah, V., Zhong, B., Nawaz, M. A., Barrow, C. J., Dunshea, F. R., & Suleria, H. A. (2020). Screening of phenolic compounds in Australian grown berries by LC-ESI-QTOF-MS/MS and determination of their antioxidant potential. *Antioxidants*, 10(1), 26.

Teneva, D., Pencheva, D., Petrova, A., Ognyanov, M., Georgiev, Y., & Denev, P. (2022). Addition of medicinal plants increases antioxidant activity, color, and anthocyanin stability of black chokeberry (*Aronia melanocarpa*) functional beverages. *Plants*, 11(3), 243.

Tolić, M. T., Landeka Jurčević, I., Panjkota Krbavčić, I., Marković, K., & Vahčić, N. (2015). Phenolic content, antioxidant capacity and quality of chokeberry (*Aronia melanocarpa*) products. *Food technology and biotechnology*, 53(2), 171-179.

Tolic, M. T., Krbavcic, I. P., Vujevic, P., Milinovic, B., Jurcevic, I. L., & Vahcic, N. (2017). Effects of weather conditions on phenolic content and antioxidant capacity in juice of chokeberries (*Aronia melanocarpa* L.). *Polish journal of food and nutrition sciences*, 67(1).

Valero, D., & Serrano, M. (2013). Growth and ripening stage at harvest modulates postharvest quality and bioactive compounds with antioxidant activity. *Stewart Postharvest Rev*, 3(5), 1-8.

Wang, M., Zheng, Q., Shen, Q., & Guo, S. (2013). The critical role of potassium in plant stress response. *International journal of molecular sciences*, 14(4), 7370-7390.

Xie, L., Lee, S. G., Vance, T. M., Wang, Y., Kim, B., Lee, J. Y., Chun, O. K., & Bolling, B. W. (2016). Bioavailability of anthocyanins and colonic polyphenol metabolites following consumption of aronia berry extract. *Food chemistry*, 211, 860-868.

Xie, L., Vance, T., Kim, B., Lee, S. G., Caceres, C., Wang, Y., Hubert, P. A., Lee, J. Y., Chun, O. K., & Bolling, B. W. (2017). Aronia berry polyphenol consumption reduces plasma total and low-density lipoprotein cholesterol in former smokers without lowering biomarkers of inflammation and oxidative stress: A randomized controlled trial. *Nutrition research*, 37, 67-77.

Yang, H., Kim, Y. J., & Shin, Y. (2019). Influence of ripening stage and cultivar on physicochemical properties and antioxidant compositions of aronia grown in South Korea. *Foods*, 8(12), 598.

Zhao, Y. W., Wang, C. K., Huang, X. Y., & Hu, D. G. (2021). Anthocyanin stability and degradation in plants. *Plant signaling & behavior*, 16(12), 1987767.

Zagoskina, N. V., Zubova, M. Y., Nechaeva, T. L., Kazantseva, V. V., Goncharuk, E. A., Katanskaya, V. M., & Aksenova, M. A. (2023). Polyphenols in plants: Structure, biosynthesis, abiotic stress regulation, and practical applications. *International Journal of Molecular Sciences*, 24(18), 13874.

Zambelli, B., & Ciurli, S. (2013). Nickel and human health. Interrelations between essential metal ions and human diseases, *Metal Ions in Life Sciences,* 13, 321-357.

Zoratti, L., Karppinen, K., Luengo Escobar, A., Häggman, H., & Jaakola, L. (2014). Light-controlled flavonoid biosynthesis in fruits. *Frontiers in plant science*, 5, 534.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

CONTRIBUTIONS TO THE KNOWLEDGE OF THE DISTRIBUTION OF SOME RARE TAXA IN THE FLORA OF OLTENIA (ROMANIA)

Daniel RĂDUȚOIU

University of Craiova, 13 A.I. Cuza Street, Craiova, Romania

Corresponding author email: radutoiudaniel@gmail.com

Abstract

Oltenia is a region located in southwestern Romania and it is characterized by great climatic, pedological, geomorphological, and floristic diversity. In this part of Romania, many southern elements find their habitat, some of them being rare in the flora of the country (e.g., Cirsium creticum). Climate changes in recent years have led to obvious changes among plant taxa, both in terms of distribution in nature and especially in their phenology. For some taxa, these changes led to the widening of the area (e.g., the psammophilous elements: Erodium hoefftianum subsp. neilreichii, Mollugo cerviana, Bassia laniflora, Achillea ochroleuca, Salvia sclarea, Silene borysthenica, Limonium tomentellum, Polycarpon tetraphyllum, Hordeum bulbosum), while for others on the contrary, to its restriction (Aldrovanda vesiculosa, Pisum sativum subsp. elatius).

Key words*: flora, ecology, distribution, Oltenia, Romania.*

INTRODUCTION

Year by year, the impacts of climate change worsen and affect plants worldwide, shifting plant communities, resulting in changes in flora biodiversity and disturbing their life cycles and function (Sărățeanu et al., 2023). In this context, measures are required for restoring ecosystems as efficient tools for reducing climate related risks (Acatrinei et al., 2024; Durău et al., 2021; Sălceanu et al., 2023; Velea et al., 2021).

The study of rare taxa in the Romanian flora has drawn the attention of numerous researchers. This interest is demonstrated by the specialized works that have tackled this category of plants (Buia & Păun, 1958, 1960a, 1960b, 1964; Buia, 1959; Buia et al., 1960; Buia & Maloș, 1963; Popescu, 1971, 1974, 1979; Popescu et al., 2001, 2003; Costache, 2004b; Oprea, 2005; Dihoru & Negrean, 2009; Boruz et al., 2013; Răduțoiu & Costache, 2012; Negrean & Ciortan, 2012; Răduțoiu et al., 2013a, 2013b, 2023; Răduțoiu, 2014, 2022; Răduțoiu et al., 2014, 2016a, 2016b; Răduțoiu & Stan, 2019; Răduțoiu & Ștefănescu, 2021; Răduțoiu & Măceșeanu, 2021; Răduțoiu & Niculescu, 2023). An additional element of interest is that Oltenia comprises the range limit of plants with different sozological degrees. Thus, the two above-mentioned elements enable us to state that this part of Romania will represent an attractive territory for numerous researchers.

A simple analysis concerning the presence of spontaneous vascular plants in the Oltenia region, as compared to other regions in Romania, leads to the conclusion that Oltenia occupies a leading place (about 2/3 of Romania's spontaneous flora is located within this region). The climatic, geomorphological, and pedological conditions that characterize this part of the country also contribute to its high floristic diversity. A special peculiarity is represented by the southern elements, which are present in several areas within Oltenia, having a better representation in the southwestern part (e.g. *Hordeum bulbosum* L.).

Moreover, certain species mentioned as rare at national level are also found in Oltenia, although in this part of the country they form phytocenosis on representative surfaces (e.g., *Medicago arabica* (L.) Huds., *M. polymorpha* L.) (Buia, 1959). Furthermore, in Oltenia there are reported genera that have all the spontaneous species within Romania (e.g., *Quercus*, *Tilia*, *Acer*), or species that find their range only in this part of the country (e.g., *Trifolium michelianum* Savi, *T. squamosum* L., *Ranunculus garganicus* Ten., *Dianthus kladovanus* Degen.) (Buia, 1959).

MATERIALS AND METHODS

Area under study

Oltenia is located in southwestern Romania and, taking into account its characteristics related to relief, climate, soil, etc., as well as its borders, this region also constitutes a well-defined natural unit. Oltenia is delimited to the east by the Olt River, to the south and west by the Danube, and to the north by the Southern Carpathians. The Jiu River flows across the region on a general north-south direction and divides Oltenia into two approximately equal parts (Buia et al., 1961).

Three climatic regimes are characteristic to the territory of Oltenia: Pontic, Central European, and Sub-Mediterranean.

Materials

In order to visualize the distribution of the analyzed taxa within Oltenia, numerous field-trips were conducted over time, in different vegetation phases, with the aim of collecting all the necessary information for correct identifications and for a comprehensive distribution. In order to observe the changes that occurred under the influence of climate changes during the recent period, the collected data were compared with those existing in the specialized literature.

For each taxon under study, the collected data comprised the coordinates, the ecological conditions in which it grows in Oltenia, and the chorological toponymy. The distribution of each taxon is also represented on two maps that correlate its location with the average annual temperatures in the country and, respectively, with the average annual precipitation in Romania. The Corolog 2010 Program, developed at the Institute of Biology in Bucharest, was used to achieve these maps. The above-mentioned Program uses an Access database (RoBioAtlas, 2023), which integrates information from the specialized literature, the herbaria, and the field. The data originating in literature and herbaria are marked in red on the maps, while the new settlements where the taxa were identified are marked in green.

The abbreviations of the authors of the analyzed species were consistent with Brummit and Powell (1992) and the species names were in accordance with Euro+Med (2006-).

RESULTS AND DISCUSSIONS

Achillea ochroleuca Ehrh. – *Asteraceae*

Ecology: it grows on sandy, xeric meadows, or on sandy-gravelly substrate.

General distribution: it is a southern element of Pontic-Pannonic origin.

Distribution in Oltenia: it is known within Oltenia from the sandy surfaces located on the left side of the Jiu River, from the Obedeanu fountain – Craiova (Buia & Popescu-Mihăilă, 1952), from the settlements of Ghindeni, Apele Vii, Celaru, Ogrin, and Dăbuleni (Buia & Păun, 1964).

The species was identified between Sadova and Piscu Sadovei settlements, in Ciupercenii Noi and Desa settlements (Dolj County) (Figures 1, 2).

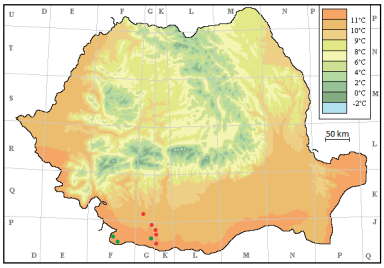


Figure 1. Distribution of the species *Achillea ochroleuca* in Oltenia, correlated with the average annual temperatures

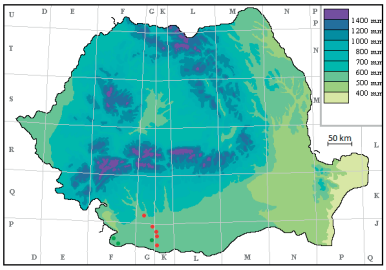


Figure 2. Distribution of the species *Achillea ochroleuca* in Oltenia, correlated with the average annual precipitation

Aldrovanda vesiculosa L. – *Droseraceae*

Ecology: it is found in ponds with stagnant, shallow water and with muddy bottom (Țopa, 1955).

General distribution: it is a cosmopolitan element, encountered in aquatic habitats. At the European level, it is protected by the Council Directive 92/43/EEC 1992, Annex II, while in Romania it is critically endangered (Dihoru &

Negrean, 2009). It is also on the IUCN Red List (Bilz et al., 2011).

Distribution in Oltenia: in the ponds located between Craiova and Breasta, as well as in those between Cernele and Atârnați settlements (Buia & Păun, 1964).

At the beginning of botanical research in Oltenia, this plant was mentioned from the Danube and the Jiu floodplains (Păun et al., 1967; Păun & Popescu, 1969, 1972), where it formed compact phytocenosis, whereas the present situation shows that this taxon has greatly reduced its distribution area because of natural causes (reduction of water level and lack of precipitation for a significant period in this part of the country), or anthropogenic ones (drainage actions in order to change the land use categories of the respective terrains).

In recent years, the species has been identified in the ponds located near the settlements of Izvoarele, Ceplea, Sărdănești, Bâlteni, and Pește ana-Jiu (Gorj County) (Figures 3, 4).

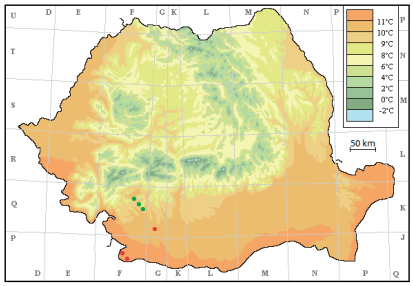


Figure 3. Distribution of the species *Aldrovanda vesiculosa* in Oltenia, correlated with the average annual temperatures

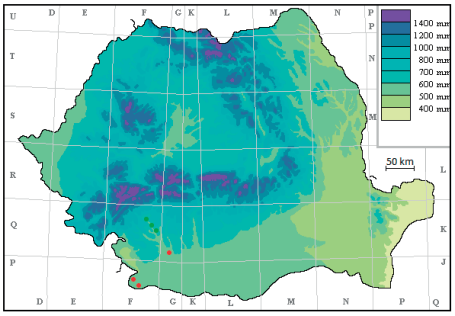


Figure 4. Distribution of the species *Aldrovanda vesiculosa* in Oltenia, correlated with the average annual precipitation

Bassia laniflora (S.C. Gmel.) A.J. Scott – *Chenopodiaceae*

Ecology: it is found on sand dunes, loess or sandy terrain located in the plain region.

General distribution: continental Eurasia.

It is known as a rare plant in almost all specialized field guides in the country (Ciocârlan, 2009, Sârbu et al., 2013).

Distribution in Oltenia: within this region, it was found in Craiova, on the Obedeanu dunes, on the outskirts of Malu Mare, Secui, Ghindeni, and Apele Vii settlements (Buia & Păun, 1964), as well as in Dăbuleni (Dihoru & Negrean, 2009).

The author of the present study found it on xerophilic areas that are characterized by the presence of sand in different degrees and are located on the outskirts of Lișteava, Sadova, and Piscu Sadovei settlements (Figures 5, 6).

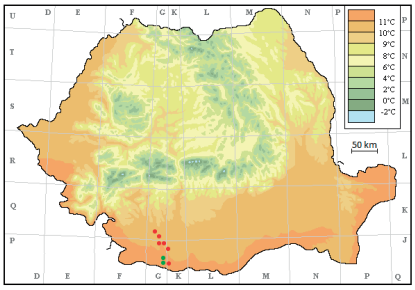


Figure 5. Distribution of the species *Bassia laniflora* in Oltenia, correlated with the average annual temperatures

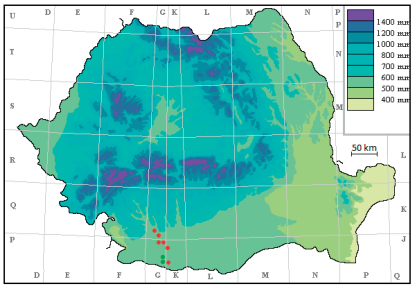


Figure 6. Distribution of the species *Bassia laniflora* in Oltenia, correlated with the average annual precipitation

Cirsium creticum (Lam.) d'Urv. – *Asteraceae*

Ecology: it is present in marshy areas or on the banks of water bodies, either in small groups of a few specimens, or on variable surfaces, ranging between 20 and 500 sq m.

General distribution: it is a Mediterranean element, the development range of which extends in the Balkan Peninsula, southern Italy, and Sicily (Dihoru & Negrean, 2009).

It is an endangered taxon, with the northern range limit on the territory of our country, being mentioned only from the southwestern part of Romania, from the plain area to the lower mountainous region, in marshy places or on the banks of water bodies (Ciocârlan, 2009; Costache 2004a, 2005, 2011; Răduțoiu & Costache 2009; Răduțoiu & Stan, 2019).

Distribution in Oltenia: The research conducted over the last 15 years has provided valuable information regarding the chorology of the species *Cirsium creticum* in Oltenia. In numerous locations, this plant has been identified under the form of scattered specimens found in the floodplains of certain rivers or hillside streams within Dolj, Gorj, and Mehedinți counties, while in other locations, it forms well-represented populations.

Following his studies in the lower catchment of the Motru River (2005), Costache I. is the first to attest to the presence of phytocenosis whose physiognomy is given by this species. Subsequent research led to the conclusion that in Oltenia, the areas dominated by this species will extend into the floodplains of the main rivers and of certain streams flowing on slopes (e.g., on the outskirts of Fratoștița village) (Răduțoiu & Ștefănescu, 2021; Răduțoiu & Măceșeanu, 2021), in the settlements of Șimian, Hinova, Vârciorova, Porțile de Fier, Strehaia, and Valea Topolniței in Mehedinți County, Motru (Gorj County), Cilieni (Olt County), and Calafat, Malu Mare, Tâmburești, Ciuperceni, Botoșești Paia (Dolj County).

It was identified in the Jiu River floodplain, near the settlements of Rojiște, Valea Stanciului, Ostroveni, Padea, Potmelțu, Tâmburești, Gângiova, Comoșteni, Horezu-Poenari, and Țuglui (Figures 7, 8).

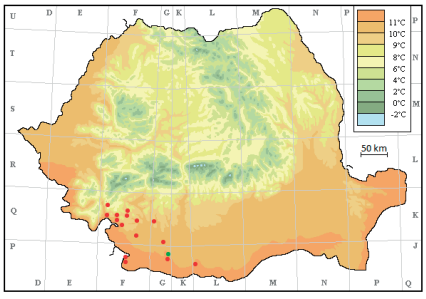


Figure 7. Distribution of the species *Cirsium creticum* in Oltenia, correlated with the average annual temperatures

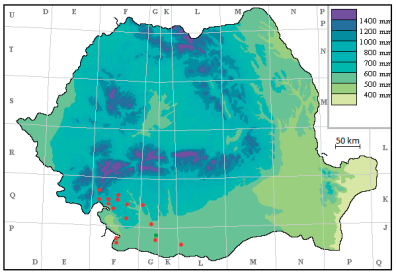


Figure 8. Distribution of the species *Cirsium creticum* in Oltenia, correlated with the average annual precipitation

Erodium hoefftianum C.A. Mey. subsp. ***neilreichii*** (Janka) P. H. Davis – *Geraniaceae*

Ecology: it is a critically endangered plant that grows on psammophilous, dry, sunny, thermophilic areas.

General distribution: East European-Anatolian-Turanian element.

Distribution in Oltenia: it is mentioned from the sandy areas located around Craiova (Buia & Păun, 1964; Răduțoiu & Costache, 2009; Cârțu & Cârțu, 1972), Tâmburești (Cârțu & Cârțu, 1972), Ciupercenii Noi (Dolj County), Ianca (Olt County), and Bistrețu (Mehedinți County) (Dihoru & Negrean, 2009). It was identified on significant areas on the outskirts of Malu Mare, Secui, and Rojiște settlements (Figures 9, 10).

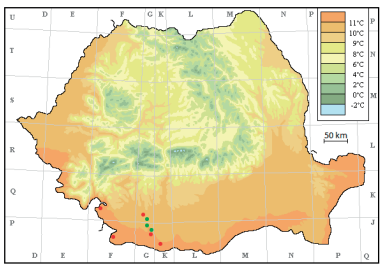


Figure 9. Distribution of the species *Erodium hoefftianum* subsp. *neilreichii* in Oltenia, correlated with the average annual temperatures

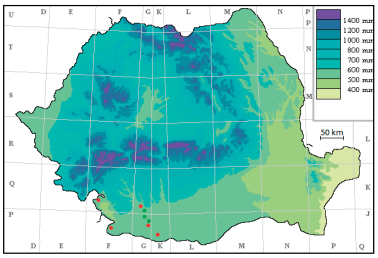


Figure 10. Distribution of the species *Erodium hoefftianum* subsp. *neilreichii* in Oltenia, correlated with the average annual precipitation

Hordeum bulbosum L. – *Poaceae*

Ecology: pastures, sunny orchards, and shrub edges.

General distribution: Mediterranean element (Euro-North African-Asian).

Distribution in Oltenia: the specialized literature mentions it from the following settlements: Coțofenii din Față, Craiova (Mofleni, Lascăr Catargiu, and Popoveni), Verbița, Verbicioara, Desa, and Nebuna (Dolj County) (Buia, 1959), Perișor, Giubega (Răduțoiu & Costache, 2009), Valea Topolniței, Schitul Topolniței (Mehedinți County) (Negrean & Ciortan, 2012), Radovan, Drăgoaia, Mărăcine, Ciutura, Răpa Roșie, and Terpezița (Dolj County) (Răduțoiu & Stan, 2019). It was identified on the side of the roads in the field, near wheat crops and orchards, between Vârtop and Caraula settlements (Figures 11, 12).

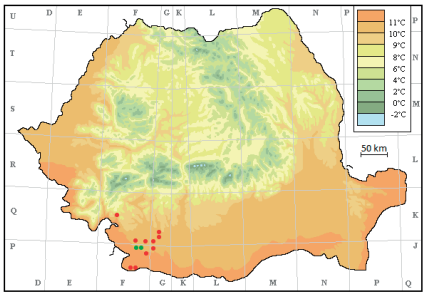


Figure 11. Distribution of the species *Hordeum bulbosum* in Oltenia, correlated with the average annual temperatures

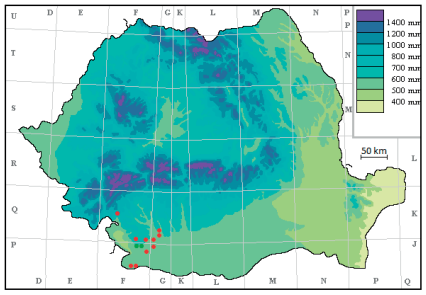


Figure 12. Distribution of the species *Hordeum bulbosum* in Oltenia, correlated with the average annual precipitation

Limonium tomentellum (Boiss.) Kuntze – *Plumbaginaceae*

Ecology: in saline, usually humid meadows located within the Oltenia Plain.

General distribution: Continental element. Southern and Eastern Ukraine, Eastern Russia, Kazakhstan.

Distribution in Oltenia: in most specialized papers, this taxon is mentioned from the classic location, i.e., Bratovoiești settlement (Ciocârlan, 2009; Sârbu et al., 2013; Răduțoiu et al., 2016b). Subsequently, it was also found under the form of scarce populations in Osica de Sus settlement (Olt County) (Răduțoiu, 2013), or as isolated specimens in Seaca de Câmp settlement (Răduțoiu & Costache, 2009), or herborized in the herbarium of the University of Craiova (CRA): the settlements of Afumați, Gura Jiului, Poiana Mare, Urzicuța, Tâmburești, Craiova – Lascăr Catargiu, between Malu Mare and Bratovoiești (Răduțoiu, 2014).

Recently, it was found under the form of isolated specimens in the Jiu floodplain, at the edge of some bushes situated on the outskirts of *Quercus robur* forests; near Rojiște settlement (Figures 13, 14) and in a subspontaneous form, in vegetative stage, near some flower shops. It must be noted that the inflorescences of this plant are frequently used in floral arrangements in some flower shops located in the municipality of Craiova.

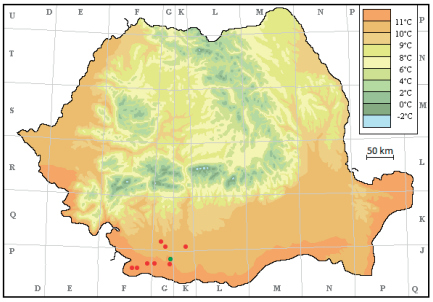


Figure 13. Distribution of the species *Limonium tomentellum* in Oltenia, correlated with the average annual temperatures

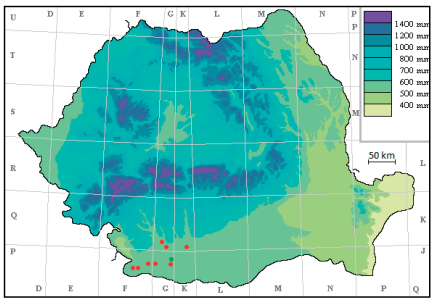


Figure 14. Distribution of the species *Limonium tomentellum* in Oltenia, correlated with the average annual precipitation

Mollugo cerviana (L.) Ser. – *Molluginaceae*
Ecology: it is a vernal plant that is encountered on sandy or sandy-clayey, dry soils, but also in more humid habitats located in various poorly maintained crops.
General distribution: Southern Europe.
Distribution in Oltenia: in this region, it is known from the settlements of Apele Vii, Craiova, Ghindeni, Secui, Cernele, Malu Mare, Teasc, Bratovoiești, Daneț, Mârșani, Tâmburești, Rojiște, Ogrin, Dăbuleni (Dolj County), and Ianca (Olt County) (Buia, 1959; Buia & Păun, 1964), Poiana Mare, Desa, and Nebuna (Buia, 1959).
The only other location where it is recorded at national level concerns the sands of southern Moldavia (Buia, 1959).
It was identified by the author of this study in the Jiu River floodplain, on sandy terrain, on the outskirts of Sadova settlement (Figures 15, 16), around Lake Victoria, located at Adunații de Georgmane.

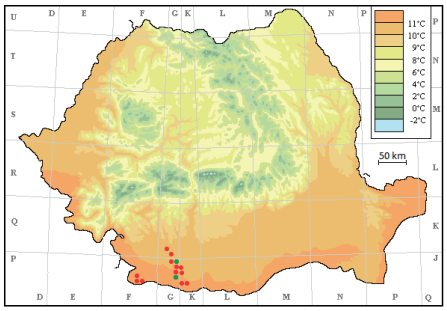


Figure 15. Distribution of the species *Mollugo cerviana* in Oltenia, correlated with the average annual temperatures

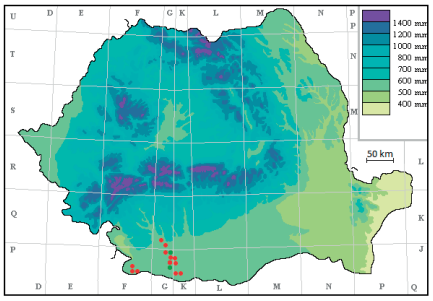


Figure 16. Distribution of the species *Mollugo cerviana* in Oltenia, correlated with the average annual precipitation

Pisum sativum L. subsp. ***elatius*** (M. Bieb.) Asch. & Graebn. – *Fabaceae*
Ecology: it is rarely found in fields, pea crops, shrub edges and ruderal places.
General distribution: it is a Mediterranean element.
Distribution in Oltenia: in Romania, it is known only from Mehedinți County, i.e. from the Danube Gorges, at Iron Gates (Ro: *Porțile de Fier*), Gura Văii, Vârciorova, and Schela Cladovei. With respect to its ecology, it is stated that this plant is found in thickets, on limestone rocks, rarely on sowed areas or in ruderal places (Țopa 1957, in Săvulescu et al. 1957; Ciocârlan, 2009).
This is the first mention of this subspecies in Dolj County, namely in Preajba settlement, on the edge of a pea crop (Figures 17, 18).

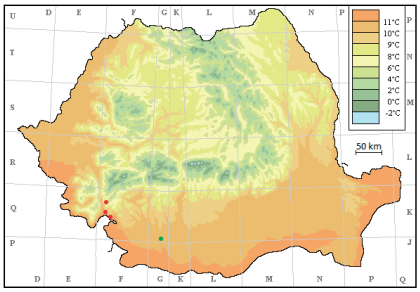


Figure 17. Distribution of the species *Pisum sativum* subsp. *elatius* in Oltenia, correlated with the average annual temperatures

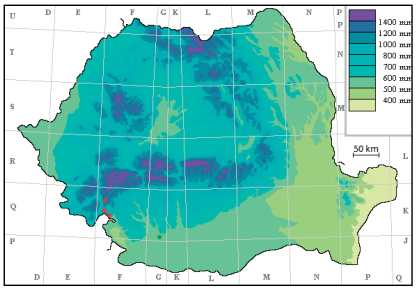


Figure 18. Distribution of the species *Pisum sativum* subsp. *elatius* in Oltenia, correlated with the average annual precipitation

Polycarpon tetraphyllum (L.) L. - *Caryophyllacaeae*
Ecology: it is a heliophilous, thermophilic taxon, which prefers ruderal places or areas with varying degrees of human-induced changes.
General distribution: Atlantic-Mediterranean-Sub-Mediterranean element.
Distribution in Oltenia: it is mentioned from Orșova (Mehedinți County) (Morariu, 1963;

Beldie & Vaczy 1976), Valea Stanciului (Costache & Răduțoiu, 2010, in Răduțoiu et al., 2023), Craiova (Cârțu & Cârțu, 1989; Răduțoiu et al., 2023).
Recent field research has led to the conclusion that the range of this species will increase in the future. This is also supported by the fact that it was identified in rich populations in other areas located in the municipalities of Bucharest (Anastasiu et al., 2024) and Craiova.

Salvia sclarea L. – *Lamiaceae*

Ecology: it is present on dry soils, in arid meadows and in grassy areas located on the sides of roads within the plain region.

General distribution: it is a Mediterranean element.

Distribution in Oltenia: the specialized literature indicates it from dry places located on Ciuperceni and Maglavit settlements (Buia, 1959), and from the outskirts of Băilești town (Răduțoiu & Stan, 2019). It is frequently cultivated in the settlements of the Danube floodplain (Răduțoiu & Ștefănescu, 2021).
It was identified as subspontaneous on the outskirts of Vârtop village, in grassy places located on the roadside, in the direction of Caraula settlement (Figures 19, 20).

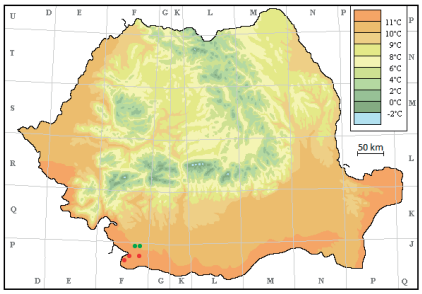


Figure 19. Distribution of the species *Salvia sclarea* in Oltenia, correlated with the average annual temperatures

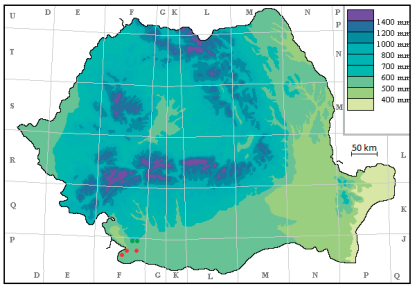


Figure 20. Distribution of the species *Salvia sclarea* in Oltenia, correlated with the average annual precipitation

Silene borysthenica (Gruner) Walters – *Caryophyllacaeae*

Ecology: it is a thermophilic, heliophilous species, which prefers sandy areas.

