



UNIVERSITY OF AGRONOMIC SCIENCES
AND VETERINARY MEDICINE OF BUCHAREST
FACULTY OF HORTICULTURE



SCIENTIFIC PAPERS

SERIES B. HORTICULTURE

VOLUME LXX, No. 1



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FRUIT GROWING



MINERAL COMPOSITION, NUTRITIONAL AND FUNCTIONAL VALUE OF SOME NEW BLUEBERRY VARIETIES AT USAMV OF BUCHAREST

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Abstract

*The purpose of this study was to evaluate the mineral and biochemical composition of fruits from 17 cultivated blueberry varieties (*Vaccinium corymbosum* L.), grown under the pedoclimatic conditions specific to the Bucharest area. The biological material analyzed included varieties of horticultural importance, and the determinations targeted macroelements (Mg, P, K, Ca), microelements (Mn, Fe, Zn, Cu, Al), as well as protein and oil content. The results confirm that the 17 analyzed varieties largely fall within the parameters specific to cultivated blueberries, demonstrating good agronomic adaptation and appropriate nutritional quality. The identified deviations are limited and can be explained by genetic variability and environmental conditions, without indicating major imbalances. Overall, the mineral and biochemical profile of the studied varieties is representative of the species *Vaccinium corymbosum* L. and confirms their value for both consumption and processing. The 'Duke', 'Legacy' and 'Blue Ribbon' cultivars stood out due to their superior mineral and biochemical profiles and are recommended for producing fruits with high nutritional value under the conditions of the Bucharest area.*

Key words: *Vaccinium corymbosum* L., cultivar, micro and macronutrients, proteins, lipids.

INTRODUCTION

Cultivated blueberry (*Vaccinium corymbosum* L.) is one of the most important berry species grown worldwide, valued both for its organoleptic properties and its high nutritional value. It is considered a horticultural crop of significant economic importance, with multiple benefits for human health (Liu et al., 2025).

The mineral and nutritional composition of blueberry fruits can vary considerably depending on several factors, including the cultivated variety, pedoclimatic conditions, soil type, level of fertilization, and stage of fruit maturity. Genetic differences among cultivars can lead to significant variations in mineral element concentrations, as highlighted in numerous studies on the nutritional diversity of cultivated blueberries (Drózdź et al., 2017; Krishna et al., 2023; Lachowicz-Wisniewska et al., 2024; Bujor et al., 2025).

Although blueberries are not a major source of proteins or fats in the diet, they contribute to overall nutrient intake and are considered functional foods due to their high content of

bioactive compounds and antioxidants. Regular consumption of blueberries is associated with multiple health benefits, including antioxidant, anti-inflammatory, and cardioprotective effects (Kalt et al., 2019).

Blueberry fruits are characterized by a complex chemical composition that includes carbohydrates, dietary fiber, vitamins, minerals, phenolic compounds, and small amounts of proteins and lipids. In recent years, researchers' interest in analyzing the biochemical composition of blueberries has increased significantly due to the role of these nutrients in human nutrition and in the development of functional food products (Rashwan et al., 2024). In addition to their high antioxidant content, these fruits are also an important source of macro- and microelements essential for human metabolism. Among the mineral elements present in blueberries, potassium, calcium, magnesium, and phosphorus stand out as essential macroelements for proper body function. In smaller amounts, but with important biological roles, microelements such as iron, manganese, zinc, and copper are also

present, participating in numerous metabolic and enzymatic processes (Lachowicz-Wisniewska et al., 2024; Cabrera et al., 2021).

Potassium is considered one of the most abundant mineral elements in blueberry fruits, playing an important role in maintaining electrolyte balance and regulating cardiovascular functions. Calcium and magnesium are also important mineral components of blueberries. Calcium contributes to the structural stability of plant tissues and influences fruit firmness, while magnesium is involved in numerous enzymatic reactions related to plant and human metabolism (Aşık et al., 2019).

Among microelements, manganese is of particular importance, being involved in the activity of antioxidant enzymes and in carbohydrate and lipid metabolism. Studies show that blueberries can be considered a valuable source of manganese, as well as iron and copper, elements involved in oxygen transport processes and immune system function (Cabrera et al., 2021). Some research indicates that wild blueberries may exhibit higher levels of certain minerals compared to cultivated ones, with differences influenced by environmental conditions and the genetic characteristics of species within the *Vaccinium* genus (Negrusier et al., 2024).

Some studies on the chemical composition of blueberries have shown that protein and lipid levels can vary significantly among different parts of the fruit, such as the skin, pulp, and seeds. Analyses of these components indicate that seeds contain higher concentrations of lipids and proteins compared to the fruit pulp (Li et al., 2023). Blueberry fruits contain relatively small amounts of proteins and fats compared to other plant-based foods; however, these components contribute to the overall nutritional value of the fruit (Ashique et al., 2024).