General distribution: it is a central and eastern European element. In the Romanian field guides, it is mentioned as a rare plant from sandy places (Ciocârlan, 2009; Sârbu et al., 2013) and the Red Book of Vascular Plants of Romania mentions it as an endangered taxon (Dihoru & Negrean, 2009).

Distribution in Oltenia: it was recorded on the sand dunes within the Obedeanu area – Craiova (Popescu et al., 2003), on the Ciuperceni-Desa area (Răduțoiu et al., 2016b), and on the outskirts of Secui settlement, Dolj County (Răduțoiu & Stan, 2019).
It is frequently found on the sands from Dăbuleni (Figures 21, 22).

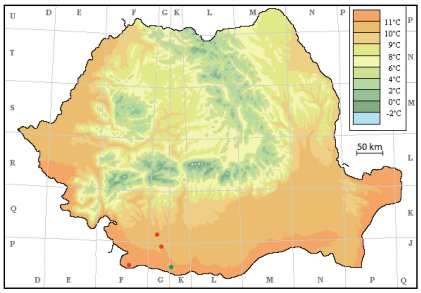


Figure 21. Distribution of the species *Silena borysthenica* in Oltenia, correlated with the average annual temperatures

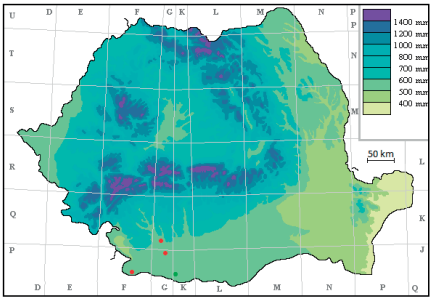


Figure 22. Distribution of the species *Silena borysthenica* in Oltenia, correlated with the average annual precipitation

CONCLUSIONS

The analysis of the plants tackled in this paper shows that those preferring dry, psammophilous places are gradually expanding their range (e.g.

Silene borysthenica, Erodium hoefftianum subsp. *neilreichii*, *Achillea ochroleuca*, *Bassia laniflora*), which is explainable if we take into account the constantly changing climatic conditions in this part of Romania (temperatures are considerably increasing and precipitation is decreasing).

At the national level, the twelve taxa under study display different sozological degrees: rare (e.g., *Achillea ochroleuca*, *Mollugo cerviana*, *Bassia laniflora*, *Pisum sativum* subsp. *elatius*), vulnerable (e.g., *Limonium tomentellum*), endangered (*Cirsium creticum*, *Salvia sclarea*, *Silene borysthenica*), or critically endangered (e.g., *Erodium hoefftianum* subsp. *neilreichii*, *Polycarpon tetraphyllum*, *Hordeum bulbosum* and *Aldrovanda vesiculosa*).

ACKNOWLEDGEMENTS

Part of the data presented in this paper was supported by the following project of the University of Craiova: "*Revizuirea Planului de Management al Ariilor Naturale Protejate ROSC I0045 Coridorul Jiului, ROSPA0023 Confluenta Jiu-Dunăre, ROSPA0010 Bistreț și rezervațiile natural Locul Fosilifer Drănic-2391și Pădurea Zăval- IV.33*".

REFERENCES

Acatrinei S., Stavarache, M., Gavrilă, C.S., Vacarciuc, E.M., Dudău, A.M., Bouruc, D., Stavarache, E., Paraschivu M., Sălceanu, C., Brezuleanu, S. (2024). Improving economic efficiency on degraded permanent meadows by various management systems. *Scientific Papers. Series Management, Economic Engineering in Agriculture and Rural Development,* Vol. 24, Issue 1: 17-23.

Anastasiu, P., Camen-Comănescu, P., Sîrbu I., M., Urziceanu M. (2024). Urban Flora: new reports for Bucharest'flora. *Acta Horti Bot.*, 50: 41-58.

Beldie, Al., Váczy, C. (1976). Taxoni noi pentru flora României, publicați după editarea volumelor respective din "Flora R.S.R.", 13: 35-53. In: Săvulescu Tr. (edit.), Pop E. (coord.). *Flora României*. Edit. Acad. Române.

Bilz, M., Kell, S.P., Maxted, N., Lansdown, R.V. (2011). *European Red List of Vascular Plants*. Publications Office of the European Union, Luxembourg.

Boruz, V., Dihoru, Gh., Răduțoiu, D. (2013). Areal limit in the Romanian territory: 6. *Secale strictum*. *Annals of the University of Craiova – Agriculture, Montanology, Cadastre series.* Vol. XLIII: 78-82.

Brummitt, R.K., Powell, C.E. (1992). *Authors of plant names,* 732 pag. Royal Botanic Gardens. Kew. (EDS.).

Buia, Al., Popescu-Mihăilă, A. (1952). Contribuţii la flora regiunii Craiova. *Buletin Ştiinţific. Secţiunea de Ştiinţe Biologice, Agronomice, Geologice şi Geografice.* Tom. IV. nr. 3: 519-536. Bucureşti.

Buia, Al., Păun, M. (1958). Plante spontane pe nisipurile din stânga Jiului. *Lucr. Şti. Inst. Agron*. Craiova, 2: 79-96.

Buia, Al. (1959). Plante rare pentru flora R.P.R., existente în Oltenia. *Ocr. Nat*., 4: 13-42.

Buia, Al., Păun, M. (1960a). Plante noi şi rare din Oltenia. *Contrib. Bot*.: 141-148. Cluj.

Buia, Al., Păun, M. (1960b). Materiale pentru flora şi vegetaţia împrejurimilor oraşului Craiova. SSNG. *Com. de Bot*., 1957-1959, Bucureşti, 281-296.

Buia, Al., Păun, M., Maloş, C. & Olaru, M. (1960). Contribuţii noi pentru flora Olteniei. *Lucr. Şti. Inst. Agron*. Craiova, 4: 121-130.

Buia, Al., Păun, M., Maloş, C. (1961). Pajiştile naturale din Regiunea Craiova şi îmbunătăţirea lor (II). *Probleme Agricole*. Anul XIII: 31-40.

Buia, Al., Maloş, C. (1963). Raritäţi floristice din Oltenia şi importanţa lor. *Lucr. Şti. Inst. Agron*. Craiova, 6: 3-26.

Cârțu, D., Cârțu, M. (1972). Date noi pentru flora cormofită din Oltenia. *Studii şi Comunic*. Muz. De Științele Naturii, Bacău: 113-118.

Cârţu, D., Cârţu, M. (1989). Semina plantarum spontanearum anno 1988 in regione Dolj, Gorj & Mehedinţi lecta. *Index Seminum*, *Hort. Bot. Craiovensis*, 31: 61.

Ciocârlan, V. (2009). *Flora ilustrată a României*. *Pteridophyta et Spermatophyta.* Edit. Ceres, Bucureşti. 1041 pp.

Costache, I. (2004a). Contribution to the Romanian Flora. *Acta Horti. Bot. Bucurest*. 31: 91-96.

Costache, I. (2004b). Ecologia, corologia și cenologia speciilor rare și vulnerabile din Bazinul Inferior al Motrului. *Annals of the University of Craiova. Biology, Horticulture, Food products processing technology, Environmental Engineering*. Vol. VII (XLII), nr. 2, Cluj Napoca: 239-257.

Costache, I. (2005). *Flora şi vegetaţia bazinului hidrografic inferior al râului Motru*. Teza de doctorat. Bucureşti. 290 pp.

Costache, I. (2011). *Flora şi vegetaţia Bazinului Hidrografic Inferior al râului Motru*. Vol. I. *Flora*. Edit. Universitaria. Craiova. 215 pag.

Dihoru, Gh., Negrean, G. (2009). *Cartea Roşie a plantelor vasculare din România*. Edit. Acad. Romane. 630 pag.

Durău, C.C., Sărățeanu, V., Cotuna, O., Paraschivu M. (2021). Impact of the grassland management planning application on some features of the grassland vegetation from Western Romania – Case Study. *Scientific Papers. Series Management, Economic Engineering in Agriculture and Rural Development*, Vol. 21, Issue 3: 325-331.

Euro+Med (2006-): Euro+Med PlantBase - the information resource for Euro-Mediterranean plant diversity. Published on the Internet http://ww2.bgbm.org/EuroPlusMed/ (accessed February 18, 2025).

Morariu I. (1963). Două spermatofite noi pentru flora R.P.R. *Com. Acad.Rom*. XIII, nr. 5: 427-431.

Negrean G., Ciortan I. (2012). New and rare plants for the Geopark Platoul Mehedinți (Oltenia, Romania). *Contrib Bot*., vol. XLVII: 13-24.

Oprea, A. (2005). *Lista critică a plantelor vasculare din România*. Edit. Univ. "Al. I. Cuza" Iași, 668 pp.

Popescu, G. (1971). Plante noi și rare pentru flora Olteniei identificate în bazinul Bistriței, jud. Vîlcea. *Anal. Univ. Craiova*. Vol. III (XIII): 35-42.

Popescu, G. (1974). *Studiul floristic şi geobotanic al Bazinului hidrografic al Bistriţei-Vîlcii.* 303 pag. Teza de doctorat, Bucureşti.

Popescu, G. (1979). Noutăţi floristice şi de vegetaţie din Oltenia. *Stud. Cercet. Biol. Veget., Ser. Bot.* Tom 31. 1: 13-21.

Popescu, G., Costache, I., Răduţoiu, D., Boruz, V. (2001). Conspectul florei şi vegetaţiei acvatice din Oltenia [Overview of the flora and aquatic vegetation of Oltenia]. *Analele Univ. din Oradea. Fascicula Silvicultură*. Tom. VI. 107-128.

Popescu, G., Costache, I., Răduţoiu, D., Boruz, Violeta. 2003. The ecology, coenology and chorology of the endemic and subendemic plant taxa in the region of Oltenia (Romania). *Contrib. Bot*. XXXVIII (2): 147-156.

Răduțoiu, D., Costache, I. (2009). New chorologic data in the region of Oltenia (Romania) (II). *Acta Horti Botanici Bucurest.* 36: 75-78.

Răduțoiu, D., Costache, I. (2012). Contribution to rare taxa chorology of the Romanian flora. *Muzeul Olteniei Craiova. Oltenia. Studii și comunicări. Științele Naturii*. Tom. 28, no. 1: 37-40.

Răduțoiu, D., Costache, I., Simeanu, C.G. (2013a). Contributions to the Romanian vascular flora. *Muzeul Olteniei Craiova. Oltenia. Studii și comunicări. Științele Naturii*. Tom. 29, no. 1: 151-154

Răduțoiu, D., Simeanu, C.G., Stan I. (2013b). Contributions to some taxa chorology of the Romanian flora. (II). *Annals of the University of Craiova. Annals of the University of Craiova. Biology, Horticulture, Food products processing technology, Environmental Engineering*. Vol. XVIII (LIV): 645-650.

Răduțoiu, D. (2014). New data about *Limonium tomentellum* in Oltenia, Romania. Muzeul Olteniei Craiova. *Oltenia. Studii şi comunicări. Ştiinţele Naturii*. Tom. 30, No. 2/: 78-83.

Răduțoiu, D., Stan, I., Costache, I. (2014). Oenanthe pimpinelloides L. in the flora of Romania. *Contrib. Bot*. XLIX: 61-66.

Răduțoiu, D., Stan, I., Ștefănescu, D.M. (2016a). Rare species from Romania threatened by habitat destruction: I. *Ziziphora capitata*. *Annals of the University of Craiova. Biology, Horticulture, Food products processing technology, Environmental Engineering*. Vol. XXI (LVII): 617-622.

Răduțoiu, D., Ștefănescu D.M., Răduțoiu, A. (2016b). Rare plant species not listed in Natura 2000 sites from Oltenia Region (Romania). *Analele Universităţii din Craiova, Seria Agricultură – Montanologie – Cadastru (Annals of the University of Craiova - Agriculture, Montanology, Cadastre Series*) vol. XLVI: 249-254.

Răduțoiu, D., Stan, I. (2019). Vegetal taxa with high sozological degree within Oltenia, Romania (I). *Analele Universităţii din Craiova, Seria Agricultură – Montanologie – Cadastru (Annals of the University of Craiova - Agriculture, Montanology, Cadastre series*) vol. XLIX: 168-174.

Răduțoiu, D., Ștefănescu, D.M. (2021). Discussions regarding the chorology of some species from Oltenia, Romania. *Oltenia. Studii şi comunicări. Ştiinţele Naturii*. Tom. 37, No 1: 57-60.

Răduțoiu, D., Măceșeanu, D., (2021). Preliminary data regarding the flora and vegetation from Fratoștița area, Dolj county, Romania. *Oltenia. Studii şi comunicări. Ştiinţele Naturii*. Tom. 37, No. 2: 53-60.

Răduțoiu, D. (2022). The chorology of *Plantago maxima* (Plantaginaceae) in Romania. *Annals of the University of Craiova. Biology, Horticulture, Food products processing technology, Environmental Engineering.* Vol. XXVII (LXIII): 335-340.

Răduțoiu, D., Niculescu, M. (2023). *Polypogon monspeliensis* (L.) Desf. from Oltenia, Romania. *Acta Horti Bot. Bucurest*., 49: 25-32.

Răduțoiu, D., Sîrbu, C., Oprea, A. (2023). *Polycarpon tetraphyllum* in Romanian flora. *Scientific Papers. Series B. Horticulture*, Vol. LXVII, No. 1: 833-837.

RoBioAtlas, 2023. Romanian Atlas Web App for Biology - Open Access. Available online: https://www.teon.ro/robioatlas/index.html (accessed on 2025.2.25)

Sălceanu, C., Paraschivu, M., Olaru, A.L. (2023). The perfomance and economic efficiency of new genotypes of Alfalfa (*Medicago sativa*). *Scientific Papers Series Management, Economic Engineering in Agriculture and Rural Development*. Vol. 23, Issue 4: 747-754.

Sărățeanu, V., Cotuna, O., Paraschivu, M., Cojocariu, L., Horablaga, M.N., Rechițean, D., Mircov, V.D., Sălceanu, C., Urlică, A.A., Copăcean, L. (2023). Features of natural succession of ex-arable forest-steppe grass-land (from western Romania) under the influence of climate. *Plants*, 12(6): 1204.

Țopa E. (1955). Aldrovanda. In Săvulescu et al. *Flora R.P.R.* vol. III. Edit. Acad. Române: 545-547.

Țopa, E. (1957). Genul *Pisum*. In Săvulescu et al., *Flora R.P.R*. (pp. 445-450). București, România: Edit. Acad. Române.

Velea, L., Bojariu, R., Burada, C., Udristioiu, M.T., Paraschivu, M., Burce R.D. (2021). Characteristics of extreme temperatures relevant for agriculture in the near future (2021-2040) in Romania. *Scientific Papers. Series E. Land Reclamation, Earth Observation & Surveying, Environmental Engineering*. Vol. X: 70-75.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

PRECISION AGRICULTURE EXTENSION MODEL BASED ON INTERDISCIPLINARY COLLABORATION

Dragoș-Ioan SĂCĂLEANU[1], Adrian ASĂNICĂ[2], Abigail BORRON[3], Daniela FAUR[1], Horia-Nicolae CIOCAN[2], Gary L. HAWKINS[3], Ioan-Ștefan SACALĂ[1], Dan POPESCU[3], Mihai-Gabriel MATACHE[4]

[1]National University of Science and Technology Politehnica Bucharest, 313 Splaiul Unirii, District 6, 060042, Bucharest, Romania
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăști Blvd, District 1, 011464, Bucharest, Romania
[3]University of Georgia, 147 Cedar St, 30602, Athens, GA, USA
[4]National Institute of Research–Development for Machines and Installations Designed for Agriculture and Food Industry Bucharest, 6 Ion Ionescu de la Brad Blvd, District 1, 013813, Bucharest, Romania

Corresponding author email: ciocan.horia@managusamv.ro

Abstract

This article presents the technical development of an autonomous irrigation system designed to optimize water delivery and support precision agriculture. Developed through a collaboration between the National University of Science and Technology Politehnica Bucharest and the National Institute of Research–Development for Machines and Installations Designed for Agriculture and Food Industry Bucharest and tested by the University of Agronomic Sciences and Veterinary Medicine of Bucharest, the system integrates advanced engineering principles and agronomic expertise to address challenges in modern agriculture. The system architecture includes an embedded control unit and wireless sensor nodes with microcontrollers, wireless communication transceivers, and soil-crop interaction sensors such as capacitive moisture probes. Real-time data acquisition enables adaptive irrigation management using predictive algorithms, ensuring precise water application aligned with crop phenological stages and environmental variability. The system's modularity ensures scalability across various farm sizes, from smallholders to large-scale operations. This initiative strengthens a cooperative agricultural extension framework, facilitating knowledge transfer between students and faculty from the involved universities. By bridging technical and agronomic expertise, the system represents a leap forward in integrating precision agriculture into sustainable farming practices.

Key words*: precision agriculture, cooperative extension, interdisciplinary collaboration, wireless sensors, irrigation.*

INTRODUCTION

The increasing demand for sustainable agricultural practices and the urgent need for climate-adaptive solutions have highlighted the importance of integrating precision technologies into modern farming systems. In this context, interdisciplinary collaboration among engineering, agronomic, and extension education institutions plays a crucial role in fostering innovation and ensuring the practical implementation of advanced technologies in the field (Teodorescu & Alexandrescu, 2007). This article presents a case study of such collaboration, developed between the National University of Science and Technology Politehnica Bucharest, the University of Agronomic Sciences and Veterinary Medicine of Bucharest, the National Institute of Research–Development for Machines and Installations Designed for Agriculture and Food Industry Bucharest, and the University of Georgia, USA. Building upon the century-old Cooperative Extension model from the United States, this initiative demonstrates how joint efforts among academic and research institutions can lead to impactful outcomes for rural communities and agricultural stakeholders (Borron et al., 2019).

The project focuses on the design, development, and testing of an autonomous irrigation system tailored for precision agriculture.

This c system integrates embedded technologies, wireless sensor networks (WSN), and adaptive control mechanisms to optimize water use based on real-time environmental data and crop-specific needs.

The principle of wireless technology applied to monitoring and control for environmental parameters started more than 20 years ago when multiple studies analyzed this possibility (Shen et al., 2001; Akylidiz et al., 2004). Many solutions are proposed these days due to the advantages that these systems bring. The challenges and opportunities offered to small-scale farmers by the latest technological advancements are reviewed by (Dhillon et al., 2023). To support them in enhancing productivity and sustainability (Ding et al., 2019) discuss the need for affordable technological solutions of monitoring and controlling. (Le Van et al., 2022) proposes a WSN with low-cost, low-power sensor nodes that operate using solar power. A controller was also developed to provide IoT connectivity. (Placidi et al., 2021) designed a low-power wireless sensor network based on the LoRaWAN protocol by using commercial components and free or open-source software libraries. A real-time, LoRaWAN-based sensor network is also proposed by (Kumar et al., 2020). Many solutions for WSN based systems for environmental parameters involves mainly the monitoring part (Rezvani et al., 2020; Săcăleanu et al., 2018). The solutions that include the control part usually use low cost and low reliability relays (Tatas et al., 2022; Kirci et al., 2022). The proposed solution contains both monitoring and control parts and, further, offers an independent operation based on an autonomous algorithm (Codeluppi et al., 2020).

Beyond its technical value, the system also brings clear economic benefits. By applying water only when and where it is needed, the technology reduces resource waste and operational costs. Moreover, by automating irrigation decisions and scheduling, the system significantly decreases the time spent by farmers on monitoring and manual intervention. This allows labor to be redirected toward higher-value tasks, increasing overall farm efficiency (Sofwan et al., 2020). In the long term, such innovations contribute to improved yield stability, better input management, and a higher return on investment (Săcăleanu et al., 2023).

The collaborative effort underpinning the development of the autonomous irrigation system also serves as a pilot for a broader extension strategy, in which students, faculty, and researchers engage directly in technology development, testing, and knowledge transfer (Ilvento, 1997). This academic-practical feedback loop strengthens both the educational experience and the responsiveness of scientific research to real-world agricultural challenges. By adopting an interdisciplinary and cross-institutional approach, the project not only addresses technical and agronomic needs, but also promotes economic sustainability, time efficiency, and scalable solutions for precision agriculture across diverse farming contexts (Ciocan et al., 2024b).

MATERIALS AND METHODS

Based on the Cooperative Extension model developed by the University of Georgia, this project demonstrates how interdisciplinary collaboration between engineering, agronomy, and extension sciences can yield scalable, affordable, and high-impact solutions for agriculture (Wang, 2014). The proposed autonomous irrigation system was developed not only as a technical prototype, but as a practical, user-oriented tool that can be deployed in real-world agricultural environments across Romania.

The technical architecture of the system supports the core goals of precision agriculture: real-time monitoring, data-driven decision-making, and resource optimization. Each wireless sensor node (WSn), built around the Arduino MKR WAN 1310 development board, integrates a suite of environmental sensors that monitor key parameters such as soil moisture, air temperature and humidity, light intensity, and atmospheric pressure. The use of LoRa communication protocol ensures low-power, long-range data transmission even in areas with limited network infrastructure, which is often the case in rural Romania.

Collected data are transmitted to a base station (BS) that also functions as a wireless actuator node (WAn), capable of controlling irrigation valves based on predefined thresholds and predictive algorithms. The modular structure of the system allows it to be adapted to different farm sizes and layouts, from a small greenhouse setup with two irrigation zones to a larger open-field system requiring multiple nodes and control points.

RESULTS AND DISCUSSIONS

To evaluate the practical implementation of the proposed system, a series of field tests were conducted using a modular setup that reflects the diversity of farm sizes and infrastructure commonly found in Romanian horticultural contexts. The following section presents the key technical results obtained during the deployment and testing phase, highlighting both the system's performance and its adaptability to real-world agricultural conditions. These findings are then discussed in relation to the broader goals of precision agriculture, sustainability, and rural innovation.

The system proposed in this paper is scalable, making it suitable for different scenarios. The tested implemented scenario includes four wireless sensor nodes (WSns) and a base station, serving also as a wireless actuator node. The scenario block diagram is presented in Figure 1.

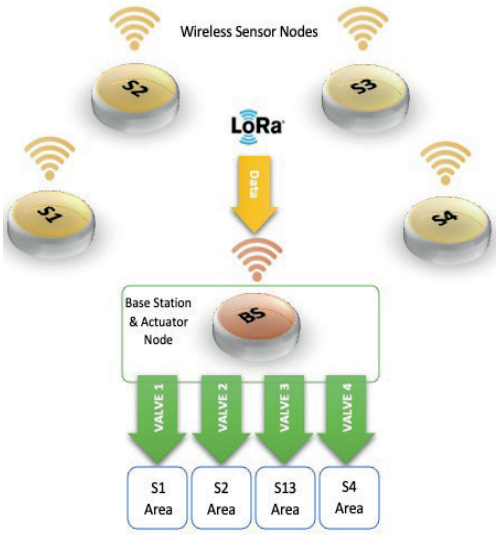


Figure 1. The architecture of the proposed system.

Data is acquired by the WSn and transmitted via a LoRa radio to the base station. There, the data are processed, stored and, depending on the values, the actuators can be activated.

Depending on the amount of area that needs to be monitored, the number of sensors nodes can be increased or decreased, as the solution can be implemented from small-scale to large-scale scenarios. The WSn and Base Station architectures were developed to be compatible with the Arduino MKR developing boards family. For the presented scenario, the Arduino MKR WAN 1310 developing board was used with LoRa communication, but other communication protocol options can be implemented, like Wi-Fi (Arduino MKR1000).

Wireless Sensor Node development

The role of the wireless sensor nodes is to acquire data from sensors, process them, and transmit the information to the base station. In Figure 2 is presented the proposed block diagram of the node including the parameters acquired.



Figure 2. Wireless sensor node block diagram.

For signal conditioning and to facilitate the connection of multiple sensors with a variety of interface protocols, a flexible node interface was developed. The interface was designed as a two-layer PCB with dimensions of 85 × 66.5 mm. The central component of the node is the microcontroller-based development board Arduino MKR WAN 1310 from the Arduino MKR family that includes the high performance and low power SAMD21 Cortex®-M0 + 32-bit ARM MCU microcontroller, the Murata CMWX1ZZABZ module with LoRa® communication and support for power supply from Li-ion or Li-Po rechargeable batteries with an integrated recharge circuit. The Arduino MKR ENV Shield personalized shield with environmental sensors, and a microSD card slot is mounted on top of the Arduino MKR WAN 1310 (Arduino MKR ENV Shield).

Based on the parameters of interest tested in this paper, a set of sensors was selected. Table 1 presents the acquired parameters together with the used sensors.

Table 1. The WSn acquired parameters and sensors

No	Parameter	Sensor	Type
1	Light Intensity	VISHAY TEMT6000	Analog
2	Air Pressure	ST LPS22HB	SPI
3	Node Temperature	ST HTS221	SPI
4	Node Humidity		
5	Air Temperature	DHT22	OneWire
6	Air Humidity		
7	Soil Tension	Watermark Sensor	Analog

The battery voltage level is acquired using an analog pin, passed previously through a ½ voltage divider. Two Li-ion 18650 rechargeable batteries of 5000 mAh were used for power supply, and a 2 W photovoltaic panel was used for battery charging. A 3D printed custom covered case was developed to protect the circuits. Figure 3 shows the final version of the wireless sensor nodes.



Figure 3. The WSn installed in the prototype scenario

Each WSn had a basic data processing software routine. After the initial setup for the microcontroller, transceiver, and individual sensors, the node acquired data from the sensors, processed them, stored them on the microSD card, and sent them to the base station. Finally, the WSn enters in a sleep mode to reduce the power consumption. Considering the application, the sleep time can be adjusted, for this scenario the parameters are acquired, and the data packets are transmitted each 1 minute. When waking up, the WSn continued acquiring a new set of data.

Base Station development

The Base Station (BS) acts as a receiver for the messages transmitted by the WSn but, also, as a wireless actuator node (WAn) to control the activation and deactiviation of the irrigation valves. Figure 4 presents the proposed block diagram for the BS with WAn.

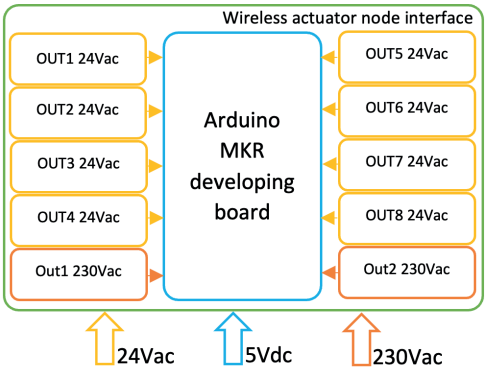


Figure 4. BS with WAn block diagram

Common equipment that can control environmental parameters (e.g., water, heat, ventilation, shadow) is powered by 24 Vac (e.g., solenoid valves for irrigation) or 230 Vac (e.g., fans and motors to control air temperature and humidity, CO2, and light intensity). The WAn was developed by considering eight channels to control 24 Vac, max. 0.5 A devices and two channels to control 230 Vac, max. 4 A devices. The Arduino MKR WAN 1310 was also used as central component for the WAn.

The designed WAn signal conditioning interface has a two-layer PCB with dimensions of 217 × 131 mm. Solid State Relay are used to control the eight 24 Vac channels and the two 230 Vac channels, together with 1 A fuse and a 5 A fuse, respectively.

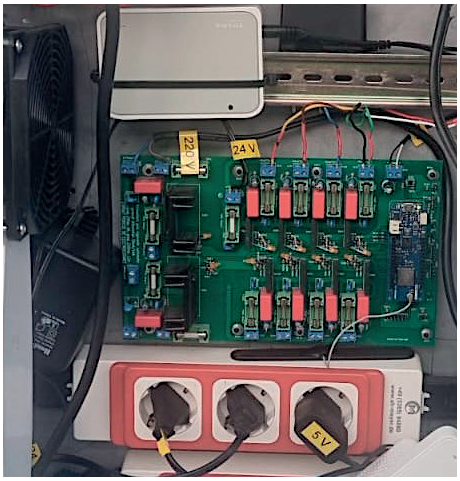


Figure 5. The installed Base Station

The BS receives the messages from the WSn and process them. The algorithm organigram can be observed in Figure 6. Each WSn has a unique ID implemented and transmitted with every message to distinguish the provenience of the data packet. Based on the value for the soil tension (Hum), compared with a defined threshold (THR), the correspondent valve is activated for 1 minute period or not. The BS is connected at the mains, and it is always in the listening state with no sleep periods.

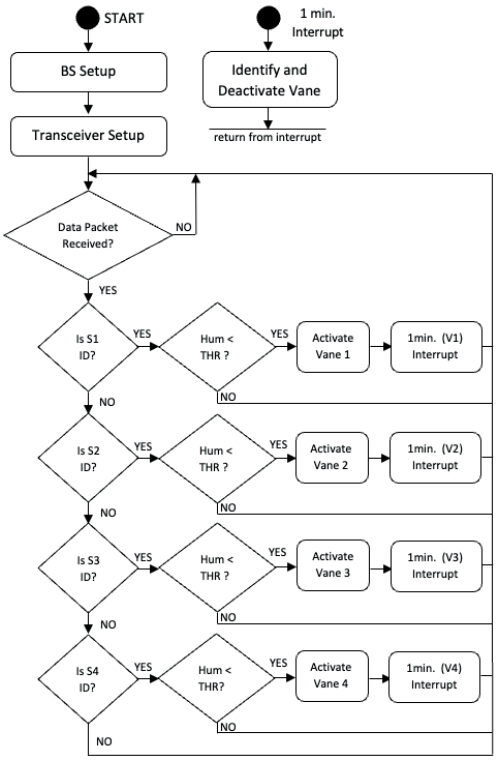


Figure 6. BS algorithm

The technical simplicity and low energy requirements (powered by photovoltaic panels and rechargeable batteries) make the system highly cost-effective and easy to maintain. This is particularly relevant in communities where farmers have limited access to high-end agricultural technology and technical support (Ciocan et al. 2024a,). In its current form, the system can be locally produced or assembled using affordable components, which opens the door for mass implementation through university-led extension programs or rural development grants (Stefanescu et al. 2013)).

From an applied perspective, the prototype addresses three fundamental challenges frequently reported by Romanian horticultural producers:

• lack of labor availability for daily irrigation tasks;

• poor water use efficiency, especially during dry seasons;

• lack of reliable data for making timely agronomic decisions.

The system was specifically designed to address the needs of small and medium-sized farmers, integrating direct input from growers regarding key aspects such as Matca (Galați County), Izbiceni (Olt County), Vidra (Ilfov County), Băleni (Dâmbovița County), and Lungulețu (Dâmbovița County). These localities represent some of the most active horticultural regions in Romania, characterized by intensive vegetable production both in greenhouses and in open fields. Farmers in these areas expressed interest in affordable, easy-to-use systems that can automate irrigation without requiring high levels of technical expertise. Discussions revealed common budget constraints (below €500 for a full system), irregular internet access, and a strong need for reliable solutions that could reduce water waste and manual labor (Teodorescu & Alexandrescu 2007).

In response to these constraints, the system was designed to be energy-independent, capable of operating offline, and structured around open-source hardware and software. It supports scalability, so it can grow with the farm's needs, and includes simple maintenance routines that can be learned quickly by end-users (Rezgui et al., 2007).

The benefits observed in the prototype phase include:

• up to 30% reduction in water usage compared to manual irrigation methods;

• potential reduction in fertilizer runoff through synchronized fertigation;

• labor savings by eliminating the need for constant field monitoring;

• consistent moisture control leading to healthier crops and potentially increased yields;

• extended equipment lifespan by optimizing valve cycles and reducing overuse.

These outcomes directly contribute to rural development goals by increasing farm profitability, lowering input costs, and improving resilience to environmental stress. Moreover, by embedding the system in a cooperative academic extension framework, it creates opportunities for local capacity building. Students and researchers become active participants in solving local agricultural problems, while farmers receive customized support and knowledge transfer based on scientific data and applied research (Ciocan et al., 2024b).

Finally, this initiative lays the foundation for a broader digital transformation of Romanian horticulture. The system can serve as a gateway for integrating other smart agriculture technologies such as remote diagnostics, weather forecasting, or crop health monitoring (Ding & Chandra, 2019). In time, such technologies can become central to national strategies aimed at increasing competitiveness, sustainability, and innovation in the agricultural sector.

CONCLUSIONS

The autonomous irrigation system presented in this study demonstrates the potential of interdisciplinary collaboration to generate practical, scalable solutions for precision agriculture. By combining engineering innovation with agronomic knowledge and cooperative extension principles, the cooperation initiative addresses both technological and economic challenges faced by small and medium-sized farms in Romania.

The system's modular design, low energy consumption, and compatibility with open-source platforms enhance its affordability and deployment, particularly in rural areas with limited infrastructure.

Engagement with farmers from major horticultural regions - including Matca, Izbiceni, Vidra, and Lunguleț u - ensured that the proposed system is aligned with real-world requirements regarding cost-effectiveness, ease of use, and adaptability to field conditions.

Economically, this prototype reduces water use, labor input, and operational costs, contributing to more sustainable and efficient horticultural practices (Placidi et al., 2021). Strategically, this initiative reinforces the role of academic institutions in rural development through hands-on extension activities and digital innovation.

In the next stage, the system will be tested on a wide range of horticultural crops, and the data collected will be used to refine the irrigation algorithms and support further scientific research. This will strengthen the system's adaptive capacity and open new opportunities for integrating precision agriculture into broader digital farming strategies.

This initiative embodies not only a technical innovation, but also a scalable model of institutional collaboration aimed at promoting sustainable and digitally enabled agricultural development.

ACKNOWLEDGEMENTS

This research was carried out with the support of Ministry of Agriculture and Rural Development, and it was financed from Project ADER 6.1.3/18.07.2023.

The work of Dragoș-Ioan Săcăleanu was supported by a grant from the National Program for Research of the National Association of Technical Universities – GNAC ARUT 2023.

REFERENCES

Akyildiz, I. F., & Kasimoglu, I. H. (2004). Wireless sensor and actor networks: Research challenges. *Ad Hoc Networks*, 2(4), 351–367.

Borron, A., Lamm, K., Darbisi, C., & Randall, N. (2019). Social impact assessment in the cooperative extension system: Revitalizing the community capitals framework in measurement and approach. *Journal of International Agricultural and Extension Education*, 26(2), 75-88.

Ciocan, H. N., Batrina, S. L., Balta, I., Moraru, P. I., Săcăleanu, D. I., & Borron, A. (2024). A proposed agricultural extension model for Romania: Leveraging universities' resources to create a cost-efficient and impactful knowledge transfer system inspired by the U.S. model. *Scientific Papers. Series Management, Economic Engineering in Agriculture and Rural Development*, 24(4), 191–204. https://www.managementjournal.usamv.ro/index.php/scientific-papers/vol-24-issue-4

Ciocan, H. N., Popescu, A., Dinu, T. A., Stoian, E., & Moldoveanu, I. D. (2024). Filling the knowledge gap: How the absence of an agricultural extension system drives Romanian farmers to seek advice and informal consultancy on social media, increasing the misinformation risk. *Scientific Papers. Series Management, Economic Engineering in Agriculture and Rural Development*, 24(3), 213–228.

https://www.managementjournal.usamv.ro/index.php/scientific-papers/vol-24-issue-3

Codeluppi, G., Cilfone, A., Davoli, L., & Ferrari, G. (2020). LoRaFarM: A LoRaWAN-based smart farming modular IoT architecture. *Sensors*, 20(7), 2028.

Dhillon, R., & Moncur, Q. (2023). Small-scale farming: A review of challenges and potential opportunities offered by technological advancements. *Sustainability*, 15(18), 15478.

Ding, J., & Chandra, R. (2019, October 11). Towards low cost soil sensing using Wi-Fi. In *Proceedings of the 25th Annual International Conference on Mobile Computing and Networking* (pp. 1–16). Association for Computing Machinery. https://doi.org/10.1145/3300061.3345443

Ilvento, T. W. (1997). Expanding the role and function of the Cooperative Extension System in the university setting. *Agricultural and Resource Economics Review*, 26(2), 153-165.

Kirci, P., Ozturk, E., & Celik, Y. (2022). A novel approach for monitoring of smart greenhouse and flowerpot parameters and detection of plant growth with sensors. *Agriculture*, 12(11), 1705.

Kumar, A., Singh, V., Kumar, S., Jaiswal, S. P., & Bhadoria, V. S. (2022). IoT enabled system to monitor and control greenhouse. *Materials Today: Proceedings*, 49, 3137–3141.

Placidi, P., Morbidelli, R., Fortunati, D., Papini, N., Gobbi, F., & Scorzoni, A. (2021). Monitoring soil and ambient parameters in the IoT precision agriculture scenario: An original modeling approach dedicated to low-cost soil water content sensors. *Sensors*, 21(15), 5110.

Rezgui, A., & Eltoweissy, M. (2007). Service-oriented sensor–actuator networks: Promises, challenges, and the road ahead. *Computer Communications*, 30(13), 2627–2648.

Rezvani, S. M.-e., Abyaneh, H. Z., Shamshiri, R. R., Balasundram, S. K., Dworak, V., Goodarzi, M., Sultan, M., & Mahns, B. (2020). IoT-based sensor data fusion for determining optimality degrees of microclimate parameters in commercial greenhouse production of tomato. *Sensors*, 20(22), 6474.

Săcăleanu, D. I., & Kiss, I. K. (2018, October). Monitoring walnut orchards with LoRa technology. In 2018 IEEE 24th International Symposium for Design and *Technology in Electronic Packaging (SIITME)* (pp. 76–79). IEEE.

Săcăleanu, D. I., Manciu, I. P., Roşu, Ş. G., Perişoară, L. A., Tannouche, A., & Creţu, L. E. S. (2023, June). GSM wireless sensor node prototype for infield environmental parameters acquisition. In *15th International Conference on Electronics, Computers and Artificial Intelligence (ECAI)* (pp. 1–6). IEEE.

Shen, C. C., Srisathapornphat, C., & Jaikaeo, C. (2001). Sensor information networking architecture and applications. *IEEE Personal Communications*, 8(4), 52–59.

Sofwan, A., Sumardi, S., Ahmada, A. I., Ibrahim, I., Budiraharjo, K., & Karno, K. (2020, March). Smart Greetthings: Smart greenhouse based on Internet of Things for environmental engineering. In 2020 *International Conference on Smart Technology and Applications (ICoSTA)* (pp. 1–5). IEEE.

Stefanescu, S. L., Steriu, S., & Dumitraşcu, M. (2013). Public and Private Players on the Market of Agricultural Advice and Extension in Romania. *Extension Education Worldwide*, 239.

Tatas, K., Al-Zoubi, A., Christofides, N., Zannettis, C., Chrysostomou, M., Panteli, S., & Antoniou, A. (2022). Reliable IoT-based monitoring and control of hydroponic systems. *Technologies*, 10(2), 26.

Teodorescu, G., & Alexandrescu, D. (2007). Improvement of fruit production and precision horticulture opportunities in Romania. *Scientific volume*, (50).

Van, T. L., Thi, P. N., Minh, P. V., Thi, L. D., Cong, H. L., & Phuong, T. N. T. (2022). Design and implementation of a wireless sensor network for smart greenhouse controller. *CommIT (Communication and Information Technology) Journal*, 16(1), 1–8.

Wang, S. L. (2014). Cooperative extension system: Trends and economic impacts on US agriculture. *Choices*, 29(1), 1-8.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

FRUITING BIOLOGY OF *STAPHYLEA PINNATA* L. (STAPHYLACEAE) IN THE REPUBLIC OF MOLDOVA

Irina SFECLĂ[1], Elena TOFAN-DOROFEEV[1], Victor SFECLĂ[2], Vasilii SLIVCA[1]

[1]"AlexandruCiubotaru" National Botanical Garden (Institute) of the State University of Moldova, 18 Pădurii Street, Chisinău, Republic of Moldova
[2]Technical University of Moldova, 48 Mirceşti Street, Chişinău, Republic of Moldova

Corresponding author email: irina.sfecla@gb.usm.md

Abstract

The study presents the results of the fruiting biology in the interaction with the environmental factors of the Staphylea pinnata L species, a rare species for the Republic of Moldova, protected by law (1998). At the European level, the species is included in the national red lists of Switzerland, France, the Czech Republic and Poland, while in Armenia and Ukraine, it is even included in the Red Cards with different degrees of endangerment. European bladdernut is a relict species with a fragmented distribution area and includes the Mediterranean, Central and East Europe regions. It grows under the canopy, in forest phytocenoses: of oak with lime and ash; of pedunculate oak with hornbeam. During the study, the morphometric parameters of the fruits and seeds of the european bladdernut were studied to determine the productivity of the species in different stations. The collected seed samples are to be preserved in the seed collection `Rare Plants of the Republic of Moldova`, within the research project `The safe conservation of the germplasm of rare plants from the Republic of Moldova` (24.80012.7007.08TC).

Key words*: Republic of Moldova, Staphylea pinnata L., fruiting biology, morphometry, rare species.*

INTRODUCTION

Staphylea pinnata L. – European bladdernut (Staphylaceae) (Figure 1) is a rare species for the territory of the Republic of Moldova, protected by the *Fund of State Protected Natural Areas Law* (LAW No. 1538, 1998). At the European level, the species is included in the national red lists of Switzerland, France, the Czech Republic and Poland (The IUCN Red List of Threatened Species). It is even included in the Red Books of Armenia and Ukraine, with different degrees of endangerment (Tamanjan et al., 2010; Mel'nik et al., 2009), while in Greece it is considered extinct due to industrial impact (Raus, 2006). The European bladdernut is a relict species with a fragmented distribution area which includes the Mediterranean Region, Central and South-Eastern Europe. The habitat of the species is represented by the thermophilic deciduous forests of Central Europe. In the spontaneous flora of the Republic of Moldova, it grows in the forest areas of the centre and north of the country as an understory in forest phytocenoses dominated by sessile oak (*Quercus petraea* (Matt.) Liebl.) with linden (*Tilia platyphyllos* Scop.) and ash (*Fraxinus excelsior* L.), but also by pedunculate oak (*Quercus robur* L.) with hornbeam (*Carpinus betulus* L.) and sycamore (*Acer platanoides* L.). It can also be occasionally found in beech forests (*Fagus sylvatica* L.) with hornbeam (*Carpinus betulus* L.), especially in the edges, clearings, along paths, avoiding the deep shade under their canopy. The European bladdernut is a light-loving plant, which usually grows in small groups with the predominance of vegetative individuals in the understory. On the slopes, in the upper half of the slopes with southwest and southeast exposure, where the density of the oak crown decreases to 0.5-0.6, the European bladdernut dominates in the undergrowth, and with a canopy closure of 0.3-0.4, it blooms and bears fruit abundantly. At the same time, in the conditions of the Republic of Moldova, the European bladdernut occasionally grows in the phytocenoses of dry forests of downy oak (*Quercus pubescens* Willd.) with European smoketree (*Cotinus coggygria* Scop.) and Cornelian cherry (*Cornus mas* L.), also it is a drought-resistant species that tolerates prolonged lack of precipitation (Gheideman, 1986).

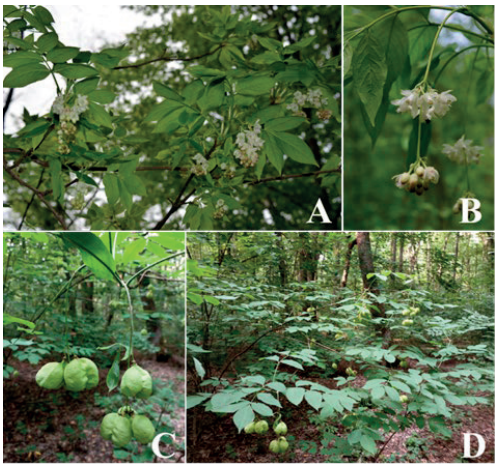


Figure 1. *Staphylea pinnata* L. in the "Dobrușa" Landscape Reserve, where: A, B – flowering phase; C, D – fruiting phase

Brief description. Shrub 1.5-4 m tall. Brown, glabrous branches, with whitish striations, young green shoots, glabrescent. Conical-ovoid buds, green to reddish-brown. Opposite leaves, imparipinnate-compound, 25-35 cm long, long-petiolate, with 2-3 pairs of leaflets, the lateral ones subsessile, and the terminal leaflet petiolate. Leaflets 5-7, oblong-ovate to elliptical, 6-12 cm long and 4-5 cm wide, acuminate, on finely serrate margins, adaxially dark green, glabrous, abaxially green glaucous, glabrescent at the base, initially thin and fine, coriaceous at maturity. Inflorescence – pendent panicle, 7-14 cm long, composed of 7-18 (26) flowers. Flowers on type 5, bisexual, actinomorphic. Calyx composed of 5 sepals, petaloid, ovate to ovate-lanceolate, concretion at the base, white-ochroleucous, abaxial with shades of pink-reddish. Campanulate corolla, 5 petals, free, spatulate, white-ochroleucous, abaxially towards the apex, with shades of pink, subequal to the sepals. 5 stamens, subequal to the petals, golden yellow anthers. Ovary superior. 3-4 styles, concretions, stigmas capitate. Fruit – capsule, 2 (4) chambered, obovoid to spherical, swollen, vesicular, 3-4 cm long, with membranous walls, flexible even at maturity. 1-2 seeds in each lodge, subspherical, about 0.8-0.9 cm in diameter, light brown, glabrous, with a hard and shiny integument. Leafing occurs in early May, and by mid-month most plants are in flower. The fruits ripen and open in July-August.

Limiting factors. The main threats to *Staphylea pinnata* populations are habitat fragmentation through silvicultural works, poor forest management, introduction of non-native tree species, and tourism and recreational activities. These threats may cause changes in habitat quality and a future reduction in *Staphylea pinnata* populations.

Conservation measures. Conservation of the species' habitats, prevention of introduction of non-native species. *Ex situ* conservation in Botanical Gardens. Introduction into cultivation as an ornamental plant.

For the successful implementation of biodiversity conservation tasks, it is necessary to know the reproductive biology of species and the specific generative traits that, in interaction with a variety of environmental factors, ensure seed production. In rare species, the study of reproductive capacity is particularly important and allows the identification of optimal and limiting factors for the perpetuation of the species.

The essential characteristic of the individual - environment relationship includes ensuring the survival, perpetuation and, possibly, prosperity of the respective species (Stugren, 1982). The study of the biology of fruiting of *Staphylea pinnata*, in the 4 populations, was carried out in 2024 and included the establishment of the following indices: seed productivity (P_s); morphology, morphometry and mass (m_{100}) of the formed seeds.

MATERIALS AND METHODS

The present study was carried out based on the population of *Staphylea pinnata* L., a rare species for the territory of the Republic of Moldova. The working material consisted of the data collected in 2024. The exact location of the site was determined using the Garmin GPSMAP® 64s GPS model. The fruiting biology of *Staphylea pinnata* was analyzed from: Rososeni Forest, Briceni district (one population); "Călărașeuca" Landscape Reserve, Ocnița district (one population); "Dobrușa" Landscape Reserve, Șoldănești district (two populations) (Figure 2).

Population parameters were established on site according to accepted methods, and the detailed description is based on the live plants collected. The general distribution, chorology, ecology

and phytocenology of the species *Staphylea pinnata* were established by the authors, supported by data from the literature.

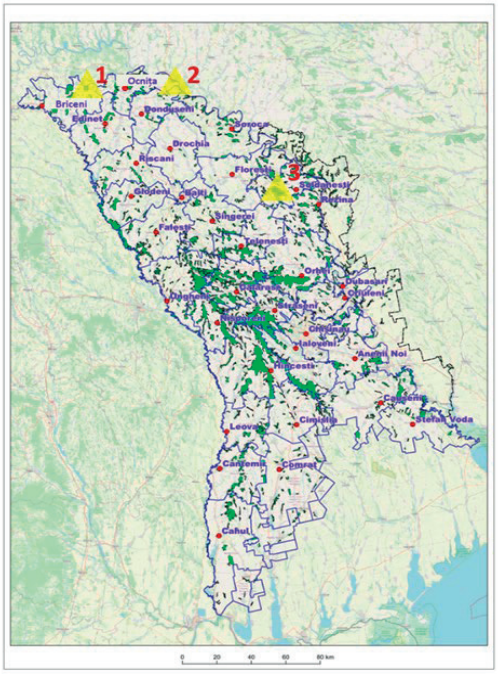


Figure 2. Geographic location of the *Staphylea pinnata* L. studied populations, where: 1 – Rososeni Forest, Briceni district; 2 – "Călărașeuca" Landscape Reserve, Ocnița district; 3 – "Dobrușa" Landscape Reserve, Șoldănești district

The climate of 2024 was characterized by a high thermal regime and a significant deficit of precipitation in the summer period. The average annual air temperature in the studied territory was 13.0-13.3°C (Table 1).

Table 1. Characterization of weather conditions in 2024

Average monthly air temperature (°C)													
*	Monthly												**
	1	2	3	4	5	6	7	8	9	10	11	12	
A	-1.6	5.3	5.6	12.9	16.0	21.5	23.5	23.1	18.9	9.8	2.5	0.9	13.3
B													
C	-0.6	6.4	6.9	14.5	17.1	23.1	25.7	25.3	20.0	11.4	3.7	2.0	13.0
Monthly and annual precipitation amount (mm)													
*	monthly												***
	1	2	3	4	5	6	7	8	9	10	11	12	
A	40	24	31	64	30	51	71	40	71	27	38	64	551
B	22	21	31	49	10	29	90	51	84	26	44	60	517
C	50	12	43	89	22	80	12	28	87	27	43	31	524

* A - Rososeni Forest, Briceni District; B - "Călărașeuca" Landscape Reserve, Ocnița District; C - "Dobrușa" Landscape Reserve, Șoldănești District
**average; ***annual

Meteorological data are taken from the Statistical Yearbook for 2024, National Bureau of Statistics of the Republic of Moldova.

The summer was hot and with a significant deficit of precipitation in the months of July-August, a period that coincides with the development and ripening of *Staphylea pinnata* fruits. The significant deficit of precipitation and the high thermal regime contributed to the occurrence of atmospheric and pedological drought in the region of the "Dobrușa" Landscape Reserve (July-August - 12-28 mm of precipitation). In the far northern districts such as Briceni and Ocnița (Rososeni Forest and the "Călărașeuca" Landscape Reserve), precipitation amounted to 40-70 and 50-90 mm, respectively.

The seed productivity coefficient, potential and real productivity of the study objects (4 populations of *Staphylea pinnata*) were studied in accordance with the "*Research Methodology of Plant Seed Productivity and Calculation Recommendations*" developed by I. Vajnagij (1974). Potential seed productivity was calculated based on the general quantity of seeds produced by the same unit (in our case the basic unit is the flower), the real one from the number of normally developed seeds, using the same unit of record (fruit). The seed productivity coefficient (C_{ps}) was determined by the ratio of the exponent of real seed productivity (P_{sr}) to that of potential productivity (P_{sp}) expressed in percentages, according to the formula:

$$Cps = \frac{Psr}{Psp} * 100\%$$

The morphological description of the seeds was compiled according to the terminology proposed by: "*Descriptive morphological atlas*" (Artjushenko, 1990); "*The Kew plant glossary*" (Beentje, 2016) and "*Polyglot Botanical Dictionary*" (Vaczy 1980). Biometric measurements were carried out according to the method proposed by G. Lakin (1990).

The mass of 100 seeds and their dry mass were determined in the *Research and Extension Center for Agriculture, Biodiversity, and Rural Development "GreenTech" (Technical University of Moldova)*. Seed lots (in three repetitions) were dried in an oven and the dry mass was determined by weighing with an analytical balance.

The water content is the difference between the fresh mass (m_{fr}) and the dry mass (m_{usc}). The percentage of water in the seeds (C_{um}) was calculated by applying the formula:

$$C_{um} = \frac{C_{um}\,(g)}{m_{fr}} * 100\%$$

The map regarding the location of the studied populations on the territory of the Republic of Moldova was developed using the free open-source application QGIS (*GIS – Geographic Information System*).

RESULTS AND DISCUSSIONS

Description of the studied populations of *Staphylea pinnata* L.

The European bladdernut population in the Rosoșeni forest (48°21'23.4"N 27°00'09.1"E), grows on a slightly inclined slope with uniform relief, with eastern exposure, altitude 262 m, in a natural-fundamental stand consisting of *Quercus robur* L. with *Cerasus avium* (L.) Moench and *Tilia tomentosa* Moench. The shrub layer is represented by *Staphylea pinnata* L., *Viburnum lantana* L., *Cornus mas* L., *Corylus avellana* L., *Rhamnus cathartica* L. and *Euonymus verrucosus* Scop. The degree of coverage of the herbaceous layer is 60-80% with the representtative species such as: *Pulmonaria obscura* (Dumort), *Veratrum nigrum* L., *Carex brizoides* L., *Potentilla alba* L., *Digitalis grandiflora* Mill., *Asarum europaeum* L., *Campanula trachelium* L., *Melampyrum nemorosum* L., *Aegopodium podagraria* L., *Lilium martagon* L. Soil type: gray forest soil (Ursu et al., 2022).

In the "Dobrușa" Landscape Reserve, *Staphylea pinnata* L. grows in stands dominated by *Quercus petraea* (Matt.) Liebl. with *Fraxinus excelsior* L., *Tilia cordata* Mill. and *Carpinus betulus* L. Among the scattered tree species encountered are *Quercus robur* L., *Acer campestre* L., *Acer platanoides* L. and *Prunus avium* L. The seeds were collected from 2 well-developed populations with an abundance of 20-25% (Sfeclă, 2024).

The first population is located in the "*Dobrușa Mică*" forest, plot 15H (47°48'896"N 28°39'498"E) and grows on a slightly inclined North-East facing slope, at an altitude of 294 m. The grove is formed by *Quercus petraea* (Matt.) Liebl. with *Fraxinus excelsior* L. and *Carpinus betulus* L. The shrub layer is predominantly represented by *Staphylea pinnata* L. accompanied by other species such as *Cornus mas* L., *Crataegus monogyna* Jacq. and *Viburnum lantana* L. The herbaceous layer is well-developed with a coverage of 40-60%, and among the dominant species we can mention: *Veronica hederifolia* L., *Corydalis cava* (L.) Schweigg. & Körte, *Anemonoides ranunculoides* (L.) Holub, *Arum orientale* M.Bieb., *Stellaria holostea* L., *Carex brevicollis* DC., *Carex pilosa* Scop., *Hypericum hirsutum* L., *Dactylis glomerata* L., *Scopolia carniolica* Jacq. Type of soil are grey forest soil (Ursu et al., 2022).

The second population is in the "*Rădi*" forest, plot 31H (47°49'367"N 28°36'945"E) positioned on a North-East facing slope with an inclination of approximately 30°, the altitude between 266-294 m. The main tree species that make up the stand are *Quercus petraea* (Matt.) Liebl., *Fraxinus excelsior* L., *Acer campestre* L., *Carpinus betulus* L. and *Tilia cordata* Mill. The understory is formed by *Staphylea pinnata* L. with an abundance of 20-30%. The herbaceous layer is made up of the following predominant species: *Corydalis solida* (L.) Clairv., *Corydalis cava* (L.) Schweigg. & Körte, *Galium aparine* L., *Stellaria holostea* L., *Geranium robertianum* L., *Lunaria annua* L. The soil type is gray forest soil (Ursu et al., 2022).

The population of *Staphylea pinata* L. from the "Călărașeuca" Landscape Reserve (48°24'25"N 27°50'29"E), grows on a slope with a North-East exposure, in a natural stand formed mainly by *Quercus robur* L., *Carpinus betulus* L., *Acer platanoides* L. The shrub species are represented by: *Staphylea pinnata* L., *Corylus avellana* L., *Cornus mas* L., *Cornus sanguinea* L., *Viburnum lantana* L., *Euonymus europaeus* L., H*edera helix* L. The herbaceous layer is made up of: *Phyllitis scolopendrium* L., *Polypodium vulgare* L., *Asplenium trichomanes* L., *Urtica dioica* L., *Hepatica nobilis* Mill., *Stellaria holostea* L., *Lunaria rediviva* L., *Asarum europaeum* L., *Pulmonaria officinalis* L., *Galanthus nivalis* L., *Galium aparine* L., *Aegopodium podagraria* L., *Anemonoides ranunculoides* L., grows on gray forest soil (Ursu et al., 2022).

Seed productivity. One of the most important stages of an individual's development is reproduction. Seed productivity is an indicator of the stability of the plant population and its perpetuation. This notion was introduced by I. Vajnagij (1973) and constitutes one of the

important criteria for assessing the viability of a plant in specific living conditions. In the methodical indication for studying seed productivity, I. Vajnagij (1990) stated some rules, such as: highlighting seed productivity proceeds differentially; when calculating ovules and seeds, the elementary unit of seed productivity is determined; the calculation of ovules and seeds is carried out on the same elementary units.

In the present study, the elementary unit of calculating the seed productivity used was the flower/fruit. Dickison (1986) mentions that in *Staphylea pinnata*, the number of ovules in each carpel can be up to eight, but very rarely more than one matures. When calculating the potential seed productivity (P_{sp}), the maximum potential value was used – 8 ovules in a carpel and 4 carpels in the fruit, respectively, P_{sp} for the elementary flower unit is 32.

Table 2 presents the data obtained on the potential (P_{sp}) and real seed productivity (P_{sr}), as well as the seed productivity coefficient (C_{ps}) of *Staphylea pinnata* under different seasonal conditions, in 2024.

Table 2. Seed productivity of *Staphylea pinnata* L. under the conditions of the Republic of Moldova

Population origin		No. of fruit lodges	No. of seeds in the box	No. of seeds in fruit (P_{sr})	Maximum number of ovules in fruit (P_{sp})	Seed productivity coefficient (C_{ps})
The European bladdernut population from the Rosoșeni forest (48°21'23.4"N 27°00'09.1"E)	*min.-max.*	1-3	0-2	1-4	32	6.696
	arith mean	2.429	1.111	2.143		
	dispersion	0.571	0.693	0.901		
	standard dev.	0.756	0.832	0.949		
	coef. of var.	31.126	74.912	44.299		
	standard error	0.202	0.196	0.254		
	confid. interval	0.396	0.385	0.497		
Population No. 1 of European bladdernut from the "Dobrușa" Landscape Reserve (47°48'896"N 28°39'498"E)	*min.-max.*	1-4	0-2	1-5	32	6.137
	arith mean	2.889	1.111	1.964		
	dispersion	0.861	0.575	0.925		
	standard dev.	0.928	0.758	0.962		
	coef. of var.	32.122	68.256	48.952		
	standard error	0.309	0.179	0.182		
	confid. interval	0.606	0.350	0.356		
Population no. 2 of European bladdernut Landscape Reserve "Dobrușa" (47°49'367"N 28°36'945"E)	*min.-max.*	1-4	0-2	1-4	32	7.872
	arith mean	2.833	1.143	2.519		
	dispersion	0.697	0.629	0.567		
	standard dev.	0.835	0.793	0.753		
	coef. of var.	29.465	69.372	29.897		
	standard error	0.241	0.173	0.145		
	confid. interval	0.472	0.339	0.284		
The population of European bladdernut from the "Călărașeuca" Landscape Reserve (48°24'25"N 27°50'29"E)	*min.-max.*	1-4	0-2	1-5	32	8.594
	arith mean	2.231	1.000	2.750		
	dispersion	0.859	0.667	1.065		
	standard dev.	0.927	0.816	1.032		
	coef. of var.	41.547	81.650	37.531		
	standard error	0.257	0.226	0.211		
	confid. interval	0.504	0.444	0.413		

The achievement of real seed productivity is in direct accordance with environmental factors. The number of seeds formed in a fruit varies from 1 to 5 and it presented variations in the averages of different origins. The highest real seed productivity 2.75 was recorded in the seeds of population from the Călărașeuca Landscape Reserve, the lowest – 2.143 in the population from the Rosoșeni forest. Of the four provenances studied by *Staphylea pinnata*, the highest seed productivity coefficient (C_{ps}) was recorded in the population from the "Călărașeuca" Landscape Reserve (8.594%) and population no. 2 from the "Dobrușa" Landscape Reserve (7.872%), followed by the seeds of population from the Rosoșeni forest (6.696%) and population no. 1 from the "Dobrușa" Landscape Reserve (6.696%).