Regarding lipid content, blueberries are considered fruits with a very low fat content. Most lipids are located in the seeds and are mainly represented by unsaturated fatty acids (Uribe et al., 2024).

In this context, analyzing the chemical composition of blueberries, including protein and lipid content, is an important aspect for evaluating the nutritional value of the fruits and

for characterizing different cultivars grown in the Bucharest cultivation area.

MATERIALS AND METHODS

In this paper, 17 blueberry varieties (*Vaccinium corymbosum* L.) from the Experimental Field of the Faculty of Horticulture in Bucharest were analyzed within the blueberry variety collection established in 2018 (Bujor et al., 2025). To evaluate the 17 studied varieties, determinations were carried out for the following qualitative characteristics: micro- and macronutrient analysis, determination of total nitrogen content, determination of protein content, and determination of lipid content. For the purpose of comparing the studied elements across the 17 analyzed varieties (Table 1), presents the reference ranges reported in the scientific literature for cultivated blueberry (*Vaccinium corymbosum* L.).

Micro and macronutrients analysis

The fresh blueberry samples were ground, and three samples were subjected to microwave extraction as follows: 0.250 g, 8 mL HNO₃ and 2 mL H₂O₂. The control consisted of 8 mL HNO₃ and 2 mL H₂O₂. The samples were subjected to 725 ICP-MS analysis Agilent 7700X (Bashdar & Rasul, 2023; Constantin et al., 2023; Barbu et al., 2023). The analysis was carried out based on the calibration curve of the multi-element standard Agilent Technologies solution.

Determination of total nitrogen content - Dry combustion method (Dumas method)

The development and validation of the analytical method were performed using an automated CHNS elemental analyzer (Eurovector EA 3100) with the following accessories: - Eurovector CHN combustion tube for 250 sample analyses of C, N, and H; - double autosampler (carousel) with 40+40 positions; - Mettler Toledo microbalance (precision 0,000001 g); - H₂O trap for water elimination; - PTFE GC column, outer diameter 8 mm, length 2 m; - Weaver.NET 1.8.0.0 software.

The determination of total nitrogen content was carried out using the Dumas method, employing the Eurovector EA 3100 elemental analyzer. This method involves the complete

combustion of dried and ground samples at high temperatures in an oxygen atmosphere. The nitrogen oxides formed are reduced to molecular nitrogen (N₂), which is subsequently separated and quantified using a thermal conductivity detector (TCD). The results were expressed as a percentage of nitrogen relative to the dry weight of the sample (Mot et al., 2022).

Determination of protein content

The determination of protein content was carried out indirectly, based on the total nitrogen content determined by the Dumas method. The resulting nitrogen value was quantified and converted into protein content by applying the general conversion factor of 6.25 (Barbu et al., 2023; Constantin et al., 2023).

Determination of lipid content - Soxhlet method

Lipid content was determined by Soxhlet extraction, using an organic solvent (such as petroleum ether or hexane) under continuous reflux over the dried and ground sample. After solvent evaporation, the lipid residue was weighed, and the results were expressed as a percentage of fat relative to the sample mass.

Natural (physico-geographical) conditions of the research area

The Municipality of Bucharest is located in the Vlăsia Plain, the central subdivision of the Romanian Plain has altitudes ranging from 100-115 m in the northwest to 50-60 m in the southeast, in the Dâmbovița floodplain. The climate is temperate-continental, characterized by moderate thermal extremes caused by high urban density, heavy traffic, and industrial activities. While the annual mean temperature is 10.7°C in peripheral areas, the central zone records an average of 12°C.

Soil pedological and agrochemical characterization

The predominant soil in the study area is a reddish preluvosol, characterized by a low humus content of 2.5%, a loam-clay texture, a pH of 6.2, and low to medium nutrient supply (N, P, K). Blueberry requires medium-textured, well-drained soils without gleiing to a depth of 40-50 cm and with an acidic reaction. The optimal soil pH range is between 4.5 and 5.2.

Lowering soil pH before planting by adding acidic peat is essential.

RESULTS AND DISCUSSIONS

Table 1 presents the reference ranges reported in the scientific literature for *Vaccinium corymbosum* L. and the content of the 17 blueberry varieties studied.

Table 1. Limits of mineral element content in the 17 cultivated blueberry varieties vs. reference values from the scientific literature (*Vaccinium corymbosum* L.)