In general, the fact that European bladdernut fructified and developed seeds in all the populations studied demonstrates its flexibility

and the provision of generative multiplication under various stationary conditions.

Seed biology. Generative self-propagation of a species ensures the presence of individuals at different ontogenetic stages within the population, which directly leads to its ecological stability. The continuity of the species and the exchange of generations take place through the seed. In the morphological description of the seed, such indices as: size; shape; surface of the tegument and location of the hilum are important. The seeds of European bladdernut are uniform and do not present distinctive taxonomic characters. Well-developed seeds were selected for the morphological description. They are oval-elongated, brown in colour, shiny, with a smooth surface. The cross-section along the entire length is circular. The seeds of European bladdernut are structured in: tegument 0.7-1.45 mm thick, endosperm and embryo. The circular hilum, light brown in colour, 2.69-4.24 mm in diameter. The endosperm is solid and friable. The embryo is positioned apically.

Seed size is an important feature of plant life history, related to the mechanisms of dispersal, germination, survival and reproductive success. There are several macro- and microevolutionary studies on seed size that have demonstrated a reduced specific and even generic variability (Leishman et all, 2000). A correlation of plant distribution with seed size and global environmental conditions is also demonstrated. Species with larger seeds grow in tropical forests and their size decreases sharply in species that make up non-forest vegetation.

The size and weight of *Staphylea pinnata* seeds are presented in Table 3 and vary little depending on the provenance.

Table 3. Morphometry and weight of *Staphylea pinnata* L seeds

Population origin		Morphometric indices						Quantitative indices		
		L, mm	W, mm	Th, mm	L/W	Sk-Th, mm	Hil ⌀, mm	m_{fr}, g	m_{usc}, g	C_{um}, %
The European bladdernut population from the Rosoșeni forest	*min.-max.*	9.06-11.21	9.27-10.85	7.83-8.98	0.935-1.152	0.77-1.16	2.69-3.24	21.67-22.066	20.309-20.689	6.240-6.280
	arith mean	10.006	9.738	8.139	1.030	0.942	3.029	21.868	20.499	6.260
	dispersion	0.532	0.184	0.114	0.006	0.017	0.041	-	-	-
	standard dev.	0.730	0.429	0.338	0.079	0.129	0.202	-	-	-
	coef. of var.	7.293	4.402	4.148	7.630	13.672	6.668	-	-	-
	standard error	0.231	0.136	0.107	0.026	0.043	0.067	-	-	-
	confid. interval	0.452	0.266	0.209	0.051	0.084	0.132	-	-	-
Population No. 1 of European bladdernut from the "Dobrușa" Landscape Reserve	*min.-max.*	9.27-10.47	9.74-10.51	8.12-8.85	0.932-1.006	0.77-1.45	3.12-4.24	22.556-23.23	21.119-21.734	6.370-6.453
	arith mean	9.652	10.060	8.412	0.960	0.967	3.501	22.938	21.465	6.421
	dispersion	0.054	0.075	0.061	0.001	0.051	0.109	-	-	-
	standard dev.	0.233	0.274	0.247	0.033	0.226	0.331	-	-	-
	coef. of var.	2.416	2.728	2.935	3.449	23.333	9.451	-	-	-
	standard error	0.078	0.091	0.082	0.011	0.075	0.110	-	-	-
	confid. interval	0.152	0.179	0.161	0.022	0.147	0.216	-	-	-
Population no. 2 of European bladdernut Landscape Reserve "Dobrușa"	*min.-max.*	9.04-10.56	9.51-10.38	7.63-8.78	0.909-1.067	0.77-1.14	3.37-4.08	21.806-23.357	20.448-21.871	6.228-6.362
	arith mean	9.450	9.774	8.072	0.967	0.981	3.623	22.699	21.266	6.313
	dispersion	0.292	0.080	0.082	0.003	0.045	0.073	-	-	-
	standard dev.	0.540	0.283	0.286	0.057	0.213	0.270	-	-	-
	coef. of var.	5.719	2.893	3.540	5.891	21.678	7.458	-	-	-
	standard error	0.180	0.094	0.095	0.019	0.071	0.090	-	-	-
	confid. interval	0.353	0.185	0.187	0.037	0.139	0.177	-	-	-
The population of European bladdernut from the "Călărașeuca" Landscape Reserve	*min.-max.*	8.18-10.19	9.12-10.49	7.64-8.61	0.852-1.005	0.76-1.07	3.09-4.16	22.75-25.74	21.347-24.191	6.167-6.017
	arith mean	9.141	9.692	8.103	0.944	0.892	3.412	24.519	23.031	6.072
	dispersion	0.345	0.271	0.114	0.004	0.012	0.136	-	-	-
	standard dev.	0.587	0.521	0.338	0.061	0.110	0.369	-	-	-
	coef. of var.	6.424	5.372	4.172	6.443	12.288	10.809	-	-	-
	standard error	0.196	0.174	0.113	0.020	0.037	0.123	-	-	-
	confid. interval	0.384	0.340	0.221	0.040	0.072	0.241	-	-	-

L – length; **W –** width; **Th** – thickness; **L/W –** ratio between length and width; **Sk-Th** – skin thickness; **Hil ⌀** – hil diameter; **m_{fr}, g** – fresh mass; **m_{usc}, g** – dry mass; **C_{um}, %** – moisture coefficient.

Seeds with larger parameters (length/width) are characteristic of European bladdernut populations from the "Dobruşa" Landscape Reserve (Table 3). Based on the mass of 100 seeds, the provenance from the "Călărașeuca" Landscape Reserve proved to be the most productive, demonstrating a reserve of nutrients accumulated in the endosperm superior to the other European bladdernut provenances.

Determining the water content of seeds is an extremely important test, as it provides information on the accumulated nutrients, the potential for harvesting, cleaning and planting, as well as for long-term storage of seeds (Copeland and McDonald, 2001). A small change in the water content of seeds can have a large effect on the storage life of seeds. Accurate determination of water content is essential to make fairly accurate predictions regarding the storage potential of seeds (Hanson, 1985). Ellis R.H. et al. (1990), in the process of researching the relationship between water content and seed longevity, came to the conclusion that seeds containing less than 10-11% water, are more long-lived. The water content of the European bladdernut seeds is within the limits of 6.16-6.37% (Table 3). Seed samples collected from *Staphylea pinnata* with the provenances mentioned in the work were preserved in the Germplasm Collection: "*Rare Plants from the Republic of Moldova*" of the National Botanical Garden (Institute) "Al. Ciubotaru", USM.

CONCLUSIONS

This study highlighted the need to research the fructification biology of rare species both in the context of germplasm conservation and for the analysis of the variability of the seed productivity within different populations with specific seasonal conditions.

Analysis of seed productivity of the studied populations demonstrated a significant variation from 6.1% (Population no. 1 from the "Dobruşa" Landscape Reserve) to 8.5% (Population from the "Călărașeuca" Landscape Reserve), under different climatic conditions, especially the amount of precipitation in the key months for the fructification process, which leads us to conclude that the real seed productivity is directly influenced by the specific conditions of the growing season.

The variation of morphometric indices and seed mass of *Staphylea pinnata* depending on the provenance highlights the ecological adaptability of the species.

The seeds collected from the "Călărașeuca" Landscape Reserve were distinguished by superior morphometric indices to the other European bladdernut populations and lower humidity coefficient, suggesting that they accumulated a greater amount of reserve substance. This provenance is the most suitable as a source of seeds used for species multiplication and conservation strategies in germplasm collections.

ACKNOWLEDGEMENTS

This research work was carried out with the financing support of Project 24.80012.7007.08T "*The safe conservation of the germplasm of rare plants from the Republic of Moldova*" (CONSERVGERM).

REFERENCES

Anuarul statistic al Republicii Moldova. (2024). Biroul Național de Statistică al Republicii Moldova. Chișinău: Statistică, F.E.-P. "Tipografia Centrală", 579 p. ISBN 978-9975-177-39-9 (PDF), from https://statistica.gov.md/files/files/publicatii_electronice/Anuar_Statistic/2024/Anuarul%20_statistic_2024.pdf

Artjushenko, Z. (1990). *Atlas po opisatel'noj morfologii vysshih rastenij: Semja*. Leningrad: Nauka, 204 s.

Beentje, H. (2016). *The Kew Plant Glossary: An Illustrated Dictionary of Plant Terms*. UK: Royal Botanic Gardens Kew, 192 p. ISBN 978-1-84246-605-6.

Copeland, L., McDonald, M. (2001). *Principles of Seed Science and Technology*. 4th Edition. USA, Massachusetts: Kluwer Academic Publishers, 467 p. ISBN 978-1-4615-1619-4.

Dickison, W., C. (1986). Floral Morphology and Anatomy of Staphyleaceae. In: *Botanical Gazette*, Vol. 147, No. 3, pp. 312-326

Dimopoulos, P., Raus, Th., Bergmeier, E., Constantinidis, Th., Iatrou, G., Kokkini, S., Strid, A., Tzanoudakis, D. (2013). *The Flora of Greece Web - Vascular plants of Greece: An annotated checklist*, from http://portal.cybertaxonomy.org/flora-greece/intro

Ellis, R., Hong, T., Roberts, E., Tao, K.-L. (1990). Low-moisture-content limits to relations between seed longevity and moisture. In: *Annals of Botany*, Volume 65, Issue 5, pp.493-504, from https://doi.org/10.1093/oxfordjournals.aob.a087961

Gheideman, T. (1986). Forest Plants (Vascular). In: *The Plant Kingdom of Moldova.* [Lesnye Rasteniya

(Sosudesnye). In: Rastitel'nyy mir Moldavii]. Kishinev: Shtiintsa. T. 1, p. 98.

Hanson, J. (1985). *Procedures for Handling Seeds in Genebanks.* Practical Manuals for Genebanks. Italy, Rome: International Board for Plant Genetic Resources, 115 p.

Heiss, A.G., Filipovic, D., Nedelcheava, A., Ruß-Popa, G., Wanninger, K., Schramyr, G., Perego, R., Jacomet, S. (2014). A Fistful of Bladdernuts: The Shifting Uses of *Staphylea pinnata* L. as Documented by Archaeology, History, and Ethnology. *Journal of ethnological studies* 52(2), 95-136, from https://www.tandfonline.com/doi/abs/10.1179/0430877814Z.00000000031

Holub, J., Procházka, F. (2000). *Red List of vascular plants of the Czech Republic* – 2000. Preslia, 72, pp. 187- 230.

Lakin, G. (1990). *Biometrija*. Moskva: Vysshaja Shkola, 350 s. ISBN 5-06-000471-6.

LEGE Nr. 1538 privind fondul ariilor naturale protejate de stat. (1998), from https://www.legis.md/cautare/getResults?doc_id=141074&lang=ro

Leishman, M., Wright, I., Moles, A., Westoby, M. (2000). The Evolutionary Ecology of Seed Size. In: *Seeds: The Ecology of Regeneration in Plant Communities*, 2nd edition,, pp. 31-57. ISBN 9780851999470, from http://bio.mq.edu.au/~iwright/pdfs/l00seed.pdf

Mel'nik, V., Peregrim, M., Kagolo, O. (2009). *Staphylea pinnata* L. *Red data book of Ukraine. Plant Kingdom*. Globalconsalting, Kyiv, p. 606.

Micevski, K., Matevski, V. (2005). Flora na Republika Makedonija. In: *MANU* 1(6): 1433-1715.

Moser, D., A. Gygax, B. Bäumler, N. Wyler, PALESE, R. (2002). *Rote Liste der gefährdeten Farn- und Blütenpflanzen der Schweiz*. 118 p, from file:///C:/Users/User/Downloads/rote_liste_der_gefaehrdetenartenderschweizfarn-undbluetenpflanze.pdf

Raus, Th. (2006) Found and lost – Staphyleaceae in Greece. In: *Willdenowia 36* (Special Issue): 311-315. – BGBM Berlin-Dahlem ISSN 0511-9618., from https://doi.org/10.3372/wi.36.36124v

Sfeclă, V. (2024) Corologia unor plante vasculare rare din Rezervația peisagistică „Dobrușa". In: *Instruire prin cercetare pentru o societate prosperă,* Chișinău: CEP UPSC, 2024, Ediția 11, Vol.1, pp. 287-293. ISBN 978-9975-46-716-2. ISBN (pdf) 978-9975-46-902-9.

Staphylea pinnata L. [on line]. On: BGCI PlantSearch. Botanic Gardens Conservation International, from https://plantsearch.bgci.org/taxon/1713

Staphylea pinnata L. [on line]. On: Online Atlas of the British and Irish Flora, from: *Staphylea pinnata* L. in BSBI Online Plant Atlas 2020

Staphylea pinnata L. [on line]. On: Global Biodiversity Information Facility (GBIF) Data Portal, from https://www.gbif.org/species/3686235

Staphylea pinnata L. [on line]. On: The IUCN Red List of Threatened Species. Version 2025, from https://www.iucnredlist.org/species/83827703/123855346

Stugren, B. (1982). *Bazele ecologiei generale*. București: Editura Științifică și Enciclopedică, 205 p.

Tamanjan, K. G., Fajvush, G. M., Vardanjan, Zh. A., Danieljan, T. S. (2010). *Krasnaja Kniga Armenii (Rastenija)*, Erevan, 598 s.

UICN France, FCBN and MNHN. (2012). *La Liste rouge des espèces menacées en France - Chapitre Flore vasculaire de France métropolitaine: premiers résultats pour 1000 espèces, sous-espèces et variétés*. 1-23 pp, from https://inpn.mnhn.fr/docs/LR_FCE/Dossier_presse_Liste_rouge_Flore_vasculaire_de_metropole_5_Nov_2012.pdf

Ursu, A., Overcenco, A., Curcubăt, S., Miron A. (2022). *Solurile pădurilor din Republica Moldova*, Chișinău, 132 p., ISBN 978-9975-3586-3-7, from https://ieg.md/sites/default/files/2023-06/Solurile_Padurilor-2022.pdf

Váczy, C. (1980). *Lexicon Botanicum Polyglottum*. *Dicționar botanic poliglot*. București: Editura Ştiinţifică şi Enciclopedică, 1017 p.

Vajnagij, I. (1973). Metodika statisticheskoj abrabotki materiala po semennoj produktivnosti rastenij na primere *Potentilla aurea* L. V: *Rast. Resur*. T. 9. Vyp. 2., cc. 287-296.

Vajnagij, I. (1974) O metodike izuchenija semennoj produktivnosti rastenij. V: *Botan. Zhurn*., T. 59, N 6, cc. 826-831.

Vajnagij, I. (1990). Metodika opredelenija semennoj produktivnosti predstavitelej semejstva Ljutikovyh. V: *Bjuleten' Glavnogo Botanicheskogo Sada*, Vypusk 155. Moskva: Nauka, 1990, cc. 86-90.

Weaver, R.E. (1980). The Bladdernuts. In: *Arnoldia* 40(2): 76-93, from https://www.biodiversitylibrary.org/page/53646745#page/85/mode/1up

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

DEVELOPMENT OF PLANT TISSUE CULTURE AND INDOOR FARMING SYSTEMS IN CONTROLLED ENVIRONMENTAL CONDITIONS USING LIGHT-EMITTING DIODES (LEDs) FOR THE PRODUCTION OF HIGH-VALUE CROPS

Sreeramanan SUBRAMANIAM[1, 2, 3, 4, a], Ayyagari RAMLAL[1, 5, b]

[1]School of Biological Sciences, University Sains Malaysia (USM), Georgetown, Penang, Malaysia-11800
[2]Centre for Chemical Biology (CCB), University Sains Malaysia (USM), Bayan Lepas, Penang, Malaysia-11900
[3]Department of Biology, Faculty of Science and Technology, Universitas Airlangga, Surabaya, Indonesia-60115
[4]Institute of Nano Optoelectronics Research and Technology, University Sains Malaysia (USM), Bayan Lepas, Penang, Malaysia-11900
[5]Division of Genetics, ICAR-Indian Agricultural Research Institute (IARI), Pusa Campus, New Delhi, Delhi, India-110012

Corresponding author emails: sreeramanan@gmail.com, sreeramanan@usm.my
[a]0000-0003-4392-285X and [b]0000-0002-1093-9877

Abstract

The plant tissue culture system makes it possible to produce plants in a controlled environment under sterile conditions in large quantities and at a constant and faster rate. Light is one of the most important variables that can affect the growth and development of plant tissue culture, influencing photosynthesis, morphogenesis and physiological processes. Light-emitting diodes (LEDs) are useful in this scenario as they allow the irradiation of a narrow spectrum of light that meets the requirements of plants for their growth and development. Different LED-generated spectra with uniform intensity are used in plant tissue culture to enhance plant growth, development and proliferation. In a vertical farming system, LEDs can be used as energy-efficient light sources to produce high-quality products with high nutritional value. Commercially viable growing systems for high-value vegetables such as premium lettuces, herbs, microgreens, edible flowers and strawberry plants have been developed. The continuous acquisition of new concepts and knowledge in photobiology and plant morphogenesis, complemented by the rapid development of LED technology, will make the application of solid-state lighting more remunerative.

Key words*: Indoor Farming, Light-emitting diodes, Plant Tissue Culture, Secondary Metabolites.*

INTRODUCTION

Plant tissue culture (PTC) is a fundamental science from the field of plant biotechnology that contributes to the understanding of plant growth and development at the cellular level (Sudheer et al., 2022; Nautiyal et al., 2024; Raj et al., 2025). Plant tissue culture is defined as an *in vitro* method for growing or manipulating plant cells, tissues, organs or whole plantlets under sterile and controlled environmental conditions such as temperature, light and humidity on a culture medium. This method is based on totipotency (each living plant cell has the genetic potential to regenerate into a whole plant) and plasticity (the ability of cells to change their developmental pathway in response to external stimuli) (Bidabadi and Jain, 2020). PTC was first proposed in 1902 by the German botanist Gottlieb Haberlandt, who is widely regarded as the father of plant tissue culture (Gaikwad et al., 2017). He cultured palisade tissue in Knop's solution with sucrose and observed cell growth. In 1904, Hanning successfully cultured excised crucifer embryos on a mineral sugar medium, a technique that was later further developed by Overbeck in 1941. An important milestone was reached in 1972 when Carlson et al. created the first somatic hybrid by fusing protoplasts of *Nicotiana glauca* Graham (Solanaceae) and *N. langsdorffii* Weinm. (Evans and Flick, 1983). Tissue culture was first

used by the orchid industry for mass propagation in the 1950s.

PTC overcomes the limitations of conventional propagation, especially for species with limited or unprofitable seed production, by providing a platform for the rapid clonal propagation of elite genotypes and the production of genetically uniform stocks (Smith and Drew, 2000). Any plant organ, such as roots, stems, buds, apical meristems, cotyledon nodes, leaves, anthers or ovaries, can be used for culture. Meristem tip culture provides pathogen-free planting material, which is crucial for vegetatively propagated plants such as banana and potato (Krishna et al., 2022). The technique also supports *in vitro* conservation and cryopreservation protocols, protecting the germplasm of rare or endangered taxa. In addition, totipotent regeneration systems are essential for modern biotechnology, as they serve as a regenerative backbone for genetic transformation, genome editing and the production of double haploids, which together accelerate breeding cycles and trait introgression (Altman, 2003).

Some of the applications of PTC are summarised in Table 1. From a sustainability perspective, closed, aseptic culture systems require significantly lower inputs of land, water and agrochemicals than field propagation (El-Sherif, 2018). The agronomic impact is illustrated in Bihar, India, where the use of tissue culture-derived, virus-indexed banana plantlets increased yields from ~20 t ha^{-1} in 2004-2005 to ~45 t ha^{-1} in 2022-2023 (Kumar et al., 2024). However, there are various challenges associated with *in vitro* cultivations (Ramlal et al., 2024; Bishnoi et al., 2025) and there is a need to include PTC in modern research due to its wide application and need (Negi et al., 2024). Light and physical conditions (temperature, relative humidity) are very important for the proper growth and development of plants, as they regulate the basic processes (Cavallaro et al., 2022). Incandescent lamps (IL), fluorescent tubes (FL), high-pressure sodium lamps (HP-Na) and metal halide lamps (MH) used to be common; however, these are being replaced by more efficient and promising technologies (Cavallaro et al., 2022). Light-emitting diodes (LEDs) are an emerging tool with promising benefits for *in vitro* cultures, especially for plant improvement and secondary metabolite production (Livadariu et al., 2023; Sena et al., 2024). Maintaining controlled environmental conditions is also crucial for the continuous supply of healthy and good biomass of crops. Therefore, given the prevailing irregularities in climatic changes and the loss of agricultural land, alternative approaches should be utilised.

This article provides an overview of the importance of plant tissue culture, the importance of light, including light-emitting diodes, and vertical rearing in the mass propagation of tissue-cultured plants.

Table 1. Areas of application of plant tissue culture

Areas of application	Tissue culture	Typical outcome/benefit	References
Micro-propagation	Multiplication via nodal, shoot-tip, or somatic embryo culture	Uniform, true-to-type plants (oil-palm, banana, ornamental foliage)	Butt et al., 2015
Virus elimination	Meristem culture + thermotherapy/chemotherapy	Clean stock of potatoes, sweet potatoes, and cassava	Naik and Buckseth, 2018
Haploid & doubled-haploid production	Anther/ovule or microspore culture	Completely homozygous lines in one generation for cereals, rapeseed	Niazian and Shariatpanahi, 2020; Ramlal et al., 2023
Somaclonal variation/*in vitro* mutagenesis	Callus culture followed by variant screening	Novel traits such as low-caffeine coffee, dwarf ornamentals, grapevine, sugarcane	Bairu et al., 2011
Genetic transformation & genome editing	*Agrobacterium*-mediated or biolistic gene delivery followed by regeneration	Transgenic/herbicide-tolerant or CRISPR-edited crops	Altpeter et al., 2016
Protoplast fusion	Fusion of somatic protoplasts, regeneration of hybrids	Interspecific hybrids (e.g., tomato × potato "pomato")	Karp et al., 1987
Secondary-metabolite production	Cell-suspension or hairy-root cultures in bioreactors	Constant supply of paclitaxel (Taxus), shikonin (Lithospermum)	Dörnenburg and Knorr, 1995
Conservation & seed-sector back-ups	Slow-growth storage, cryopreservation	Long-term safekeeping of coconut, yam, and *Musa* germplasm	Rajasekharan and Sahijram, 2015

Areas of application	Tissue culture	Typical outcome/benefit	References
Physiology & stress studies	Controlled *in vitro* assays	Rapid screening for salt- or drought-tolerant lines or embryogenesis-related studies	Sahu et al., 2023; Nautiyal et al., 2024
Cryopreservation	Orchid preservation in liquid nitrogen for long-term storage	Long-term preservation of germplasm using liquid nitrogen	James Antony et al., 2019
Suspension cultures	Biomass increase of certain cells for the production and enhancement of secondary metabolites	Secondary metabolite production	Lim et al., 2025

LIGHT-EMITTING DIODE (LED) TECHNOLOGY IN PLANT TISSUE CULTURE

Light, both quantity and quality as well as photoperiod, are the most important factors influencing the light-induced responses in plants responsible for growth and development under artificial culture conditions. Illumination is essential for the induction of photosynthesis and photomorphogenic responses in a controlled environment (Batista et al., 2018; Murthy et al., 2024). Artificial light sources have long been used in PTCs and growth rooms, including FL, such as cold cathode fluorescent lamps (Ding et al., 2010), high pressure sodium lamps (HP-Na) and plasma lamps were used for comparison of cucumber production by Guo et al. (2016), MHL and IL light by Dutta Gupta and Jatothu, (2013). However, they were replaced by LED technology due to several disadvantages, which include the generation of more heat, energy consumption and differences in absorption spectra that hinder plant growth under *in vitro* conditions (Xu et al., 2016).

On the other hand, the use of LED technology in PTC is advantageous in terms of longevity, space saving, lower heat dissipation and no use of mercury (Shukla et al., 2017; Batista et al., 2018; Ying et al., 2020; Gnasekaran et al., 2021). LEDs have been proposed as a primary light source for space-based plant research chambers and as a potential replacement light source for plant growth and development in vitro to achieve the success of commercial LED lighting (Bello-Bello et al., 2017; Barceló-Muñoz et al., 2022). So far, many plants have been tested under LED, for example Momordica cochinchinensis, *Ficus carica* var. 'Black Jack', *Zingiber officinale* var. rubrum Theilade, *Hylocereus costaricensis*, *Cucumis metuliferus* and white strawberry (Lim et al, 2020; Gnasekaran et al, 2021; Parab et al, 2021; Winson et al, 2021; Lai et al, 2022; Pang et al, 2023a, 2023b), the spectra produced by LEDs will have uniform intensity and quality, which is a better source for plant growth and development. Developing an ideal lighting system with optimal light intensity, best lighting position and lighting times while saving energy is the most important goal in this field.

INDOOR VERTICAL FARMING

One of the global threats at present is global warming and the associated climate change. Greenhouse gas emissions such as carbon dioxide (CO_2), which is released when fossil fuels are burned, and other gases such as nitrous oxide, methane and chlorofluorocarbons contribute to global warming. As a result, the temperature and precipitation in the Earth's atmosphere are disturbed, which is exacerbated by anthropogenic activities (Malhi et al., 2021). This in turn affects agriculture and its production, leading to lower yields, higher prices and reduced food availability, which in turn leads to food insecurity. This is exacerbated by the unprecedented increase in population at an alarming rate and the loss of agricultural land to urbanisation.

It will therefore become even more difficult in the coming years to find suitable land for agriculture to meet the growing demand for food (Arora, 2019). Therefore, alternatives should be explored to maximise yield in a sustainable and promising way. Controlled environment farming systems, such as vertical farming (VF) and indoor farming, have the potential to increase production in the most sustainable way possible without compromising yield and quality (Avendaño-Abarca et al., 2022). VF can be defined as a multi-tiered production system where yield is a function of area. Sharathkumar et al (2020): “A multi-tiered indoor crop production system in which all growth factors such as light, temperature, humidity, CO_2 concentration, water and nutrients are precisely

controlled to produce high quantities of high-quality fresh produce throughout the year, completely independent of sunlight and other outdoor conditions". Depending on the needs and requirements, VFs have different facilities such as plant factories with artificial lighting, container farms, in-store farms and equipment farms (Butturini and Marcelis, 2020). Avendaño-Abarca et al. (2022) have shown that vertical indoor farming increases strawberry production in conjunction with LED technology, especially with green and red LEDs.

CONCLUSIONS

The field of plant tissue culture plays an important role in the maintenance of germplasm and mass propagation. LED technology also offers immense potential for plants grown *in vitro*. These technological approaches, including LEDs and indoor vertical farming, are therefore recommended to explore further possibilities and ways to maximise yield.

Author contributions

S. Subramaniam conceived the study and supervision. Ay. Ramlal and S. Subramaniam were involved in editing and revisions. Both authors have read and approved the final manuscript.

Data availability statement

Not applicable.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publishing

Not applicable.

Competing interests

The authors declare no competing interests.

Funding

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

REFERENCES

Altman A., 2003. From plant tissue culture to biotechnology: scientific revolutions, abiotic stress tolerance, and forestry. *In Vitro* Cellular & Developmental Biology-*Plant*, 39, 75-84.

Altpeter F., Springer N. M., Bartley L. E., Blechl A. E., Brutnell T. P., Citovsky V., et al., 2016. Advancing crop transformation in the era of genome editing. *The Plant Cell*, 28, 1510-1520.

Arora N. K. 2019. Impact of climate change on agriculture production and its sustainable solutions. *Environmental Sustainability*, 2, 95-96.

Avendaño-Abarca V. H., Alvarado-Camarillo D., Valdez-Aguilar L. A., Sánchez-Ortíz E. A., González-Fuentes J. A., Cartmill A. D., 2022. Response of strawberry to the substitution of blue light by green light in an indoor vertical farming system. *Agronomy*, 13(1), 99.

Bairu M. W., Aremu A. O., Van Staden J., 2011. Somaclonal variation in plants: causes and detection methods. *Plant Growth Regulation*, 63, 147-173.

Batista D. S., Felipe S. H. S., Silva T. D., de Castro K. M., Mamedes-Rodrigues T. C., Miranda N. A., *et al*. 2018. Light quality in plant tissue culture: does it matter?. *In Vitro* Cellular & Developmental Biology-Plant, 54, 195-215.

Bello-Bello J. J., Pérez-Sato J. A., Cruz-Cruz, C. A. Martínez-Estrada, E., 2017. Light-emitting diodes: Progress in plant micropropagation. In: Jacod-Lopes, E., Zepka, L.Q., & Queiroz, M. I. (Eds) 6(1), IntechOpen, Croatia, 93-103.

Bidabadi S. S., Jain S. M., 2020. Cellular, molecular, and physiological aspects of *in vitro* plant regeneration. *Plants*, 9, 702.

Bishnoi P., Ramlal A., Raju D., Saini M., Mallikarjuna B. P., Pushpa R., Rajendran A., 2025. *In vitro* culture of soybean (*Glycine max* (L.) Merr.): challenges and solutions. *Journal of Crop Improvement*, 1-26.

Butt S. J., Varis S., Nasir I. A., Sheraz S., Shahid, A. 2015. Micro propagation in advanced vegetable production: a review. *Advancements in Life Sciences*, 2, 48-57.

Butturini M., Marcelis L. F., 2020. Vertical farming in Europe: Present status and outlook. In: Kozai, T., Niu, G., & Tokagaki, M. (Eds), *Plant factory*, Elsevier, Amsterdam, The Netherlands, 77-91.

Cavallaro V., Pellegrino A., Muleo R., Forgione I., 2022. Light and plant growth regulators on *in vitro* proliferation. *Plants*, 11, 844.

Ding Y., He S., da Silva J. A. T., Li G., Tanaka M. 2010. Effects of a new light source (cold cathode fluorescent lamps) on the growth of tree peony plantlets *in vitro*. *Scientia Horticulturae*, 125, 167-169.

Dörnenburg H., Knorr D., 1995. Strategies for the improvement of secondary metabolite production in plant cell cultures. *Enzyme and Microbial Technology*, 17, 674-684.

Dutta Gupta S., Jatothu, B., 2013. Fundamentals and applications of light-emitting diodes (LEDs) in *in vitro* plant growth and morphogenesis. *Plant Biotechnology Reports*, 7, 211-220.

El-Sherif N. A., 2018. Impact of plant tissue culture on agricultural sustainability. Sustainability of Agricultural Environment in Egypt: Part II: Soil-Water-Plant Nexus, 93-107.

Evans D. A., Flick C. E., 1983. Protoplast fusion: Agricultural applications of somatic hybrid plants. In: Kosuge T., Meredith C.P., Hollaender A., Wilson C.M. (Eds), *Genetic engineering of plants: An agricultural perspective*, Springer, US, Boston, MA, 271-288.

Gaikwad A. V., Singh S. K., Gilhotra R., 2017. Plant tissue culture-A review. *Journal of Pharmaceutical Research & Education*, 2, 217-220.

Gnasekaran P., Rahman Z. A., Chew B. L., Appalasamy S., Mariappan V., Subramaniam S., 2021. Development of micropropagation system of *Zingiber officinale* var. rubrum Theilade using different spectrum light-emitting diode (LED) irradiation. *Industrial Crops and Products*, 170, 113748.

Guo X., Hao X., Zheng J. M., Little C., Khosla S., 2016. Effects of plasma vs. high pressure sodium lamps on plant growth, fruit yield and quality in greenhouse cucumber production. In *VIII International Symposium on Light in Horticulture*, 1134, pp. 79-86.

James Antony J. J., Zakaria S., Zakaria R., Anak Ujang J., Othman N., Subramaniam S., 2019. Biochemical analyses of *Dendrobium* Sabin Blue PLBs during cryopreservation by vitrification. *Physiology and Molecular Biology of Plants*, 25, 1457-1467.

Karp A., Jones M. G., Ooms G., Bright S. W., 1987. Potato protoplasts and tissue culture in crop improvement. *Biotechnology and Genetic Engineering Reviews*, 5, 1-32.

Krishna R., Ansari W. A., Khandagale K., Benke A. P., Soumia P. S., Manjunathagowda D. C., *et al*. 2022. Meristem culture: a potential technique for *in vitro* virus-free plants production in vegetatively propagated crops. In: Rai A.C., Kumar A., Modi A., Singh, M. (Eds) *Advances in plant tissue culture: Current developments and future trends*, Academic Press, 325-343.

Kumar R., Singh R. K., Shah A., Srivastava A. K., Singh U. P., Agarwal A., 2024. Banana Cultivation and Micropropagation in India: Addressing Challenges and Exploring Future Prospects. *Biosciences Biotechnology Research Asia*, 21, 1383-1395.

Lai C. S., Kho Y. H., Chew B. L., Raja P. B., Subramaniam S., 2022. Organogenesis of *Cucumis metuliferus* plantlets under the effects of LEDs and silver nanoparticles. *South African Journal of Botany*, 148, 78-87.

Lim C. H., Guan T. S., Chan Hong E., Lit Chow Y., Lynn C. B., Subramaniam S., 2020. Effect of different LED lights spectrum on the *in vitro* germination of gac seed '(*Momordica cochinchinensis*)'. *Australian Journal of Crop Science*, 14, 1715-1722.

Lim W. H., Khaw M. L., Yungeree O., Hew W. H., Parab A. R., Chew B. L., *et al*., 2025. Effects of LEDs, macronutrients and culture conditions on biomass and artemisinin production using *Artemisia annua* L. suspension cultures. *Biotechnology Progress*, e70041.

Livadariu O., Maximilian C., Rahmanifar B., Cornea C. P., 2023. LED technology applied to plant development for promoting the accumulation of bioactive compounds: a review. *Plants*, 12, 1075.

Malhi G. S., Kaur M., Kaushik P., 2021. Impact of climate change on agriculture and its mitigation strategies: A review. *Sustainability*, 13, 1318.

Murthy H. N., Joseph K. S., Paek K. Y., Park S. Y., 2024. Light as an elicitor for enhanced production of secondary metabolites in plant cell, tissue, and organ cultures. *Plant Growth Regulation*, 104, 31-49.

Naik P. S., Buckseth, T., 2018. Recent advances in virus elimination and tissue culture for quality potato seed production. In: Gosal, S., Wani, S. (eds), *Biotechnologies of Crop Improvement, Volume 1: Cellular Approaches*, Springer, Cham, 131-158.

Nautiyal A., Ramlal A., Agnihotri A., Rashid A., 2023. Stress-induced somatic embryogenesis on seedlings of *Azadirachta indica* A. Juss. by thidiazuron and its inhibition by ethylene modulators. *Plant Cell, Tissue and Organ Culture*, 153, 357-366.

Negi S., Singh P., Trivedi V. L., Rawat J. M., Semwal, P., 2024. The current trends and research progress globally in the plant tissue culture: 90 years of investigation. *Plant Cell, Tissue and Organ Culture*, 157, 73.

Niazian M., Shariatpanahi M. E., 2020. *In vitro*-based doubled haploid production: recent improvements. *Euphytica*, 216(5), 69.

Pang W. Q., Tan S. T., Mad'Atari M. F., Yoong I. C. K., Subramaniam S. 2023a. Establishment of an efficient micropropagation protocol for Cameron Highlands White Strawberry (*Fragaria* × *ananassa*) using light emitting diodes (LEDs) system. *South African Journal of Botany*, 157, 189-200.

Pang W. Q., Lai C. S., Mad'Atari M. F., Pandian B. R., Ibrahim M. N. M., Tan S. T., *et al*., 2023b. Effect of graphene oxide nanoparticles on in vitro growth of *Fragaria* × *ananassa* (Cameron Highlands white Strawberry) and evaluation of genetic stability using DAMD and ISSR markers. *Plant Physiology and Biochemistry*, 204, 108104.

Parab A. R., Han K. Y., Chew B. L., Subramaniam S., 2021. Morphogenetic and physiological effects of LED spectra on the apical buds of *Ficus carica* var. Black Jack. *Scientific Reports*, 11, 1-11.

Raj D. M., Perumal K., Balakrishnan K., Subramaniam S., 2024. Revitalizing *Cocos nucifera* L var matag: unravelling new horizons in clonal propagation through organogenesis and LED illumination. *Plant Cell, Tissue and Organ Culture*, 158, 36.

Rajasekharan P. E., Sahijram, L., 2015. In vitro conservation of plant germplasm. In: Bahadur, B., Venkat Rajam, M., Sahijram, L., Krishnamurthy, K. (Eds) *Plant biology and biotechnology: volume II: plant genomics and biotechnology*, Springer, New Delhi, 417-443.

Ramlal A., Mehta S., Nautiyal A., Baweja P., Shivam Sharma D., et al., 2024. Androgenesis in soybean (*Glycine max* (L.) Merr.): a critical revisit. *In Vitro Cellular & Developmental Biology-Plant*, 60, 1-15.

Ramlal A., Sharma D., Lal S. K., Raju D., Shivam, Rajendran A., 2023. First report of ovary-derived calli induction in soybean [*Glycine max* (L.) Merr.]. *Plant Cell, Tissue and Organ Culture*, 153, 439-445.

Sahu M., Maurya S., Jha Z. 2023. In vitro selection for drought and salt stress tolerance in rice: an overview. *Plant Physiology Reports*, 28, 8-33.

Sena S., Kumari S., Kumar V., Husen A., 2024. Light emitting diode (LED) lights for the improvement of plant performance and production: A comprehensive review. *Current Research in Biotechnology*, 7, 100184.

SharathKumar M., Heuvelink E., Marcelis L. F., 2020. Vertical farming: moving from genetic to environmental modification. *Trends in Plant Science*, 25, 724-727.

Shukla M. R., Singh A. S., Piunno K., Saxena P. K., Jones A. M. P., 2017. Application of 3D printing to prototype and develop novel plant tissue culture systems. *Plant Methods*, 13, 1-10.

Smith M. K., Drew, R. A., 1990. Current applications of tissue culture in plant propagation and improvement. *Functional Plant Biology*, 17, 267-289.

Sudheer W. N., Praveen N., Al-Khayri J. M., Jain, S. M., 2022. Role of plant tissue culture medium components. In: Rai A.C., Kumar A., Modi A., Singh M., (Eds), *Advances in plant tissue culture: Current developments and future trends*, Academic Press, 51-83.

Winson K. W. S., Chew B. L., Sathasivam K., Subramaniam S., 2021. Effect of amino acid supplementation, elicitation and LEDs on *Hylocereus costaricensis* callus culture for the enhancement of betalain pigments. *Scientia Horticulturae*, 289, 110459.

Xu Y., Chang Y., Chen G., Lin H., 2016. The research on LED supplementary lighting system for plants. *Optik*, 127(18), 7193-7201.

Ying Q., Kong Y., Jones-Baumgardt C., Zheng Y., 2020. Responses of yield and appearance quality of four Brassicaceae microgreens to varied blue light proportion in red and blue light-emitting diodes lighting. *Scientia Horticulturae*, 259, 108857.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

ASSESSMENT OF *BUXBAUMIA VIRIDIS* FROM TWO NATURA 2000 SITES: RETEZAT (ROSCI0217) AND RUSCA MONTANĂ - ȚARCU - RETEZAT CORRIDOR (ROSCI0292)

Sorin ȘTEFĂNUȚ[1], Ana-Maria CORPADE[2], Claudia DANAU[3], Miruna-Maria ȘTEFĂNUȚ[1]

[1]Institute of Biology Bucharest, 296 Splaiul Independenței, District 6, Bucharest, Romania
[2]Faculty of Geography, Babeș-Bolyai University, 5-7 Clinicilor Street, Cluj-Napoca, Romania
[3]RNP Romsilva - Retezat National Park Administration, 284 Nucșoara Street, Com. Salasu de Sus, Hunedoara County, Romania

Corresponding author email: sorinstefanut@gmail.com

Abstract

Buxbaumia viridis (Moug. ex Lam. & DC.) Brid. ex Moug. & Nestl. is a moss species included in the Habitats Directive 92/43, being an umbrella species for mature forest habitats, especially spruce forest, well-conserved or with adequate forest management for long-term conservation. The results are based on field data collected in 2022-2024 and literature data. Research revealed that the species is undervalued, since at the beginning of the study there was a lack of data on the presence of the species in the Rusca Montană -Țarcu - Retezat Corridor site (ROSCI0292). Based on the field data, the number of B. viridis individuals and microhabitats (rotten wood) for the two sites were estimated.

***Key words**: Buxbaumia viridis, bryophyte, moss, threatened species, Habitats Directive 92/43.*

INTRODUCTION

Buxbaumia viridis (Moug. ex Lam. & DC.) Brid. ex Moug. & Nestl. is a moss species listed on Annex II of the European Habitats Directive (Council Directive 92/43/CEE, 1992), Natura 2000 species code: 1386. This moss is one of the easiest to identify bryophyte species of this Directive. Even if are two species of *Buxbaumia* in Romania (Ștefănuț & Goia, 2012) and in Europe (Hodgetts & Lockhart, 2020), *B. viridis* (Figure 1) and *B. aphylla* Hedw. (Figure 2), they can be easily distinguished in the field (no laboratory analysis is needed as for many bryophyte species).



Figure 1. Young sporophytes of *Buxbaumia viridis* (photo Ștefănuț S.)



Figure 2. *Buxbaumia aphylla* (photo Ștefănuț M.M.)

Moreover, the two species have different habitat preferences, *B. viridis* is found mainly on rotten wood and *B. aphylla* never on this substrate.
A number of 532 NATURA 2000 sites are designated for protection of *B. viridis* in Europe. Although it seemed to be a rare species, *B. viridis* turned out to be a frequently one in mature spruce and deciduous forests, after the increased attention given by this species' listing in the Habitats Directive.

By including it in the Habitats Directive it made *B. virdis* an umbrella species for protecting mature forest habitats, especially spruce forest, but beech forest, also.

MATERIALS AND METHODS

The research area is delimited by the two NATURA 2000 sites: Retezat – ROSCI0217 and Rusca Montană-Țarcu-Retezat Corridor – ROSCI0292 (Figure 3).

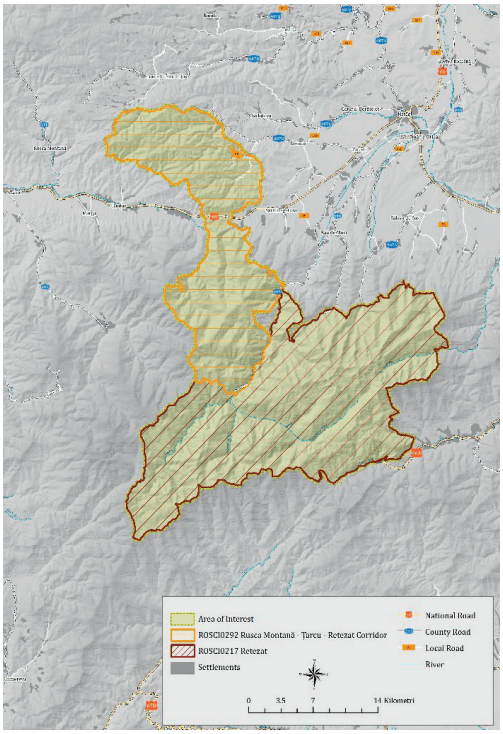


Figure 3. Retezat – ROSCI0217 site and Rusca Montană-Țarcu-Retezat Corridor – ROSCI0292 site

Because it is a species protected by Habitats Directive, no bryological material was collected, the determinations being made in the field. Photographs of the species and its habitats were taken, and the distribution data of the species were collected by GPS.

Transects were conducted along forested valleys from 600 m a.s.l. to 1850 m a.s.l. *B. viridis* was recorded on spruce or beech logs, spruce stumps, maple bark or on soil. Even though it was identified on the soil, these soils are full of rotten wood.

The bryophytes nomenclature used is according to Hodgetts et al., 2020.

The Area of Occupancy (AOO) for *B. viridis* was estimated using the 2 × 2 km grids, according to the IUCN Red List Categories and Criteria (IUCN Standards and Petitions Committee, 2024).

RESULTS AND DISCUSSIONS

The habitat of the *Buxbaumia viridis* species in the Retezat site (ROSCI0217) consists of spruce and mixed forests, spruce with beech, located between 800 m a.s.l. to 1,750 m a.s.l., with an area of 20,204.19 ha (Figure 4).

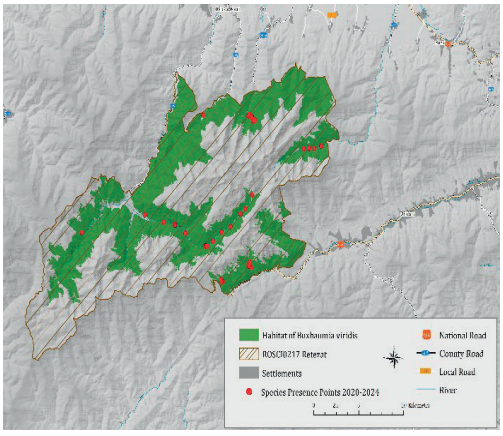


Figure 4. Habitat of *Buxbaumia viridis* in Retezat – ROSCI0217 site

To estimate the population of *B. viridis* in the Retezat site (ROSCI0217), previously published field data (Ștefănuț et al., 2023), data collected by the Retezat National Park Administration (2022-2023) and field data (2024) were used.

During the field trip on August 21-25, 2024, the species *B. viridis* was reported from 16 different localities in the Retezat Mountains, Lăpușnicul Mare Valley (Table 1: LMV-2 – Lăpușnicul Mare Valley; Figure 5; Figure 6).



Figure 5. *Buxbaumia viridis* in Retezat

In the assessment of the population of *B. viridis* from the Retezat site (ROSCI0217), distribution data of the species provided by the Retezat National Park Administration, recently collected (2022-2023) were also used (Table 1: RV-1 – Râușor Valley, BRV-1 – Bărbat River Valley and LMV-1 – Lăpușnicul Mare Valley).

Table 1. New records of *Buxbaumia viridis* in Retezat – ROSCI0217 site

Locality	Latitude	Longitude	m a.s.l.	Date
RV-1	45°24'19.07"N	22°49'20.86"E	1482	16.05.2023
BRV-1	45°22'57.90"N	22°59'27.33"E	1329	16.05.2023
BRV-1	45°22'57.58"N	22°59'26.64"E	1320	11.10.2023
BRV-1	45°22'50.61"N	22°58'51.53"E	1341	11.10.2023
BRV-1	45°22'49.68"N	22°58'25.87"E	1364	11.10.2023
BRV-1	45°22'49.48"N	22°58'25.00"E	1416	11.10.2023
BRV-1	45°22'49.15"N	22°58'24.86"E	1407	11.10.2023
BRV-1	45°22'51.19"N	22°58'23.79"E	1410	11.10.2023
BRV-1	45°22'51.19"N	22°58'23.48"E	1416	11.10.2023
BRV-1	45°22'49.14"N	22°57'58.75"E	1445	11.10.2023
BRV-1	45°22'48.42"N	22°57'57.73"E	1449	11.10.2023
LMV-1	45°18'51.51"N	22°47'8.99"E	1205	03.11.2022
LMV-1	45°18'51.07"N	22°47'8.37"E	1262	03.11.2022
LMV-1	45°18'51.60"N	22°47'8.66"E	1245	03.11.2022
LMV-1	45°18'49.71"N	22°47'8.50"E	1245	03.11.2022
LMV-1	45°18'50.27"N	22°47'9.60"E	1260	03.11.2022
LMV-1	45°17'49.80"N	22°49'57.36"E	1326	03.11.2022
LMV-2	45°19'17.10"N	22°44'34.30"E	1124	21.08.2024
LMV-2	45°18'56.60"N	22°46'12.30"E	1139	21.08.2024
LMV-2	45°18'48.60"N	22°47'7.00"E	1186	21.08.2024
LMV-2	45°17'50.70"N	22°49'47.90"E	1264	21.08.2024
LMV-2	45°17'49.50"N	22°49'49.30"E	1267	21.08.2024
LMV-2	45°17'49.30"N	22°49'49.50"E	1268	21.08.2024
LMV-2	45°17'50.60"N	22°49'49.00"E	1266	21.08.2024
LMV-2	45°18'50.10"N	22°51'52.40"E	1404	21.08.2024
LMV-2	45°20'26.61"N	22°53'38.48"E	1644	21.08.2024
LMV-2	45°19'44.30"N	22°53'4.80"E	1597	25.08.2024
LMV-2	45°19'44.40"N	22°53'5.30"E	1599	25.08.2024
LMV-2	45°19'28.70"N	22°52'42.40"E	1476	25.08.2024
LMV-2	45°19'29.50"N	22°52'41.40"E	1469	25.08.2024
LMV-2	45°18'32.80"N	22°51'7.50"E	1363	25.08.2024
LMV-2	45°18'4.90"N	22°50'24.40"E	1307	25.08.2024
LMV-2	45°18'26.80"N	22°48'3.00"E	1212	25.08.2024

A number of 63 logs, 59 spruce logs and 4 beech logs, colonized by *B. viridis* and one record on the soil were reported from the Retezat site (ROSCI0217). From these, 35 logs are new reported and 28 logs one record on the soil were published before (Ștefănuț et al., 2023).

The total number of *B. viridis* sporophytes identified in the Retezat site (ROSCI0217) is 483, with 261 sporophtes new reported here and 222 sporophtes published before (Ștefănuț et al., 2023).

For the Retezat site (ROSCI0217) a total population of 15,000 sporophytes of *B. viridis* and about 2,000 microhabitats (logs, stumps or m^2 soil) are estimated.



Figure 6. Spruce logs colonized by *Buxbaumia viridis* in Retezat – ROSCI0217 site

The habitat of the species *B. viridis* in the Rusca Montană - Țarcu - Retezat Corridor site (ROSCI0292) consists of spruce and mixed forests, spruce with beech, located between 800 a.s.l. to 1750 m a.s.l., totaling an area of 14,925.25 ha (Figure 7).

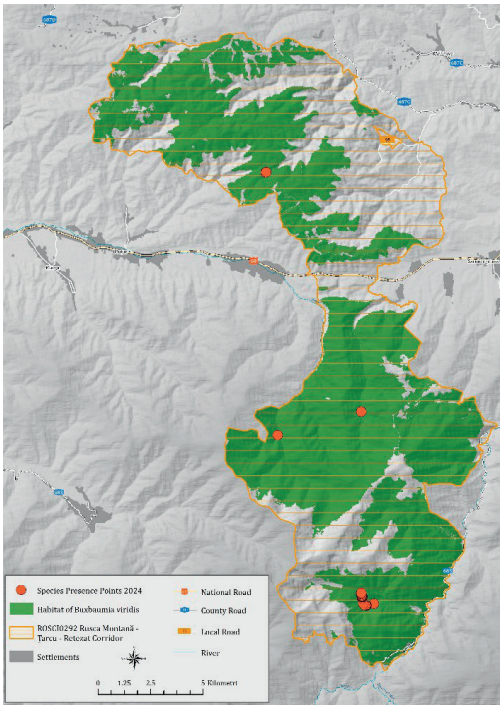


Figure 7. Habitat of *Buxbaumia viridis* in Rusca Montană-Țarcu-Retezat Corridor – ROSCI0292 site

To estimate the population of *B. viridis* in the Rusca Montană - Țarcu - Retezat Corridor site (ROSCI0292), no previously published data before 2023 were identified (Ștefănuț et al., 2023) and exclusively field data collected during August 22-26, 2024, were used (Table 2).

The reporting of *B. viridis* from the Poiana Ruscă Mountains proved to be the first record for these mountains (Sabovljević et al., 2024).

B. viridis were reported from 45 microhabitats in the Rusca Montană -Țarcu - Retezat Corridor site (ROSCI0292), 43 spruce logs, one beech log and one rotten maple (*Acer pseudoplatanus* L.) bark (Figure 11).

A total number of 239 sporophytes of *B. viridis* were identified in the Rusca Montană -Țarcu - Retezat Corridor site (ROSCI0292).

For the Rusca Montană - Țarcu - Retezat Corridor site (ROSCI0292) a total population of 5,300 sporophytes of *B. viridis* and about 1,000 microhabitats (logs, stumps or m^2 soil) are estimated.

Table 2. New records of *Buxbaumia viridis* to Rusca Montană -Țarcu - Retezat Corridor site

Locality	Latitude	Longitude	m a.s.l.	Date
NV-Ț	45°21'57.50"N	22°42'55.90"E	1232	22.08.2024
NV-Ț	45°21'54.80"N	22°42'40.00"E	1292	22.08.2024
NV-Ț	45°21'54.90"N	22°42'39.60"E	1293	22.08.2024
NV-Ț	45°21'54.80"N	22°42'38.73"E	1295	22.08.2024
NV-Ț	45°21'54.40"N	22°42'38.70"E	1295	22.08.2024
NV-Ț	45°21'54.70"N	22°42'38.90"E	1294	22.08.2024
NV-Ț	45°21'55.10"N	2°42'37.90"E	1297	22.08.2024
NV-Ț	45°21'55.20"N	22°42'35.10"E	1307	22.08.2024
NV-Ț	45°21'57.10"N	22°42'32.70"E	1315	22.08.2024
NV-Ț	45°22'5.10"N	22°42'29.90"E	1336	22.08.2024
NV-Ț	45°22'6.92"N	22°42'29.70"E	1341	22.08.2024
NV-Ț	45°22'7.70"N	22°42'29.90"E	1345	22.08.2024
NV-Ț	45°22'8.10"N	22°42'29.90"E	1347	22.08.2024
NV-Ț	45°22'8.70"N	22°42'29.50"E	1347	22.08.2024
NV-Ț	45°22'8.60"N	22°42'29.10"E	1343	22.08.2024
NV-Ț	45°22'8.50"N	22°42'30.10"E	1351	22.08.2024
NV-Ț	45°22'9.20"N	22°42'29.20"E	1346	22.08.2024
NV-Ț	45°22'9.10"N	22°42'28.70"E	1343	22.08.2024
NV-Ț	45°22'10.30"N	22°42'28.70"E	1348	22.08.2024
NV-Ț	45°22'11.70"N	22°42'27.90"E	1351	22.08.2024
NV-Ț	45°22'13.10"N	22°42'27.20"E	1354	22.08.2024
NV-Ț	45°22'13.70"N	22°42'27.80"E	1362	22.08.2024
BV-Ț	45°26'14.40"N	22°39'14.30"E	1045	23.08.2024
ZV-Ț	45°26'54.60"N	22°42'16.30"E	1099	23.08.2024
FV-PR	45°33'1.20"N	22°38'31.70"E	851	26.08.2024
FV-PR	45°33'0.60"N	22°38'30.90"E	859	26.08.2024
FV-PR	45°33'1.25"N	22°38'32.20"E	849	26.08.2024

On rotten woods, *B. viridis* was identified along with *Herzogiella seligeri* (Brid.) Z. Iwats. (Figures 8-10) in almost all localities.

During the field trip in 2024, *B. viridis* were reported from one locality in the Bistra Valley, Țarcu Mountains (Table 2: BV-Ț; Figure 8); 22 localities in the Netiș Valley, Țarcu Mountains (Table 2: NV-Ț; Figures 9,10); one locality in the Zeicani Valley, Țarcu Mountains (Table 2: ZV-Ț); 3 localities in the Fierului Valley, Poiana Ruscă Mountains (Table 2: FV- PR; Figure 11).



Figure 8. *Buxbaumia viridis* on beech log, Bistra Valley, Țarcu Mountains, Rusca Montană-Țarcu-Retezat Corridor – ROSCI0292 site



Figure 9. *Buxbaumia viridis* and *Herzogiella seligeri* on spruce stumps, Netiș Valley, Țarcu Mountains, Rusca Montană-Țarcu-Retezat Corridor – ROSCI0292 site

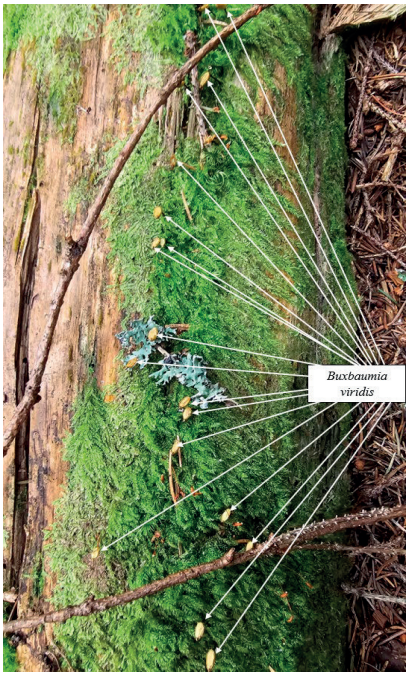


Figure 10. *Buxbaumia viridis* (18 mature sporophytes) and *Herzogiella seligeri* on spruce log, Netiș Valley, Țarcu Mountains, Rusca Montană-Țarcu-Retezat Corridor – ROSCI0292 site



Figure 11. *Buxbaumia viridis* (2 sporophytes) on rotten maple (*Acer pseudoplatanus* L.) bark, Fierului Valley, Poiana Ruscă Mountains, Rusca Montană-Țarcu-Retezat Corridor – ROSCI0292 site

CONCLUSIONS

In the present, 256 microhabitats and 2,049 sporophytes of *Buxbaumia viridis* were reported to Romania.

According our study, the Area of Occupancy (AOO) for *B. viridis* in Romania is increasing from 268 km^2 (Ștefănuț et al., 2023) to 304 km^2 and all population of *B. viridis* are estimated at more than 200,000 sporophytes and more than 25,000 microhabitats (logs, stumps or m^2 soil).

The conservation status of *Buxbaumia viridis* in Romania is unchanged at Vulnerable – VU A3c (Ștefănuț et al., 2023).

ACKNOWLEDGEMENTS

This research work was carried out with the support of Institute of Biology Bucharest, Romanian Academy, Faculty of Geography, Babeș-Bolyai University and National Forest Administration – Romsilva, Retezat National Park Administration.

REFERENCES

Council Directive 92/43/CEE (1992). Council directive 92/43/EEC of 21 May 1992 on the conservation of

natural habitats and of wild fauna and flora. *Official Journal of the European Union*, L 206/7, 22.7.1992, p.7.

Hodgetts, N.G., Lockhart, N. (2020). *Checklist and country status of European bryophytes- update 2020. Irish Wildlife Manuals*, No. 123. Dublin, Ireland: National Parks and Wildlife Service, Department of Culture, Heritage and the Gaeltacht.

Hodgetts, N.G., Söderström, L., Blockeel, T.L., Caspari, S., Ignatov, M.S., Konstantinova, N.A., Lockhart, N., Papp, B., Schröck, C., Sim-Sim, M., Bell, D., Bell, N.E., Blom, H.H., Bruggeman-Nannenga, M.A., Brugués, M., Enroth, J., Flatberg, K.I., Garilleti, R., Hedenäs, L., Holyoak, D.T., Hugonnot, V., Kariyawasam, I., Köckinger, H., Kučera, J., Lara, F., Porley, R.D. (2020). An annotated checklist of bryophytes of Europe, Macaronesia and Cyprus. *Journal of Bryology*, 42(1), 1–116. DOI: 10.1080/03736687.2019.1694329

IUCN Standards and Petitions Committee (2024). Guidelines for Using the IUCN Red List Categories and Criteria. Version 16. Prepared by the Standards and Petitions Committee. Downloadable from Available online: https://www.iucnredlist.org/documents/RedListGuidelines.pdf. (accessed on 7 March 2025).

Sabovljević, M.S., Tomović, G., Ștefănuț, S., Djordjević, V., Stanković, S., Djurović, S.Z., Frajman, B., Boycheva, P., Kaschieva, M., Szűcs, P., Sabovljević, A.D., Trbojević, I., Marković, A., Vlaović, L., Vidaković, D., Jenačković Gocić, D., Nikolić, D., Stoykov, D., Krdžić, S., Chinan, V-C., Mânzu, C.C., Assyov, B, Niketić, M. (2024). New records and noteworthy data of plants, algae and fungi in SE Europe and adjacent regions, 19. *Botanica Serbica*, 48(2), 297–307. DOI: 10.2298/BOTSERB2402297S.

Ștefănuț, S., Goia, I. (2012). Checklist and Red List of Bryophytes of Romania. *Nova Hedwigia,* 95(1-2), 59–104. DOI: 10.1127/0029-5035/2012/0044

Ștefănuț, S., Ion, C.M., Sahlean, T., Tamas, G., Nicoară, G.-R., Vladimirescu, M., Moroșanu, A.-M., Helepciuc, F.-E., Ștefănuț, M.-M., Bîrsan, C.-C. (2023). Population and Conservation Status of *Buxbaumia viridis* (DC.) Moug. & Nestl. in Romania. *Plants-Basel*, 12, Art. no. 473. DOI: 10.3390/plants12030473

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

STUDY ABOUT THE INFLUENCE OF GIBBERELLIC ACID AND NAPHTHYL ACETIC ACID ON *IN VITRO* DEVELOPMENT OF FOUR ROMANIAN POTATO VARIETIES

Andreea TICAN, Mihaela CIOLOCA, Monica POPA

National Institute of Research and Development for Potato and Sugar Beet Brasov,
2 Fundăturii Street, Braşov, Romania

Corresponding author email: tican_andreea@yahoo.com

Abstract

*In 2024 within the Research Laboratory for Plant Tissue Cultures of National Institute for Research and Development for Potato and Sugar Beet Braşov, Romania an in vitro study was performed to determine the optimum concentration of gibberellic acid and naphthyl acetic acid for growth and development of plantlets belonging of four Romanian potato varieties, created at our institute. The minicuttings were cultured in Murashige and Skoog medium (MS). Gibberellic acid and naphthyl acetic acid were applied in different concentrations. The bifactorial experience of the 4*4 type included 16 variants, on 3 repetitions. Factor a is represented by the variety with 4 graduations (Azaria, Braşovia, Cosiana and Cezarina) and factor b is the culture medium with 4 graduations (0 mg GA_3: 0.005 NAA; 3 mg GA_3: 0.005 mg NAA; 4 mg GA_3: 0.01 mg NAA; 5 mg GA_3: 0.2 mg NAA). Measurements were made for: number of leaves, vitroplants height, root length and internodes number. For the analyzed parameters, the varieties behaved differently with each hormonal treatment applied.*

Key words*: potato, in vitro, variety, gibberellic acid, naphthyl acetic acid.*

INTRODUCTION

In the rapid multiplication of clean material *in vitro*, the use of single nodal cutting is the most preferred method of propagation since it ensures higher propagation rates with maximum genetic uniformity in potato (Chandra and Naik, 1993). Tissue culture techniques are used worldwide to produce pre-basic, virus-free seed potatoes. Plant growth regulators play an important role in this progress and have been studied extensively but the interactions that take place between them are still being discovered (Ross and O'Neill, 2001; Zhang et al., 2002; Rafique et al., 2012; Rafique et al., 2013; Rasheed et al., 2013). The plant growth regulators (PGRs) modulate plant growth and development and mediate responses to both biotic and abiotic stress (Makau et al., 2022). They are of importance in regulating shoot and root development in seed potatoes *in vitro* (Badoni & Chauhan, 2010; Hoque, 2010).

Gibberellic acid (GA_3) is a diterpenoid carboxylic acid that belongs to the gibberellin's family and acts as a natural plant growth hormone. Plants and some microorganisms, such as fungi and bacteria, produce it. GA_3 has promising applications in the agro-industrial sector due to its properties related to plant development. Since it was discovered, studies have been focused on enhancing its process yield, boosting its productivity, and reducing its cost, which sometimes restricts the use of this important growth hormone (Camara et al., 2018). GA_3 is involved in cell elongation and its addition in MS medium enhances shoot growth (Camara et al., 2018; Rizza et al., 2017). The phytohormone gibberellin (GA) is a key regulator of plant growth and development (Rizza et al., 2017).

The basic Murashige &Skoog (MS) medium (Murashige & Skoog, 1962) is the most widely used media in production of potato (*Solanum tuberosum* L.). Since each individual hormone has its own unique effect on regeneration, it is vital to determine the combined effects of these on the regeneration of shoots and roots (Kumlay, 2014). The aim of this study was to establish the plant growth regulators concentration for plantlets development of four potato varieties.

MATERIALS AND METHODS

In 2024 within the Research Laboratory for Plant Tissue Cultures of NIRDPSB Brașov, Romania an *in vitro* study was performed to determine the optimum concentration of gibberellic acid and naphthyl acetic acid for growth and development of plantlets belonging to four Romanian potato varieties, created at our institute. The minicuttings were cultured in Murashige and Skoog (MS) media supplemented with sucrose; gibberellic acid and naphthyl acetic acid were applied, as follow in Table 1. Medium with 5 mg GA_3 and 0.2 mg NAA significantly enhanced shoot length, with the longest shoot (14.17 cm) as a mean for all values of varieties.

The treatment scheme followed the influence of the combination of gibberellic acid and naphthyl acetic acid on the *in vitro* development of the seedlings of the varieties under analysis. A 4 × 4 factorial design was used, in three replicates. Factor a is represented by the variety with 4 graduations (Azaria, Brașovia, Cosiana and Cezarina – control variety), and factor b is the culture medium with 4 graduations (according to treatment scheme from Table 1).

Table 1. Treatment scheme (Combination of gibberellic acid and naphthyl acetic acid)

Plant growth regulator	The amount introduced into the culture medium (mg/l)							
GA_3	0	b_1	3	b_2	4	b_3	5	b_4
NAA	0.005		0.005		0.01		0.2	

RESULTS AND DISCUSSIONS

The results of the variance analysis showed that the effect of hormonal treatment was significant for plantlets height at the level <0.05 and for root length at the level <0.01. The influence of variety was significant for root length at the level <0.05 (Table 2).

The application in the culture medium of different concentrations of gibberellic acid and naphthyl acetic acid was favourable for Cezarina variety, which recorded the highest number values the number of leaves (8.50); the height of the seedlings (12.88 cm) and the number of internodes (6.58). About number of leaves and internodes, there were no significant differences, the values being between 8.50 (Cezarina variety) and 8.08 (Azaria, Brașovia, Cosiana) for the number of leaves and 6.58 (Cezarina variety) and 5.42 (Azaria variety) for number of internodes. Regarding the height of the plants between Brașovia (12.58 cm), Cosiana (12.83 cm) and Cezarina varieties (12.88 cm) there were no significant differences instead, the recorded values of the vitroplants height for these three previously mentioned varieties they differed significantly from Azaria variety (9.38 cm). For root length, there were significant differences between Cosiana (4.29 cm) and Azaria (2.96 cm) cultivars (Table 3).

Table 2. Analysis of variance in F values for determined parameters

Source of variation	df	Leaves number	Plantlets height	Root length	Number of internodes
Variety (a)	3	0.208 ns (4.76; 9.78)	3.401 ns	5.47 *	1.209 ns
Hormonal treatment (b)	2	0.877 ns (3.01; 4.72)	4.038 *	9.077 **	1.312 ns
Variety (a) *Hormonal treatment (b)	9	1.092 ns (2.30; 3.26)	0.980 ns	0.895 ns	1.007 ns

Table 3. The influence of variety on the studied parameters under the effect of the use of gibberellic acid and naphthyl acetic acid in the culture medium

Variety	Leaves number	Vitroplants height (cm)	Root lenght (cm)	Internodes number
Azaria	8.08 A	9.38 B	2.96 B	5.42 A
Brașovia	8.08 A	12.58 A	3.25 AB	6.08 A
Cosiana	8.08 A	12.83 A	**4.29 A**	**6**.25 A
Cezarina	**8.50 A**	**12.88 A**	2.54 B	**6.58 A**

Means of each factor designated by the same letter are not significantly different at 5% level using Duncan's Multiple Range Test.

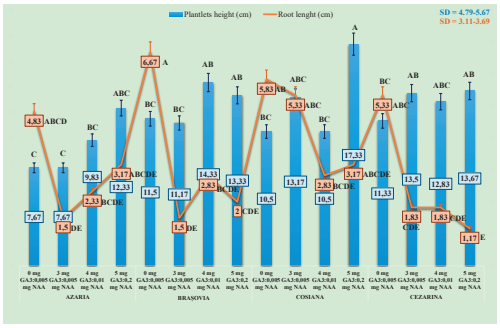
Treatment 4 (T4: 5 mg GA_3: 0.2 mg NAA) from treatment scheme was favourable for the following elements analysed in the experiment: number of leaves (8.92), but there were no significant differences between the four treatments and height seedlings (14.17 cm); for this parameter significant differences are observed between treatment 4 and treatments 2 and 3. Regarding the viroplants root, the best result was obtained for treatment 1, with significant differences compared to the other treatments that had a negative influence, leading to a decrease in the length of the root system. Combination 3 mg GA_3: 0.005 mg NAA (T2) was beneficial for the formation of a large number of internodes (6.50), but without significant differences between treatments

(Table 4). According to Pennazio and Vecchiati, (1976) higher concentration of NAA inhibited root, form Table 4 we can observe that root growth decreased with increasing NAA concentration. Increasing the concentration of GA_3 leads to plantlets development, at higher concentration of GA_3 are obtained vitroplants with the greatest height (Table 4), suitable with Salisbury and Ross (1985) study, which mentions that gibberellic acid (GA_3) is a plant growth regulator that has the unique ability to promote extensive growth of many intact plants

Table 4. The influence of hormonal treatment on the studied parameters)

Hormonal treatment applied (mg/l)			Leaves number	Vitroplants height (cm)	Root lenght (cm)	Internodes number
	GA_3	NAA				
T1	0	0.005	7.58 A	10.25 B	**5.67 A**	5.17 A
T2	3	0.005	7.83 A	11.38 B	2.54 B	**6.50 A**
T3	4	0.01	8.42 A	11.88 AB	2.46 B	6.42 A
T4	5	0.2	**8.92 A**	**14.17 A**	2.38 B	6.25 A

Means of each factor designated by the same letter are not significantly different at 5% level using Duncan's Multiple Range Test.



Means of each parameter designated by the same letter are not significantly different al 5% level using Duncan's Multiple Range Test

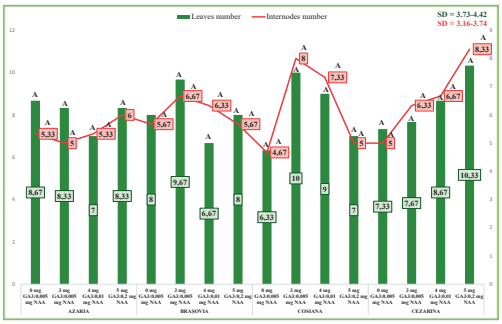
Figure 1. The influence of variety and hormonal treatment applied over plantlets height (cm) and root length (cm)

Each treatment had a different influence for the varieties studied, regarding the four parameters (Figures 1 and 2).

Combination of 5 mg GA_3: 0.2 mg NAA positively influenced Cosiana variety (Figure 1) regarding plantlets height, with the highest value being recorded for this element (17.33 cm). The lowest values of plantlets height (7.67 cm) were observed for Azaria cultivar for treatments 1 and 2.

Brașovia (6.67 cm) and Cosiana (5.83 cm) varieties presented the highest values of root system length, for treatment 1, without significant differences (Figure 1).

From the analysis of the combined influence of the variety and the applied hormone combinations, there are no significant differences in the number of leaves and the number of internodes (Figure 2).



Means of each parameter designated by the same letter are not significantly different al 5% level using Duncan's Multiple Range Test

Figure 2. The influence of variety and hormonal treatment applied over leaves and internodes number

CONCLUSIONS

For the analysed parameters, the varieties behaved differently with each hormonal treatment applied.

The application in the culture medium of different concentrations of gibberellic acid and naphthyl acetic acid was favourable for Cezarina variety, which recorded the highest number values the number of leaves (8.50); the height of the seedlings (12.88 cm) and the number of internodes (6.58).

For root length, the highest value was recorded for Cosiana variety (4.29 cm).

Medium with 5 mg GA_3 and 0.2 mg NAA significantly enhanced shoot length, with the longest shoot (14.17 cm) as a mean for all values of varieties; this hormonal combination was beneficial in forming a high number of leaves (8.92).

Combination of 5 mg GA_3: 0.2 mg ANA positively influenced Cosiana variety (regarding plantlets height, with the highest value being recorded for this element - 17.33 cm).

Brașovia (6.67 cm) and Cosiana (5.83 cm) varieties presented the highest values of root system length, for combination 0 mg GA_3: 0.005 mg NAA, without significant differences

ACKNOWLEDGEMENTS

This research work was carried out with the support of National Institute of Research and Development for Potato and Sugar Beet Brasov and also was financed by Ministry of Research, Innovation and Digitalization, from PN 23 19 01 01 Project "Research on increasing the performance process of the potato minituber production using an intelligent aeroponic system", period 2023-2026.

REFERENCES

Badoni, A., Chauhan, J. S. (2010). Potato seed production of cultivar Kufri Himalini *in vitro*. *Stem Cell*, 1(1), 7-10.

Camara, M., Vandenberghe, L., Rodriguez, C., Oliveira, J., Faulds, C., Betrand, E., Soccol C. (2018). Current advances in gibberellic acid (GA_3) production, patented technologies and potential applications. *Planta*, 248(5), 1049-1062.

Chandra, R., Naik, P. S. (1993). Potato tissue and cell culture in Potato Research in India, *Advances in Horticulture*, Chandra, K. L. and Grewel, J. S. (Eds.), pp. 113-141.

Hoque, M. (2010). *In vitro* regeneration potentiality of potato under different hormonal combination. *World Journal of Agriculture Sciences*, 6(6), 660-663.

Kumlay, A. M. (2014). Combination of the auxins NAA, IBA, and IAA with GA_3 improves the commercial seed-tuber production of potato (*Solanum tuberosum* L.) under *in vitro* conditions, *Hindawi Publishing CorporationBioMed Research International* Volume 2014, http://dx.doi.org/10.1155/2014/439259.

Makau, F. M., Mwangi, M., Oyoo, M. E., Kibe, M. A., Oggema, J. (2022). Effects of Sucrose and Gibberellic Acid on Growth and Survival of Local Potato (*Solanum tuberosum* L.) Varieties *in vitro* in Kenya, *European Journal of Biology and Biotechnology*, Vol 3, Issue 4, DOI: http://dx.doi.org/10.24018/ejbio.2022.3.4.372.

Murashige, T., Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiologia Plantarum*, 15, 473-497.

Pennazio, S., Vecchiati, M. (1976). Effect of naphthalene acetic acid on meristem tips development. *Potato Res*., 19(3): 232-234.

Rafique, R., Fatima, B., Mushtaq, S., Iqbal, M. S., Rasheed, M., Ali, M. and Zia-Ul-Hasan, S. (2012). Effect of indole-3-butyric acid (IBA) on *in vitro* root induction in dendrobium orchid (Dendrobium sabin H.). *Afr. J. Biotech*., 11(20), 4673-4675

Rafique, R., Fatima, B., Usman, M., Iqbal, M. S., Hasan, S. Z. U., Rasheed, M., Iqbal, M., Mushtaq, S., Rasheed, M. (2013). Effect of sucrose on *in vitro* Callogenesis, Embryogenesis and Organogenesis of Dendrobium sabin H. *Int. J. Modern Agri*., 2(1):43-47.

Rasheed, M., Jaskani, M. J., Rasheed, M., Iqbal, M. S., Hasan, S. Z. U., Rafique, R., Mushta, S. and Iqbal, M. (2013). Regeneration of plantlets from various explants of tetraploid watermelon. *J Bio. Agri* & Healtcare, 3(1): 27-29.

Rizza, A., Walia, A., Languar, V., Frommer, W. & Jones, A. (2017). *In vivo* gibberellin gradients visualized in rapidly elongating tissues. *Nature Plants*, 3(10), 803-813.

Ross, J. J., O'Neill, D. P. (2001). New interactions between classical plant hormones. *Trends Plant Sci*., 6: 2-4.

Salisbury, F. B., Ross, C. W. (1985). *Plant Physiology* (3rd ed.). Wadsworth Pub.

Zhang, Z. J., Zhou, J., Li, H. Z., Yoneyama, K., Takeuchi, Y. (2002). Effect of $AgNO_3$ on *in vitro* microtuber induction in potato. *Acta Physiologiae Plantarum*, 27(3): 363-369.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

STUDIES REGARDING THE CYTOTOXICITY OF A BIOPRODUCTS BASED ON POLYSACCHARIDES DERIVED FROM *PLANTAGO* SP.

Marilena-Gabriela OLTEANU ZAHARIE[1], Nicoleta RADU[1, 2], Narcisa Elena BĂBEANU[1], Marinela BOSTAN[3], Viviana ROMAN[3]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăşti Blvd, District 1, Bucharest, Romania
[2]National Institute of Chemistry and Petrochemistry R & D of Bucharest, 202 Splaiul Independentei, District 6, 060021, Bucharest Romania
[3]Center of Immunology, Virology Institute "Stefan S. Nicolau", 285 Mihai Bravu Avenue, District 3, 030304, Bucharest, Romania

Corresponding author email: nicoleta.radu@biotehnologii.usamv.ro

Abstract

Cytotoxicity studies performed on HUVEC and CaCo-2 cell lines with a polysaccharides-based bioproduct isolated from Plantago lanceolata as well with inulin (for comparison) revealed moderate cytotoxicity after 24-hour exposure to inulin and polysaccharides from Plantago lanceolata. Inulin showed a high selectivity index (SI = 11) for the CaCo-2 tumour cell line, indicating specificity for this cell line. After 48 hours, the polysaccharides from Plantago lanceolata showed no cytotoxicity on either studied cell line. Inulin showed no cytotoxicity to HUVEC cells but exhibited cytotoxicity to CaCo-2 cells, giving a higher selectivity index (SI = 25.3) after 48 hours of exposure. Overall, the polysaccharides from Plantago lanceolata showed similar physicochemical properties to inulin, without cytotoxicity on HUVEC cells and moderate cytotoxicity on CaCo-2 cells after 48 hours of exposure.

Key words*: cytotoxicity, Plantago lanceolata, selectivity index.*

INTRODUCTION

Polysaccharides from plants represent potential sources of fibre for functional foods. From this perspective, an interesting species is *Plantago lanceolata*, an indigenous plant from spontaneous flora, from which bioproducts with therapeutic properties can be obtained (Pol et al., 2021; Zaharie et al., 2022; Radu et al., 2010). At present, this plant are distributed entire the worldwide (Figure 1) (Ianovici et al., 2010; Rojas Sandoval, 2023). According to data published by Bräutigam & Franz (Bräutigam & Franz, 1985), the crude polysaccharides extract obtained from *Plantago lanceolata* contains as a major compounds D-galactose, L-arabinose, and D-galacturonic acid. The leaves of this species contain polysaccharides with units consisting of galactose (44%), arabinose (32.4%), rhamnose (6.9%), mannan (4%), glucose (9.2%), and xylose (1.9%) (Pol et al., 2021; Clifford et al., 2002). Recent studies performed on three species of *Plantago* have shown that, overall, the plant may contain 12% fibre and 11.64% carbohydrates (Alghamadi, 2018). Polysaccharides from *Plantago,* obtained through acid hydrolysis, contain sorbitol, glucitol, fructose, glucose, sucrose, and raffinose (Jiang et al., 2019). Guil-Guerrero (Guil-Guerrero, 2001) determined the mineral, fatty acid, vitamin, nitrate, and oxalic acid content for three *Plantago* species from south-eastern Spain, and the results obtained showed that these plants had a low carbohydrate content, ranging between 1.99-2.81 g/100 g fresh plant.

Polysaccharides isolated from indigenous plants can represent potential sources of fiber for new functional foods. From this perspective, an interesting species is *Plantago lanceolata*, an indigenous plant from the spontaneous flora, from which bioproducts with therapeutic properties can be obtained. Our previous studies (Zaharie et al., 2022) revealed that the polysaccharide fraction isolated from *Plantago lanceolata* exhibits prebiotic activities for probiotic microorganisms such as *Lactobacillus* sp.

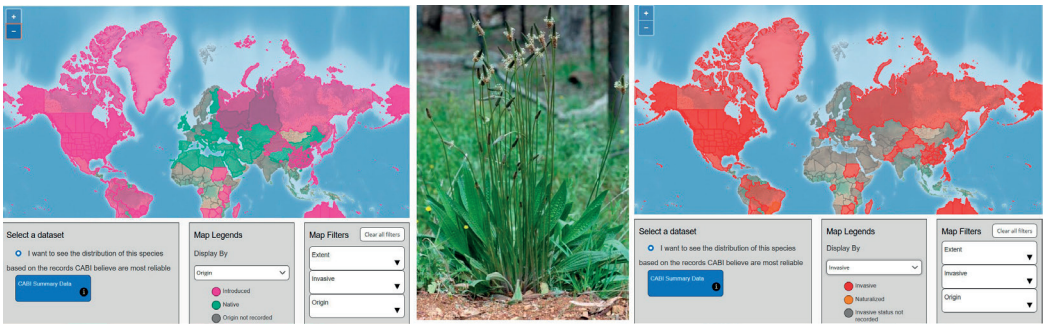


Figure1. The global distribution of the plant *Plantago lanceolata* (Sources: https://www.cabidigitallibrary.org/doi/full/10.1079/cabicompendium.41813#sec-9; https://tropical.theferns.info/image.php?id=Plantago+lanceolata

In order to assess its potential for developing functional foods, the present study aimed to: 1) characterise physical chemical this fraction; and 2) to evaluate the effect of this biomaterial on normal and/or tumoral cell lines, by studies performed *in vitro*.

MATERIALS AND METHODS

Electrospray ionisation mass spectrometry (ESI-MS) analysis of the macromolecule sizes in the polysaccharide fraction isolated from Plantago sp. was performed using high-performance liquid chromatography (HPLC) with LC-MS/TOF 6224 system (Agilent Technology, Houston, TX, USA) (Băbeanu et al., 2022; Ho et al., 2003). The polysaccharides isolated from *Plantago* sp. were injected into the ionisation chamber as 0.1% solution made in 99% DMSO (dimethyl sulfoxide, Merck, Bucharest, Romania).

Infrared spectra were recorded in the 400-4000 cm^{-1} range using a Perkin Elmer FT-IR GX spectrophotometer (Perkin Elmer Ltd., Beaconsfield, UK), equipped with an ATR device, Dynascan interferometer, and DTGS (deuterated triglycine sulfate) detector. The infrared spectra were analysed comparatively, for a sample containing polysaccharides isolated from *Plantago lanceolata*, and for an inulin sample (inulin from chicory, with average molecular mass = 3.5-5.5 kDa, Merck, Bucharest, Romania). Cell proliferation tests were conducted according to the methodology described by Zaharie et al. (2022), using two standardised human cell lines: HUVEC ATCC PCS 100-010 and CaCo-2 ATCC HTB-37, The cell lines were provided by S.C. BioZyme SRL, Cluj-Napoca, Romania. The proliferation index (PI) was calculated using the following formula:

PI = (%viability of treated cells)/% viability of untreated cells (1)

where:

Viability % of treated cells = % of viable cells in each tested experimental variant;

Viability % of untreated cells = % of viable cells in the control sample (considered as a reference)

The analysed polysaccharides were isolated from the aerial part of the *Plantago lanceolata,* collected from the spontaneous flora of Sibiu, Romania (voucher specimen deposited at INCDCF Bucharest, under the number Plal19) according to methodology of Zaharie and colaborators (Zaharie et al, 2022).

RESULTS AND DISCUSSIONS

The results obtained from ESI-MS analysis (presented in Figure 2 and Table 1) show that the polysaccharides isolated from *Plantago lanceolata* contain species with molecular mass ranging between 0.2-1.94 kDa (Figure 2).

Infrared spectroscopy analysis (Figure 3 a, b; Table 1) conducted on the studied polysaccharide fraction, in comparison with inulin, revealed the following:

a) an intense band due to the carbon-carbon double bond (νC=C) appears at 1575 cm^{-1} in the polysaccharides isolated from *Plantago* sp. The same bands it is also present in inulin but shifted to 1617 cm^{-1};

b) a high-intensity band at 1398 cm^{-1}, which appears in the polysaccharide fraction isolated

from Plantago sp., is also observed in inulin, though as a weaker band which appears at 1417 cm^{-1} and is attributed to the C-H stretching vibration (νC-H); c) an intense band appears at 1084 cm^{-1} in the sample of Plantago's polysaccharides which also was found in inulin at 1099 cm^{-1}, but as a weaker band. These bands are mainly due to the stretching of the glycosidic bond (C-O-C) and C-C or C-O bond stretching from the pyranosic ring; d)the medium-intensity band from 878 cm^{-1} which appears in the in the polysaccharides from *Plantago* sp., also appears as a medium-intensity band in inulin at 873 cm^{-1} and is attributed to the vibration of the 2-keto pyranosic ring. The both biomaterials (polysaccharides from *Plantago* sp. and inulin) exhibit: a1) C-H stretching vibrations detected in the IR spectrum at 2922 cm^{-1} (*Plantago* sp.) and 2902 cm^{-1} (inulin); a2) bands attributed to intermolecular hydrogen bonding vibrations, appearing at 3238 cm^{-1} for polysaccharides from Plantago sp. and 3257 cm^{-1} for inulin (Balan et al., 2018).

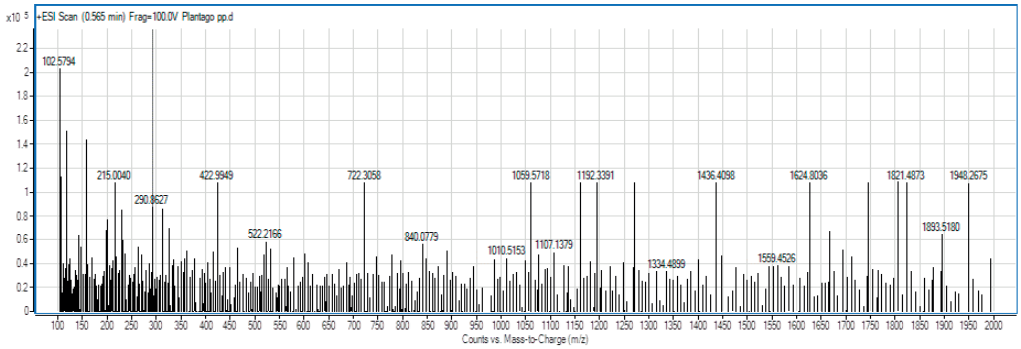


Figure 2. ESI-MS analysis for polysaccharides fraction isolated from *Plantago lanceolata*

Table 1 The infrared bands appearing in the FT-IR spectra of inulin and the polysaccharide fraction isolated from *Plantago lanceolata*

Domain	Inulin	*Plantago lanceolata* polisaccharides	Attribution	Bibliographic references
<900 cm^{-1}	817 cm^{-1} (m)	835 cm^{-1}	C-C-C; O-C-O;C-H -bending vibration of 2-cetose	Balan et al., 2018 Akram & Garud, 2020
	873 cm^{-1} (m)	878 cm^{-1} (m)	-bending vibration δ(C1-H) in the ring (2-ketofuranosic)	Akram & Garud, 2020
900-1500 cm^{-1}	1028 cm^{-1} (i)	1054 cm^{-1}	stretching vibration of the C=O; νC-O (C-O); νC-O-C	Balan et al., 2018 Akram & Garud, 2020 Melanie et al., 2015
	1099 cm^{-1} (s)	1084 cm^{-1} (i)	- stretching vibration of the C-C bond in the pyranose ring -stretching vibration of the C-O bond in the pyranosic ring -stretching vibration of the glycosidic C-O-C bond	Redondo-Cuenca et al., 2021
	1213 cm^{-1}	1194 cm^{-1}	- stretching vibration of the C-C bond from pyranosic ring -stretching vibration of the C-O bond from pyranosic ring - stretching vibration of the C-O-C from glycosidic bond	Redondo-Cuenca et al., 2021
	1319 cm^{-1}	1334 cm^{-1}	βO-H (OH) ν C-H, νO-H	Akram & Garud, 2020 Melanie et al., 2015
	1417 cm^{-1} (s)	1398 cm^{-1} (i)	δC–Hs (CH2)	Akram & Garud, 2020 Melanie et al., 2015
1550-2500 cm^{-1}	1617 cm^{-1} (s)	1575 cm^{-1} (i)	C=C	Balan si col., 2018
2500-3600 cm^{-1}	2902 cm^{-1}	2922 cm^{-1}	ν C–Hs (CH2)	Akram & Garud, 2020; Melanie et al., 2015 Redondo-Cuenca et al., 2021
	3257 cm^{-1}	3238 cm^{-1}	νO-H (OH) intermolecular hydrogen bonds	Akram & Garud, 2020 Melanie et al., 2015
(i) = intense band; (m) = medium band; (s) = weak band				

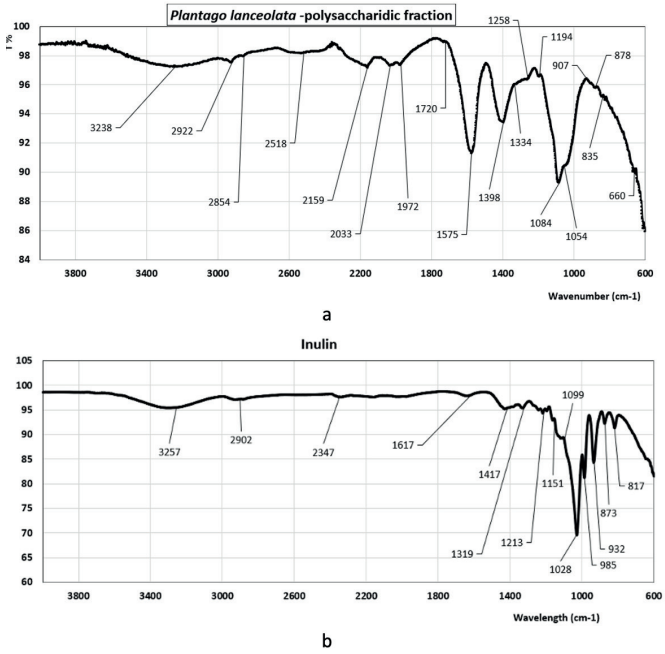
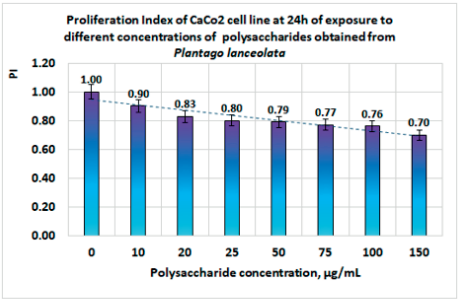


Figure 3. FT-IR Spectra for**:** a) polysaccharides fraction isolated from *Plantago lanceolata;* b) inulin

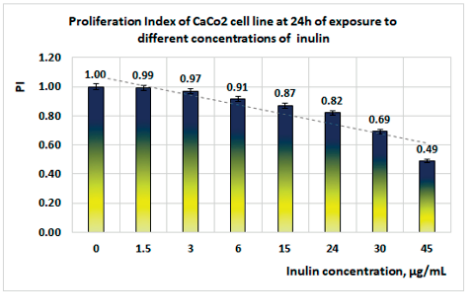
The infrared spectra confirm that both molecules contain C=C bonds and glycosidic C-O-C bonds. In inulin, the C-O-C band appears at 1028 cm^{-1}, whereas in the polysaccharides isolated from Plantago lanceolata, it appears at 1054 cm^{-1}. For biomaterial derived from *Plantago lanceolata*, this band is structured as a shoulder, which appears at 1054 cm^{-1} and as a well-defined intense band with a maximum at 1084 cm^{-1} (Balan et al., 2018).

The exposure for 24 hours of the studied cell lines to inulin and/or polysaccharides isolated from *Plantago lanceolata* (Figure 4 a, b; Figure 5 a, b) leads to the following results: a1) in the case of exposing the CaCo-2 cell line to polysaccharides from *Plantago* sp., the PI values ranging from 0.7- 1 µg/mL are obtained for polysaccharide concentrations between 0-150 µg/mL (Figure 4 a); a2) exposure of the CaCo-2 cell line to inulin concentrations between 45-0 µg/mL results in PI values ranging from 0.49-1, with inulin being cytotoxic to CaCo-2 cells starting at concentrations of 30 µg/mL (Figure 4 b); a3) when exposing the HUVEC cell line to polysaccharides from *Plantago* sp., the proliferative index (PI) obtained ranges from 0.86-1 for a concentration range between 0-150 µg/mL (Figure 5 a); a4) exposure of the HUVEC cell line to inulin results in the values of PI ranging between 0.8-1 for inulin concentrations between 45-0 µg/mL (Figure 5 b).

Exposure for 48 hours of the studied cell lines to inulin and/or polysaccharides isolated from *Plantago lanceolata* (Figure 6 a, b; Figure 7 a, b) leads to the following results: b1) in the case of exposing the CaCo-2 cell line to polysaccharides from *Plantago lanceolata* for 48 hours, PI values ranging from 0.94-1 are obtained for polysaccharide concentrations in the culture medium between 150-0 µg/mL (Figure 6 a); b2) at the exposure of the CaCo-2 cell line to inulin concentrations between 0-45 µg/mL are obtain PI values situated between 0.15-1, with inulin being cytotoxic for the CaCo-2 cell line at concentrations greater than 1.5 µg/mL (Figure 6 b); b3) the exposure of the HUVEC cell line to polysaccharides isolated from *Plantago lanceolata* generate PI values between 1-1.08 for polysaccharide concentrations sitiated between 0-150 µg/mL (Figure 7 a);

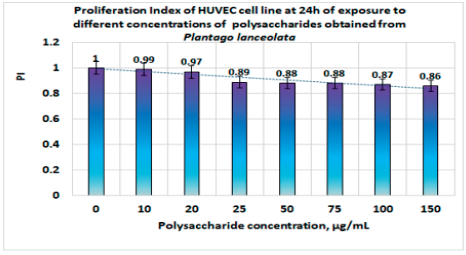


a

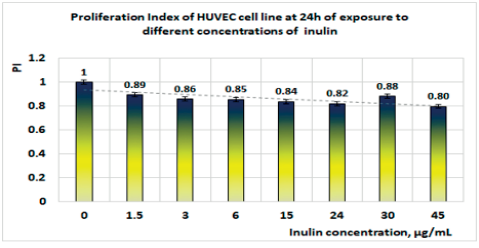


b

Figure 4. The effect of exposing the CaCo-2 cell line for 24 hours to polysaccharides isolated from: a) *Plantago lanceolata*; b) inulin



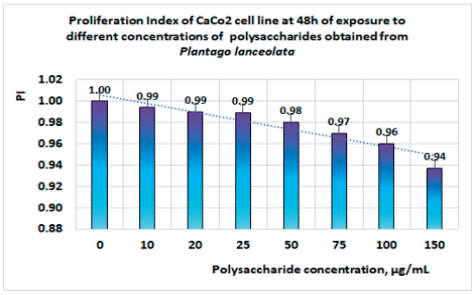
a



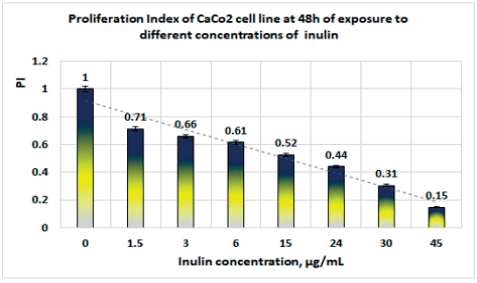
b

Figure 5. The effect of exposing the HUVEC cell line for 24 hours to polysaccharides isolated from: a) *Plantago lanceolata*; b) inulin (Source: own studies)

b4) exposure of the same normal cell line to inulin for 48 hours, for inulin concentrations ranging between 0-45 µg/mL, generates the PI values situated between 1-0.82 (Figure 7 b).

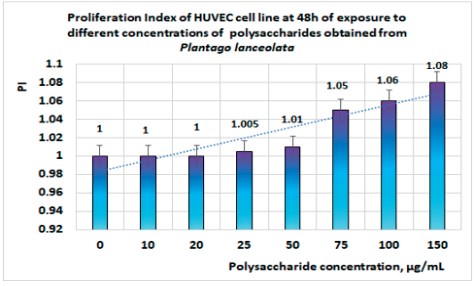


a

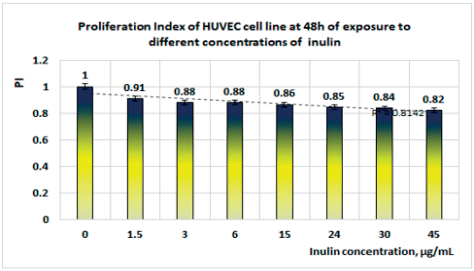


b

Figure 6. The effect of exposing the CaCo-2 cell line for 48 hours to polysaccharides isolated from: a) *Plantago lanceolata*; b) inulin



a



b

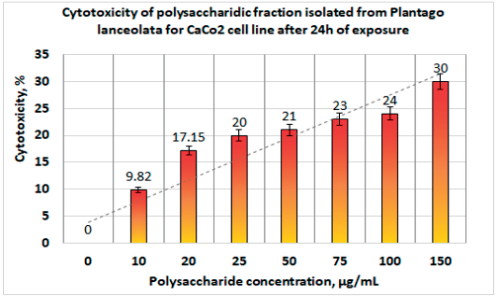
Figure 7. The effect of exposing the HUVEC cell line for 48 hours to polysaccharides isolated from: a) *Plantago lanceolata*; b) inulin

As a general observation, it is noted that, for the normal HUVEC cell line, polysaccharides isolated from *Plantago lanceolata* have a lower

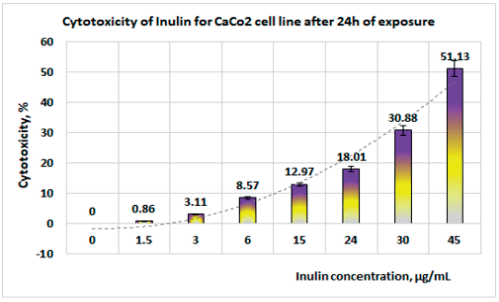
cytotoxic effect than inulin, for which PI values smaller than 1 are obtained.

Regarding the cytotoxicity calculated for the CaCo-2 and HUVEC cell lines, in the case of the two biopreparations (polysaccharides from *Plantago* sp. and inulin), the results showed that for concentrations of polysaccharides isolated from *Plantago* sp. between 0-150 µg/mL, a dose-effect relationship is obtained (Figure 8 a), with the maximum cytotoxicity reaching 30%. In the case of exposure to inulin, the cytotoxicity generated on the CaCo-2 cell line is higher.

A dose-effect relationship is also evident (Figure 8 b). The maximum cytotoxicity obtained in this case is 51.13% for 45 µg inulin/mL



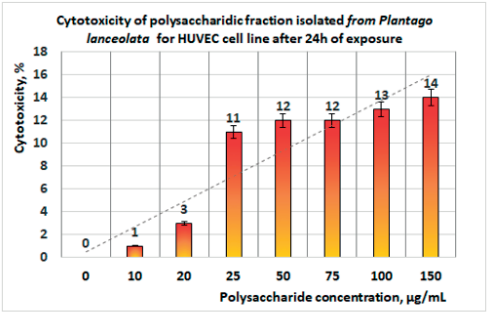
a



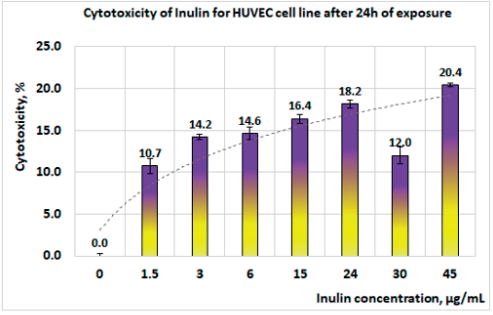
a

Figure 8. The cytotoxic effect generated by exposing the CaCo-2 cell line for 24 hours to: a) Polysaccharides isolated from *Plantago lanceolata*; b) inulin

In the case of the normal HUVEC cell line, the maximum cytotoxicity obtained at exposure to polysaccharides isolated from *Plantago lanceolata* is 14% (Figure 9 a). It corresponds to a concentration of 150 µg polysaccharides/mL. In the case of inulin, at a concentration of 45 µg/mL in the culture medium, the cytotoxicity obtained is 20.4% (Figure 9 b). In general, after 24 hours of exposure, inulin exhibits a more pronounced cytotoxic effect on the CaCo-2 and HUVEC cell lines.



b



b

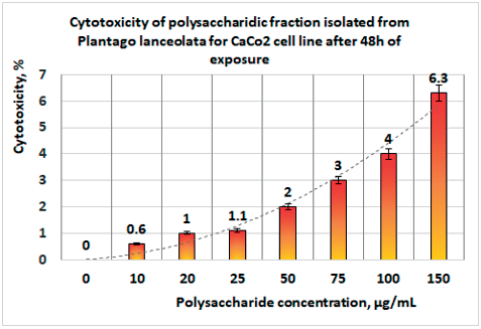
Figure 9. The cytotoxic effect generated by exposing the HUVEC cell line for 24 hours to: a) polysaccharides isolated from *Plantago lanceolata*; b) inulin (Source: own studies)

Cytotoxicity studies performed at 48 hours showed that, in the case of the CaCo-2 cell line, exposure to polysaccharides isolated from *Plantago lanceolata* resulted in a medium cytotoxicity of 6.3% for a polysaccharide concentration of 150 µg/mL in the culture medium (Figure 10 a).

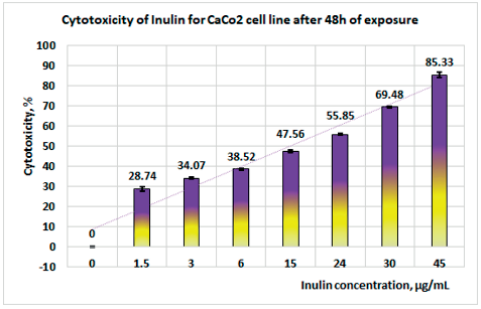
The other hand, exposure of the same cell line to 45 µg/mL of inulin in the culture medium resulted in a cytotoxicity of approximately 85.33% (Figure 10 b).

In the case of the normal HUVEC cell line, it can be observed that after 48 hours of exposure to polysaccharides derived from *Plantago lanceolata*, no cytotoxic effects are observed (Figure 11 a).

However, in the case of inulin, a cytotoxic effect of 17.9% is observed at a concentration of 45 µg/mL (Figure 11 b).

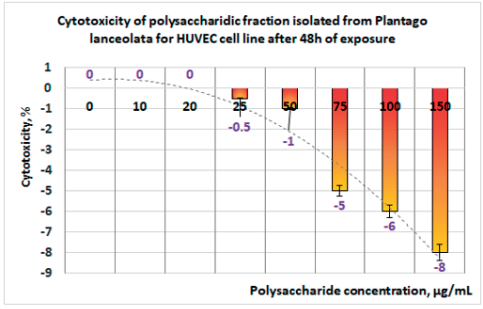


a

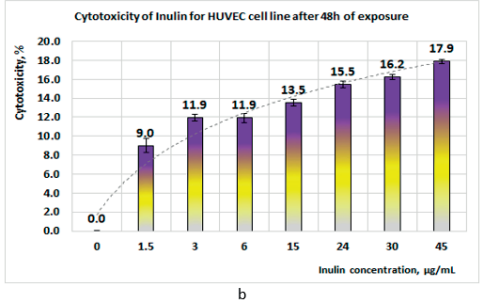


b

Figure 10. The cytotoxic effect generated by exposing the CaCo-2 cell line for 48 hours to: a) Polysaccharides extracted from *Plantago lanceolata*; b) inulin (Source: own studies)



a



b

Figure 11. The cytotoxic effect generated by exposing the HUVEC cell line for 48 hours to: a) polysaccharides extracted from *Plantago lanceolata*; b) inulin (Source: own studies)

The evaluation of the IC50 *in vitro* for inulin and polysaccharides isolated from *Plantago lanceolata* for two standardized cell lines, HUVEC and CaCo-2, at 24-hour and 48-hour exposure times showed that, generally, after 24 hours of exposure, moderate cytotoxicity was observed for both cell lines.

However, after 48 hours of exposure, inulin exhibited high cytotoxicity for the CaCo-2 cell line (Table 2). The evaluation of the selectivity index for the two studied biomaterials (Table 2) showed that the polysaccharides isolated from *Plantago lanceolata* do not exhibit selectivity *in vitro* for the CaCo-2 cell line, whereas inulin, both at 24 hours and 48 hours of exposure, shows significant *in vitro* selectivity for the CaCo-2 cell line (Selectivity - S = 11 for CaCo-2 at 24 hours and 25.36 at 48 hours of exposure, Table 2).

Table 2. Evaluation of the selectivity of the studied biomaterials on the CaCo-2 cell line at 24-hour and 48-hour exposure times. Source: own studies

Polysaccharide sources	Cells line	Exposure time, h	IC50 µg/mL±StDev	S.I.
Plantago lanceolata	CaCo-2	24	196.62±1.97	2
Chickory	CaCo-2	24	44.43±0.44	11
Plantago lanceolata	HUVEC	24	400.00±4.00	-
Chickory	HUVEC	24	427.52±4.28	-
Plantago lanceolata	CaCo-2	48	1210.65±12.11	-
Chickory	CaCo-2	48	19.83±0.20	25.36
Plantago lanceolata	HUVEC	48	-809.31±8.09	-
Chickory	HUVEC	48	502.78±5.03	-
(S.I.=Selectivity Index; S.I.=(IC50 HUVEC cell line/IC50 CaCo-2 cell line)				

The results obtained are consistent with those reported in the specialised literature for the studies carried out *in vitro*, or on lab animals. Thus, studies conducted by Lafontaine and collaborators regarding the effects of galactooligosaccharides and fructo-oligosaccharides on colon epithelial cells cultured in monolayer indicated that the polysaccharides studied have a positive effect on the integrity of the epithelial barrier (Lafontaine et al., 2020). Other studies have shown that the use of inulin as an adjunct during chemotherapy with doxorubicin results obtaining a cytotoxic response at lower concentrations of doxorubicin when used concomitantly with inulin (Schoener et al., 2013). Rahamooz-Haghighi et al. obtained

polysaccharides from *Plantago* sp. through alcoholic extraction and tested their cytotoxicity *in vitro* on standardised cell lines of colorectal adenocarcinoma HCT-116, colon cancer type SW-480, and a normal HEK-293 cell line (Rahamooz-Haghighi et al., 2021).

The tested concentration range used was ranged 25-200 µg/mL of crude extract/mL of culture medium, and the exposure times to the polysaccharides were 24 hours and 72 hours. The results obtained after 24 hours showed that the crude polysaccharide extract has cytotoxic effects on the HCT-116 cell line at concentrations higher than 400 µg/mL, at which cell viability decreases to 80%. In the case of the SW-480 and HEK-293 cell lines, no cytotoxic effects were observed after 24 hours of exposure (Rahamooz-Haghighi et al., , 2021). After 48 hours of exposure to the crude polysaccharide extract, the authors found that for the HCT-116 cell line, viability decreased to 75% at 400 µg/mL. For the HEK-293 and SW-480 cell lines, cell viability decreased slightly but remained above 80%. (Rahamooz-Haghighi et al., 2021). After 72 hours of exposure, the reduction in viability for the HCT-116 cell line was more pronounced, with cell viability reaching 85% at 25 µg/mL of crude extract in the culture medium. At 400 µg/mL of crude extract in the culture medium, after 72 hours, cell viability decreased to 60%, and the biopreparation became cytotoxic for this cell line. The SW-480 cell line ranked second in terms of cytotoxicity after 72 hours of exposure, with cell viability of 65% at 400 µg/mL in the culture medium. In the case of the HEK-293 cell line, a dose-effect relationship was observed, but the cell viability did not decrease below 80% (Rahamooz-Haghighi et al., 2021).

Regarding the cytotoxicity of the crude polysaccharides towards *Artemia salina*, the results obtained indicated no cytotoxic effects, with values of 100% achieved for all the concentration ranges studied (from 7.81 to 1000 µg/mL crude polysaccharide extract) (Rahamooz-Haghighi et al., 2021).

The tests performed on the lab animals did not indicate cytotoxic effects leading to mortality. In the case of laboratory animals, the concentrations tested were 175, 1750, and 5000 µg/kg body weight (Rahamooz-Haghighi et al., 2021). *In vitro* and *in vivo* studies conducted by Gao and collaborators (Gao et al, 2021) on the total polysaccharide fraction isolated from a Plantago species showed that:

a) the tests performed on the CD 11CT cells, derived from bone marrow exposed to polysaccharides concentrations ranging from 12.5-200 µg/mL, and exposure times of 24 h and 48 h, the measured viability was around of 90% regardless of the polysaccharide concentration in the culture medium;

b) the tests performed on the canine mammary adenocarcinoma cell lines (CIPp) with polysaccharides obtained from *Plantago* sp. at exposure times of 24 h and 48 h for polysaccharides concentrations ranging from 8-250 µg/mL, showed that the cell viability remained unaffected (viability = 100%);

c) the tests made on the murine 4T1 tumour cell lines, demonstrated that if laboratory animals with induced tumour pathology by 4T1, were treated with the polysaccharide extract isolated from *Plantago* sp., tumour growth was slightly inhibited;

d) if the crude polysaccharide extract was administered concomitantly with the chemotherapy reagent (taxol), it was found that after 20 days of treatment, the tumour volume decreased from 2000 mm^3 to 1000 mm^3, with tumour progression slowed by approximately 50%. Zhang and collaborators showed that the mechanism of action of crude polysaccharides isolated from *Plantago* sp. is based on inhibiting the migration processes of 4T1 cells, improving phagocytosis processes, and the release of reactive oxygen species by macrophage cells (Zhang et al., 2023).

The polysaccharide extract from *Plantago* sp. mediates the polarization of macrophages to the M1 phenotype, which suppresses the proliferation, migration, and invasion of 4T1 tumour cells (Zhang et al., 2023). Regarding the probiotic effect, studies by Zhang and collaborators (Zhang et al., 2021) on polysaccharides isolated from various *Plantago* species (*P. asiatica, P. ciliata, P. depressa, P. major, P. lanceolata, P. media*) led to the following conclusions:

a) in general, the polysaccharides isolated from *Plantago* sp., are resistant to the environment from digestive system;

b) under the action of digestive enzymes, the bonds between the elementary units from the polysaccharides are partially broken, forming monosaccharides such as fructose, sucrose, and lactose, which, in turn, become nutrients for the microbiota;
c) regarding biological properties, the same authors showed that these polysaccharides have immunomodulatory effects in infections and inflammatory processes, inhibiting carcinogenesis processes that can occur in the digestive system.
Considering that the polysaccharides derived from *Plantago lanceolata* do not show cytotoxicity towards the HUVEC cell line and taking into consideration a value of IC50 = 0, it results that in this case, the cytotoxicity generated *in vitro* by polysaccharides isolated from *Plantago lanceolata* on the CaCo-2 tumour cell line is practically non-existent.
The limitations of this study lie in the fact that the results are obtained solely from cell proliferation studies, where experimental methods used may generate errors. Future developments of these studies include the purification of the polysaccharide fraction obtained from *Plantago lanceolata* and conducting cytotoxicity studies in the presence or absence of an antitumour reagent to identify possible synergistic effects (Ioan et al., 2020).

CONCLUSIONS

The physicochemical tests performed on the polysaccharides obtained from Plantago lanceolata showed that they contain species with an average molecular weight ranging from 0.1-1.94 kDa. The FTIR spectra, made in comparison with inulin, demonstrated that the structure of the two biomaterials is similar, with characteristic bands of the C-O-C glycosidic bond specific to plant polysaccharides being identified, as well as bands corresponding to the stretching vibration of the C-C bond and the C-O bond in the pyranosic ring of polysaccharides.
Cytotoxicity tests performed *in vitro* for polysaccharides obtained from *Plantago lanceolata* and inulin indicated that exposure to these biomaterials for 24 hours of HUVEC or CaCo-2 cell lines, may cause moderate cytotoxicity for both cell lines.
After 24h of exposure, the selectivity index obtained for inulin for the CaCo-2 cell line was 11. This value indicates the selectivity of inulin for the CaCo-2 tumour cell line. Increasing the exposure time to 48 hours for both biomaterials resulted in high IC50 values, indicating the absence of cytotoxicity of the bioproduct derived from *Plantago lanceolata* for both HUVEC and CaCo-2 cell lines. The results obtained *in vitro* for inulin, after 48 hours of exposure, showed that it does not exhibit cytotoxicity for the HUVEC cell line, but it does present cytotoxicity for the CaCo-2 tumour cell line, with the selectivity index obtained in this case being 25.3. The cytotoxicity tests revealed that polysaccharides from *Plantago lanceolata* exhibit low cytotoxicity toward both HUVEC and CaCo-2 cell lines, especially after 48 hours of exposure, suggesting good biocompatibility. In contrast, inulin showed selective cytotoxicity toward the CaCo-2 tumor cell line, with a selectivity index of 25.3 after 48 hours, indicating its potential as a selective antitumor agent. These findings support the potential use of *Plantago lanceolata* polysaccharides in functional foods

REFERENCES

Alghamdi, A.A.A. (2018). Nutritional Value and Phytochemical Constituents of some *Plantago* sp. *University of Ha'il-Journal of Science* 1(1), 8-18.

Babeanu, N., Radu, N., Enascuta, C.-E., Alexandrescu, E., Ganciarov, M., Mohammed, M. S. O., Suica-Bunghez, I. R., Senin, R., Ursu, M., & Bostan, M. (2022). Obtaining and Characterizing Composite Biomaterials of Animal Resources with Potential Applications in Regenerative Medicine. *Polymers*, *14*(17), 3544

Balan, C., Chis , M.I., Rachisan, A., L., Baia, M., (2018). A vibrational study of inulin by means of experimental and teoretical methods. *Journal of molecular structure*, 116484-88.

Bräutigam, M., & Franz, G. (1985). Structural Features of *Plantago lanceolata* Mucilage. *Planta medica*, 51(4), 293–297.

Ciric, A., Radu, N., Zaharie, M. G. O., Neagu, G., Pirvu, L. C., Begea, M., & Stefaniu, A. (2023). Potential Antitumor Effect of Functional Yogurts Formulated with Prebiotics from Cereals and a Consortium of Probiotic Bacteria. *Foods*, *12*(6), 1250.

Clifford, S.C. , Arndt, S.K., Popp, M., Jones, H.G.(2002). Mucilages and polysaccharides in *Ziziphus* species (Rhamnaceae): localization, composition and physiological roles during drought-stress . *Journal of Experimental Botany,* 53 (366),131–138.

Cs, HO, Lam , CW K, Chan, MHM, Cheung, RC K, Law, Lit, L K W, Ng, KF, Suen MWM, Tai, HL. (2003) . Electrospray ionisation mass spectrometry: principles and clinical applications. *Clin Biochem Rev.*,24(1), 3-12.

Gao, J., Zhang, Y. N., Cui, J., Zhang, J., Ming, Y., Hao, Z., Xu, H., Cheng, N., Zhang, D., Jin, Y., Lin, D., & Lin, J. (2021). A Polysaccharide From the Whole Plant of *Plantago asiatica* L. Enhances the antitumor activity of dendritic cell-based immunotherapy against breast cancer. *Frontiers in pharmacology,*12, 678865.

Gerhäuser, C. (2005), Beer constituents as potential cancer chemopreventive agents. *Eur. J. Cancer, 41*, 1941–1954.

Guil-Guerrero, J.L., (2001). Nutritional composition of Plantago species (*P. major* L., *P. lanceolata* L., and *P. media* L.), *Ecology of Food and Nutrition*, 40(5) , 481-495.

https://www.cabidigitallibrary.org/doi/full/10.1079/cabicompendium.41813#sec-9 Accessed on 7 March 2025;

https://tropical.theferns.info/image.php?id=Plantago+lanceolata Accessed on 7 March 2025;

Ianovici,N., Ţarau, G., Todosi, A.L., Iriza, E, Danciu, A., Ţolea, L., Tudosie , D., Munteanu, F. Bogdan, D., Ciobănică V. (2010). Contributions to the characterization of *Plantago* species from Romania. *Annals of West University of Timişoara, ser. Biology,* XIII, .37-76.

Ioan, D.-C.; Rău, I.; Albu Kaya, M.G.; **Radu, N.;** Bostan, M.; Zgârian, R.G.; Tihan, G.T.; Dinu-Pîrvu, C.- E.; Lupuliasa, A.; Ghica, M.V.(2020) Ciprofloxacin-Collagen-Based Materials with Potential Oral Surgical Applications. *Polymers 12*, 1915.

Jiang, S, ,Bryan, RH, Jiao, J., TUNG, R. (2019). BRIEF COMMUNICATION: Investigation of the water-soluble carbohydrates content of Plantain (*Plantago lanceolata* L*.). New Zealand Journal of Animal Science and Production*, 79, 174-176.

Lafontaine, G. M. F., Fish, N. M., & Connerton, I. F. (2020). *In Vitro* Evaluation of the Effects of Commercial Prebiotic GOS and FOS Products on Human Colonic CaCo-2 Cells. *Nutrients*,*12*(5), 1281.

Melanie, H.,Susilowati, A.,Iskandar M.,Y., Lotulung, P. D., Andayani, D. G.S.(2015).Characterization of Inulin from Local Red Dahlia (*Dahlia* sp. L.) Tubers by Infrared Spectroscopy.*Procedia Chemistry*, vol.16,78-84.

Mishra, P., Badiyani, V.M., Jain, S., Subramanian, S., Maharaj, S.V., Kumar, A., Singh, B.N. (2023). Prebiotics: Ignored player in the fight against cancer. *Cancer Rep (Hoboken)*, 6(11), e1870.

Munjal, U., Glei, M., Pool-Zobel, B.L., Scharlau, D., (2009). Fermentation products of inulin-type fructans reduce proliferation and induce apoptosis in human colon tumour cells of different stages of carcinogenesis, *British Journal of Nutrition*, 102(5), 663-71.

Pol, M., Schmidtke, K. & Lewandowska, S. (2021). *Plantago lanceolata* – An overview of its agronomically and healing valuable features .*Open Agriculture*, 6(1), 479-488.

Radu, N., Ghita, I., Rau, I. (2010). Therapeutic Effect of Polysaccharides from *Plantago* species. *Mol*. *Cryst. Liq. Cryst.,* 523, 236-246.

Rahamooz-Haghighi, S., Bagheri, K., Danafar, H., & Sharafi, A. (2021). Anti-Proliferative Properties, Biocompatibility, and Chemical Composition of Different Extracts of *Plantago major* Medicinal Plant. *Iranian biomedical journal*, *25*(2), 106–116.

Redondo-Cuenca, A.,Herrera-Vasquez , S.E., Condezo-Hayas , L., Gomez-Ordonez, E., Recuperez , P. (2021). Inulin extraction from common inulin-containing plant sources. *Industrial Crops and Products*, 170, 113726.

Réquilé, M., Gonzàlez Alvarez, D. O., Delanaud, S., Rhazi, L., Bach, V., Depeint, F., & Khorsi-Cauet, H. (2018). Use of a combination of *in vitro* models to investigate the impact of chlorpyrifos and inulin on the intestinal microbiota and the permeability of the intestinal mucosa. *Environmental science and pollution research international*, 25(23), 22529–22540.

Rojas-Sandoval, J. (2022), *Plantago major (broad-leaved plaintain*), CABI Compendium, CABI International, 41814.

Schoener, C. A., Carillo-Conde, B., Hutson, H. N., & Peppas, N. A. (2013). An inulin and doxorubicin conjugate for improving cancer therapy. *Journal of drug delivery science and technology*,23(2), 111–118.

Zaharie, M-G.O., Radu, N., Pirvu, L., Bostan, M., Voicescu, M., Begea, M., Constantin, M., Voaides, C., Babeanu, N., & Roman, V. (2022). Studies Regarding the Pharmaceutical Potential of Derivative Products from Plantain. *Plants (Basel, Switzerland*),11(14), 1827.

Zhang, J., Cui, J., Gao, J., Zhang, D., Lin, D., & Lin, J. (2023). Polysaccharides of *Plantago asiatica* enhance antitumor activity via regulating macrophages to M1-like phenotype. *Biomedicine & pharmacotherapy* Vol.159, 114246. https://doi.org/10.1016/j.biopha.2023.114246

Zhang, S., Hu, J., Sun, Y., Tan, H., Yin, J., Geng, F., & Nie, S. (2021). Review of structure and bioactivity of the *Plantago (Plantaginaceae*) polysaccharides. *Food chemistry*: X,12, 100158.

Scientific Papers. Series B, Horticulture. Vol. LXIX, No. 1, 2025
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

COMPARISON OF MULTIANUAL CLIMATIC DATA IN NORTH BUCHAREST REGION, ROMANIA

Ana Cornelia BUTCARU[1], Cosmin Alexandru MIHAI[2], Adrian ASĂNICĂ[2], Florin STĂNICĂ[1, 2]

[1]Research Center for Studies of Food Quality and Agricultural Products, University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăști Blvd, District 1, Bucharest, Romania
[2]Faculty of Horticulture, University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăști Blvd, District 1, Bucharest, Romania

Corresponding author emails: ana.butcaru@qlab.usamv.ro, cosmin-alexandru@horticultura-bucuresti.ro

Abstract

Temperature, precipitation, and light are essential climatic parameters for fruit tree life and productivity, and they have a consistent and specific influence on each plant's growing stage. Considering the whole ecosystem, strong linkages between those parameters are highlighted. The present study analyzes multiannual climatic data (over 50 years) regarding precipitation and solar radiation at the International Meteorological Station Bucharest-Băneasa, Romania. A comparative synthesis between months and a correlation between the two parameters is presented. The study results are essential for understanding the area's climate changes more closely and for incorporating them into future strategies for orchard management.

Key words*: weather station, sensors, automation, irrigation.*

INTRODUCTION

Understanding how precipitation and surface solar radiation (SSR) have co-evolved in recent decades is important to diagnosing changes in regional hydroclimate and anticipating water-, energy-, and horticulture-related risks. The latest IPCC assessment concludes that the global hydrological cycle is intensifying, with heavy precipitation events increasing in frequency and intensity in many regions, consistent with thermodynamic scaling of atmospheric moisture (IPCC, 2021a, 2021b). Large multi-dataset studies also report widespread increases in extreme rainfall, including global analyses of annual maximum daily precipitation and the largest events in each record, while acknowledging spatial heterogeneity and methodological sensitivity (Fischer & Knutti, 2015; Papalexiou & Montanari, 2019; Westra et al., 2013). Observational syntheses further show that the intensification signal is now evident in the measured record, broadly consistent with theory and model projections (Fischer & Knutti, 2015; IPCC, 2021b; Papalexiou & Montanari, 2019; Westra et al., 2013).

Against this hydrological backdrop, the surface radiation budget has undergone pronounced multi-decadal changes known as "global dimming" (roughly the 1950s–1980s decline in SSR) followed by "global brightening" since the mid-1980s, linked to aerosol emissions, air-quality regulation, and cloud changes (Wild, 2009, 2012). Over Europe, high-quality satellite and surface datasets show a continent-wide SSR increase since the 1980s (about +1.9 to +2.4 W/m² per decade), with decadal variability and good agreement with homogenized sunshine-duration records; newer records (e.g., SARAH-3) provide consistent, long, quality-controlled series (Pfeifroth et al., 2018; Pfeifroth et al., 2024; Sánchez-Lorenzo et al., 2017). Recent analyses have further examined the causes of European brightening, highlighting the roles of aerosol reductions and cloud changes (Pfeifroth et al., 2018; Sánchez-Lorenzo et al., 2017; Wild, 2012).

The physical linkages between radiation and precipitation operate on multiple scales. At daily scales, cloudiness produces a strong negative correlation (low SSR on rainy days). In contrast, at monthly scales, co-seasonality can yield weak

positive associations in convective seasons - even as SSR and rainfall are not causally related per se. These interactions are embedded in the broader aerosol-cloud-precipitation system, where anthropogenic aerosols alter radiation directly and modify cloud microphysics and precipitation efficiency (IPCC, 2021a; Ramanathan et al., 2001a, 2001b). IPCC provides an authoritative synthesis of these aerosol-radiation-cloud interactions and their effective radiative forcing; detailed reviews and field campaigns (e.g., INDOEX) have quantified how aerosol loading can reduce SSR by ~10% seasonally and suppress precipitation in specific regimes (Ramanathan et al., 2001a, 2001b).

At regional scales in Eastern Europe, studies report significant spatial and temporal variability in precipitation trends and extremes. Several analyses have found significant changes in daily extreme indices over the post-1960 period at some stations, alongside mixed signals in totals. This underscores the need for long, homogenized local records to resolve decadal variability versus trend (Busuioc et al., 2017; Croitoru et al., 2016)

Despite substantial progress, three issues remain particularly pertinent at regional and country scales: (i) the joint evolution of SSR and precipitation has rarely been assessed using co-located, multi-decadal observations; (ii) potential regime shifts (e.g., post-1980s brightening) may alter the seasonal distribution and variability of rainfall without significant changes in annual means; and (iii) the scale dependence of radiation-precipitation relationships (daily vs. monthly vs. annual) is often overlooked in detection-and-attribution studies. By leveraging century-long precipitation records (1895-2024) and SSR measurements (since 1964), our analysis addresses these gaps, quantifying trends, variability, and timescale-dependent co-variability within a consistent observational framework, and situating local findings within the robust global context summarized.

MATERIALS AND METHODS

The Bucharest-Băneasa International Weather Station data for 1895-2024 for precipitation and 1964-2024 for solar radiation were analyzed (Ecad.eu, 2025).

Bucharest-Băneasa Weather Station is placed at the extremity of the city, at 4.3 km from the Agronomie - Herastrau University campus, in the North-West part of Bucharest city (44º28'12", 26º03'51", 86 m altitude).

Statistical analysis was performed with the R program (with RStudio 2024.04.2+764).

RESULTS AND DISCUSSIONS

Trends and dynamics in precipitation at Bucharest-Băneasa International Station

Annual and monthly precipitation quantities were compared from 1895 to 2024 (Figures 1-3).

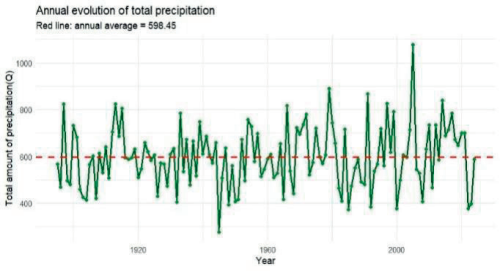


Figure 1. Annual evolution of total precipitation in the 1895-2024 period

Total precipitation has been highly variable with alternating wet and dry periods rather than an apparent long-term increase or decrease.

They can be identified as multi-year clusters: (1) drier spells: late 1920s-1930s, mid-1940s, and several years in the early 2010s and early 2020s fall below the mean (598.45 mm); (2) wetter spells: mid-1910s and especially the late 1990s-2000s show a run of above-average totals, including some of the highest peaks.

The amplitude of extremes appears larger after ~1970, with more frequent very wet years alongside some deep minima, suggesting increased variability in recent decades.

In Figure 2, there appear to be more frequent very wet months, especially in parts of the 1990s-2000s, alongside strings of dry months, suggesting higher variability rather than a steady trend.

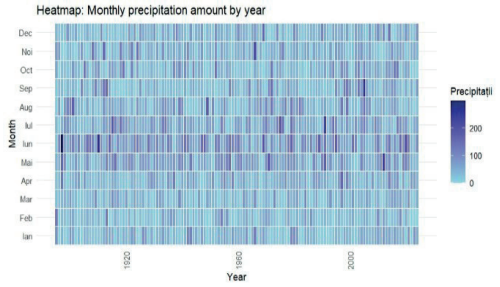


Figure 2. Heat map with monthly precipitation amounts by year

A detailed analysis is presented for each month in Figure 3. In January, generally modest totals and relatively small year-to-year differences. There are occasional wet spikes, but long dry runs appear mid-century. There was no apparent long-term trend observed. February is typically the driest month. There are frequent low precipitation amounts. Variability is low to moderate and trendless. March is a transitional month, with totals and variability increasing compared to winter. There is an alternation of wet/dry years without an apparent directional change. April marks a further step up from March. Mixed conditions are present: some dry periods punctuated by wet events. No clear trend was observed; moderate variability is presented. May is the start of the convective season. A marked increase in variability with several very wet Mays is observed. In recent decades, wet outliers seem more frequent. June is among the wettest months, with extensive interannual periods of multiple high peaks (convective storms). Post-1970 variance looks higher. July is still in the seasonal maximum, with repeated very wet Julys interspersed with very dry periods. The variability is high and the mean level broadly stable. August presents high levels, but they taper slightly from July. Significant year-to-year differences continue, and some prolonged dry runs are offset by intense wet years. September is the transition toward autumn rains. The total precipitation amounts remain relatively high. There were identified clusters of wet Septembers, particularly in the late 20th/early 21st centuries. October is the month when the autumn maximum begins. There are many wet Octobers, including some of the most significant monthly totals in the record. The variability is high. November is often part of the autumn peak. There are several very wet Novembers and multi-year wet clusters. Interannual variance is comparable to summer high-variability months. December returns toward winter levels. The totals and variability are lower than in October and November. During the analyzed period, there were occasional wet Decembers, but no clear trend.

Comparing the monthly precipitation amounts, the wettest season is a bimodal pattern - summer (May-August, especially June-July) due to convective rains, and autumn (October-November) due to frontal systems.

The driest months are February and, to a lesser extent, January and December.

The highest variability is presented in June-August and October-November (frequent extremes). The lowest variability occurs in January and February.

Regarding temporal changes, they are no strong, consistent trend in the mean apparent for individual months, but several months, especially June–July and October-November, show more frequent extreme wet months in the late 20th and early 21st centuries (i.e., increased variability rather than a steady rise or fall in averages).

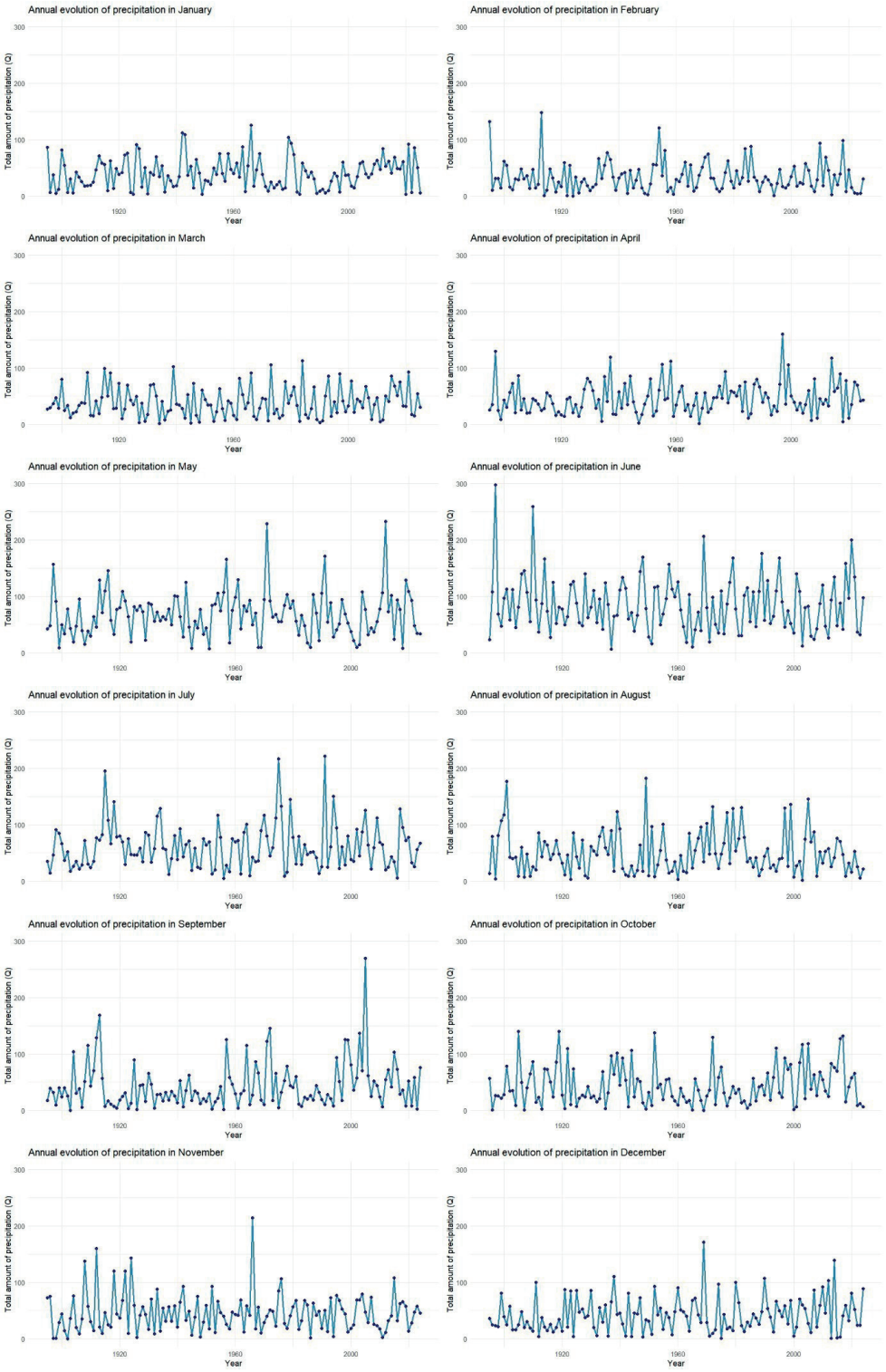


Figure 3. Monthly mean precipitation amounts (1895-2024)

Comparing the 2000-2024 period with the 1895-2024 period, the annual precipitation average increases by 5.8%, from 598.2 mm/year to 633 mm/year. Analyzing each month, it can be observed the following: January +6.7 mm (+16.7%), February −0.9 mm (−2.7%), March +4.7 mm (+12.2%), April +0.7 mm (+1.5%), May: +0.3 mm (+0.4%), June −3.9 mm (−4.6%), July −0.9 mm (−1.5%), August −5.9 mm (−11.7%), September +15.3 mm (+35.7%) – being the most significant increase, October +12.2 mm (+27.7%), November −1.3 mm (−2.9%), and December +7.8 mm (+18.5%).
Since 2000, the totals are higher overall, driven mainly by much wetter September-October and wetter mid-winter (Dec–Jan). The summer (June-August) is drier, especially in August. This points to a shift of precipitation toward autumn and winter.

Trends and dynamics in solar radiation at Bucharest-Băneasa International Station

Annual and monthly solar radiation values (W/mp^2) were compared for the period 1964 to 2024 (Figures 4-6).
When analyzing the multiannual variation of the solar radiation (Figure 4), no strong monotonic trend across the whole period is observed; instead, a regime change occurs from dimmer conditions pre-mid-1980s to brighter and more variable conditions afterward.

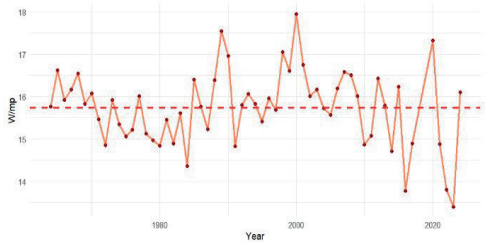


Figure 4. Annual evolution of total solar radiation in the 1964-2024 period

Comparing monthly values (Figures 5 and 6), there is a seasonal cycle: radiation climbs from December/January minima to a June-July plateau (the annual peak), then declines through autumn. The highest months are June and July with maximum energy availability. The lowest months are December, January, and then November/February.

Regarding variability, the summer months (June-August) exhibit the tightest year-to-year range, while the transition months (March-April, September) are slightly more variable. Low and steady values characterize winter.

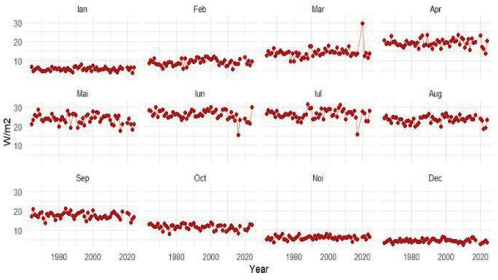


Figure 5. Monthly average solar radiation trends by year (1964-2024)

January has low winter values (~9-11 W/mp^2) and presents a small interannual spread. February is slightly higher than January (~10-12 W/m²) with modest scatter. March increases to ~13-16 W/m². April has an average of ~16-19 W/m². May goes to ~20-23 W/m². June has the near-annual maximum (~23-26 W/m²), being very stable with rare dips. July is similar to ~24-27 W/m². August starts the late-summer decline (~22-25 W/m²). September presents an average of ~18-21 W/m². October has ~13-16 W/m². November is back to winter-like levels, ~10-12 W/m². December had the annual minimum (~8-10 W/m²).

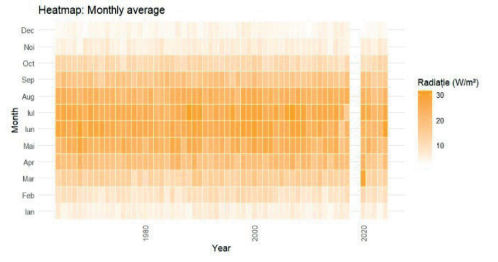


Figure 6. Heatmap - monthly solar radiation amounts by year

Comparing the 2000-2024 period with the 1964-2024 period, the annual solar radiation remains remarkably stable over decades, without significant differences. The main small changes are a brighter March and slightly dimmer October through December. For planning, the most reliable high-radiation window remains

June-July; early spring has improved marginally, while late autumn-early winter is weaker. Month-by-month changes (2000–2024 vs. 1964-2024) in W/mp^2 are January −0.04 (−0.7%), February +0.08 (+0.9%), March +0.80 (+5.6%) being the most significant increase, April +0.20 (+1.0%), May −0.30 (−1.3%), June −0.70 (−2.7%), July +0.20 (+0.8%), August +0.20 (+0.9%), September −0.40 (−2.3%), October −0.40 (−3.4%), November −0.01 (−0.2%), and December −0.19 (−4.3%), being the most significant decrease.

Correlation between precipitation and solar radiation at Bucharest-Băneasa International Station

Regarding the correlation between annual values for solar radiation and precipitation, there is a clear but weak inverse tendency: brighter years are generally drier, but many other factors drive precipitation. Hence, solar radiation is not a reliable predictor of annual rainfall in a standalone capacity. No significant correlation was observed between them (Figures 7-9).

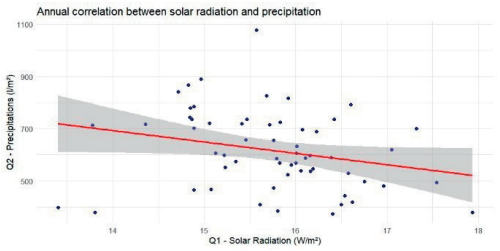


Figure 7. Annual correlation between solar radiation and precipitation

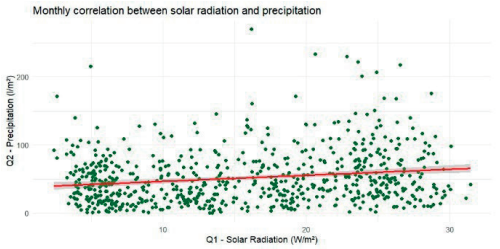


Figure 8. Monthly correlation between solar radiation and precipitation

Our station-based analysis provides a coherent image of how precipitation and surface solar radiation (SSR) have co-varied locally over the last 6-12 decades, and how these results sit within the broader literature. Since 2000, the annual total precipitation increased from ~598 to ~633 mm/year (+5.8%).

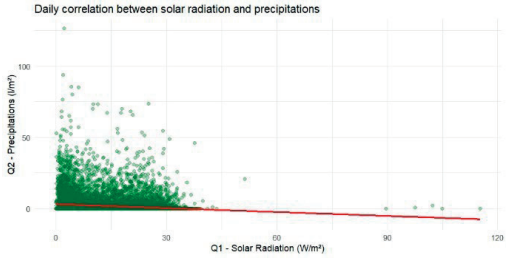


Figure 9. Daily correlation between solar radiation and precipitation

However, the signal is strongly seasonal: autumn rose the most (+19.8%, driven by September +35.7% and October +27.7%), winter also increased (+11.8%, mainly December +18.5% and January +16.7%). The long record shows significant year-to-year variability and an apparent increase in the amplitude of wet and dry years after \~1970, rather than a persistent monotonic trend. This pattern aligns with the intensification of the hydrological cycle and the rising heavy-precipitation potential assessed in the IPCC AR6, while also reflecting the firm regional heterogeneity emphasized therein (IPCC, 2021a, 2021b). It is consistent with European and Eastern European findings of mixed changes in totals but more frequent/stronger extremes at many sites (Busuioc et al., 2017; Croitoru et al., 2016; Fischer & Knutti, 2015; Papalexiou & Montanari, 2019; Westra et al., 2013).

The monthly SSR climatology retains a stable seasonal cycle (minima in December–January, maxima in June-July). The full-time series exhibits the classic pre-mid-1980s “dimming” followed by “brightening” regime change, which has been documented globally and across Europe, and is linked to aerosols and cloudiness (Wild, 2009, 2012; Pfeifroth et al., 2018, 2024; Sánchez-Lorenzo et al., 2017). Our slight autumn dimming coincides with the increase in autumn precipitation, a physically self-consistent pairing (more cloudiness, less SSR).

At the daily scale, a negative correlation was found to be non-significant, following the normal trend: rainy days cluster at low SSR, and high-SSR days are almost always dry. At the monthly scale, the slope turns weakly positive, reflecting co-seasonality (bright months

coincide with the convective season that can produce rainfall). At the annual scale, the relationship reverts to a weak negative association.

This scale dependence is expected from basic radiative-cloud physics and from the aerosol-cloud-precipitation interactions synthesized by IPCC and classic field studies (IPCC, 2021a; Ramanathan et al., 2001a, 2001b): clouds both reduce SSR and enable precipitation, while aerosols can dim SSR directly and modify cloud microphysics and rain efficiency.

CONCLUSIONS

The local hydro-radiative system shows seasonally reallocated wetting and largely stable radiation with subtle autumn dimming, changes that are physically consistent and literature-congruent, and that matter operationally for water, farming, and energy planning. Mean annual precipitation increased by ~6% in the 2000-2024 period versus the historical baseline, but the rise is not uniform: it is concentrated in autumn (most significant gains in September and October) and mid-winter (December-January), while summer totals declined slightly (especially August).

Radiation is stable, mainly with seasonal nuances. The SSR seasonal cycle (minima December-January, maxima June-July) is stable. Recent decades show minor, month-scale adjustments, a brighter March and a slightly dimmer October-December, superimposed on the well-known dimming-brightening regime change since the mid-1980s. For water management, greater cool-season inputs and more frequent wet autumns require monitoring of flood-prone windows. Irrigation stress may intensify in summer despite stable summer radiation, with the leverage of autumn-winter recharge.

ACKNOWLEDGEMENTS

This research was supported by a grant from the Ministry of Agriculture and Rural Development, Sectoral Plan 2023-2026, contract no. 613/18.07.2023, project title ADER 6.1.3 - Digitization of some technological phases in the fruit shrubs precision culture.

REFERENCES

Busuioc, A., Baciu, M., Breza, T., Dumitrescu, A., Stoica, C., & Băghină, N. (2017). Changes in intensity of high temporal resolution precipitation extremes in Romania: Implications for Clausius–Clapeyron scaling. *Climate Research, 72*, 239–249. https://doi.org/10.3354/cr01469

Croitoru, A.-E., Piticar, A., & Burada, D. C. (2016). Changes in precipitation extremes in Romania. *Quaternary International, 415*, 325–335. https://doi.org/10.1016/j.quaint.2015.07.028

Ecad.eu (2025) [Dataset]. https://www.ecad.eu/

Fischer, E. M., & Knutti, R. (2015). Anthropogenic contribution to global occurrence of heavy precipitation and high-temperature extremes. *Nature Climate Change, 5*, 560–564. https://doi.org/10.1038/nclimate2617

IPCC (2021a). Chapter 7 – The Earth's energy budget, climate feedbacks, and climate sensitivity. *In "Climate Change 2021: The Physical Science Basis" (WGI, AR6)*. Cambridge University Press. https://www.ipcc.ch/report/ar6/wg1/chapter/chapter-7/

IPCC (2021b). Chapter 11 – Weather and climate extreme events in a changing climate. *In "Climate Change 2021: The Physical Science Basis" (WGI, AR6)*. Cambridge University Press. https://www.ipcc.ch/report/ar6/wg1/chapter/chapter-11/

Papalexiou, S. M., & Montanari, A. (2019). Global and regional increase of precipitation extremes under global warming. *Water Resources Research, 55*(6), 4901–4914. https://doi.org/10.1029/2018WR024067

Pfeifroth, U., Drücke, J., Kothe, S., Trentmann, J., Schröder, M., & Hollmann, R. (2024). SARAH-3 – Satellite-based climate data records of surface solar radiation. *Earth System Science Data, 16*, 5243–5265. https://doi.org/10.5194/essd-16-5243-2024

Pfeifroth, U., Sanchez-Lorenzo, A., Manara, V., Trentmann, J., & Hollmann, R. (2018). Trends and variability of surface solar radiation in Europe based on surface- and satellite-based data records. *Journal of Geophysical Research: Atmospheres, 123*, 1735–1754. https://doi.org/10.1002/2017JD027418

Ramanathan, V., Crutzen, P. J., Kiehl, J. T., & Rosenfeld, D. (2001b). Aerosols, climate, and the hydrological cycle. *Science, 294*(5549), 2119–2124. https://doi.org/10.1126/science.1064034

Ramanathan, V., Crutzen, P. J., Lelieveld, J., Mitra, A. P., Althausen, D., Anderson, J., … Valero, F. P. J. (2001a). Indian Ocean Experiment: An integrated analysis of the climate forcing and effects of the great Indo-Asian haze. *Journal of Geophysical Research: Atmospheres, 106*(D22), 28371–28398. https://doi.org/10.1029/2001JD900133

Sánchez-Lorenzo, A., Enríquez-Alonso, A., Wild, M., Trentmann, J., Vicente-Serrano, S. M., Sánchez-Romero, A., Posselt, R., & Hakuba, M. Z. (2017). Trends in downward surface solar radiation from satellites and ground observations over Europe during 1983–2010. *Remote Sensing of Environment, 189*, 108–117. https://doi.org/10.1016/j.rse.2016.11.018

Westra, S., Alexander, L. V., & Zwiers, F. W. (2013). Global increasing trends in annual maximum daily precipitation. *Journal of Climate, 26*(11), 3904–3918. https://doi.org/10.1175/JCLI-D-12-00502.1

Wild, M. (2009). Global dimming and brightening: A review. *Journal of Geophysical Research: Atmospheres, 114*, D00D16. https://doi.org/10.1029/2008JD011470

Wild, M. (2012). Enlightening global dimming and brightening. *Bulletin of the American Meteorological Society, 93*(1), 27–37. https://doi.org/10.1175/BAMS-D-11-00074.1



ISSN 2285 – 5653
ISSN-L 2285 – 5653