Element	Obtained value (min.-max.)	Literature reference
Macroelements (mg/100 g)		
Mg	4.81-7.52	5.0-8.0
P	6.66-17.22	7.0-20.0
K	66.76-93.14	70-90
Ca	6.22-10.02	6-9
Microelements (mg/100 g)		
Mn	0.31-0.44	0.2-0.5
Fe	0.17-0.29	0.2-0.4
Zn	0.14-0.30	0.1-0.3
Cu	0.06-0.29	0.05-0.2
Al	0.25-1.02	0.1-1.5
Organic compounds (g/100 g)		
Proteins	0.38-0.91	0.4-0.7
Lipids	0.30-0.58	0.3-0.5

Micro and macronutrients

The fruits of cultivated blueberry (*Vaccinium corymbosum* L.) are characterized by a diverse chemical composition, which includes carbohydrates, dietary fiber, organic acids, vitamins, phenolic compounds, and mineral elements.

Micronutrients

The manganese (Mn) content varies within a narrow range (0.31-0.44 mg/100 g) (Figure 1), with all varieties falling within the reference ranges reported in the scientific literature for cultivated blueberry (*Vaccinium corymbosum* L.) (0.2-0.5 mg/100 g) (Table 1). This uniformity indicates stable physiological regulation and a balanced uptake of this microelement. Considering the role of manganese in activating enzymes involved in photosynthesis and metabolism, the lack of significant variation suggests good adaptation of all varieties to the cultivation conditions.

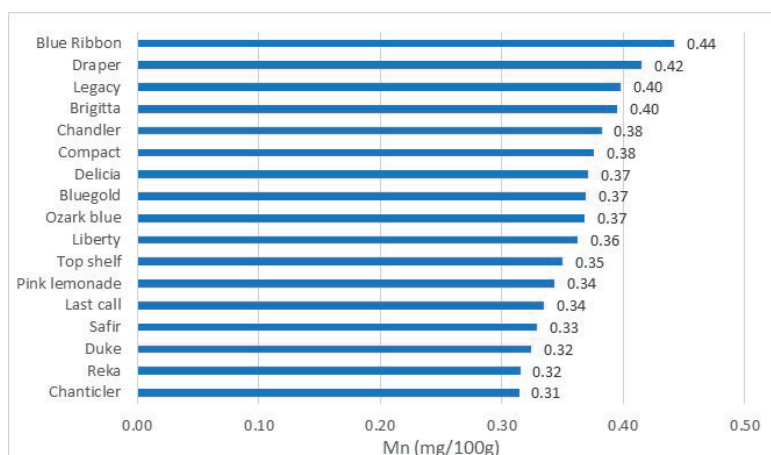


Figure 1. Variation of manganese (Mn) content in cultivated blueberry fruits

Iron (Fe) is present in concentrations ranging between 0.17 and 0.29 mg/100 g (Figure 2), with most values falling within the optimal range (0.2-0.4 mg/100 g) (Table 1). The ‘Draper’ variety shows the highest value (0.29 mg/100 g), while ‘Delicia’ (0.17 mg/100 g) is

slightly below the lower limit. The variability is moderate and reflects differences in iron absorption and transport, an element essential for chlorophyll synthesis and redox processes. Overall, the iron content contributes positively to the nutritional value of the fruits.

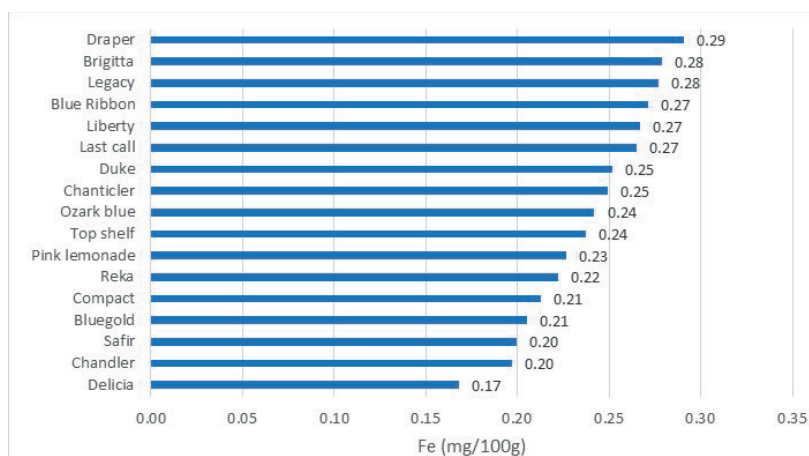


Figure 2. Variation of iron (Fe) content in cultivated blueberry fruits

Zinc (Zn) content ranges between 0.14 and 0.30 mg/100 g (Figure 3), highlighting moderate variability among the varieties. Most values fall within the optimal range (0.1-0.30 mg/100 g) (Table 1), indicating adequate mineral nutrition and good physiological balance. The ‘Chandler’, ‘Delicia’, and ‘Pink Lemonade’ varieties show the lowest

concentrations, situated in the lower part of the range, while ‘Reka’ reaches the upper limit (0.30 mg/100 g). Overall, the distribution of values indicates that zinc is well regulated in most varieties, without major deviations, and the observed differences are most likely genetically determined, with a possible contribution from edaphic factors.

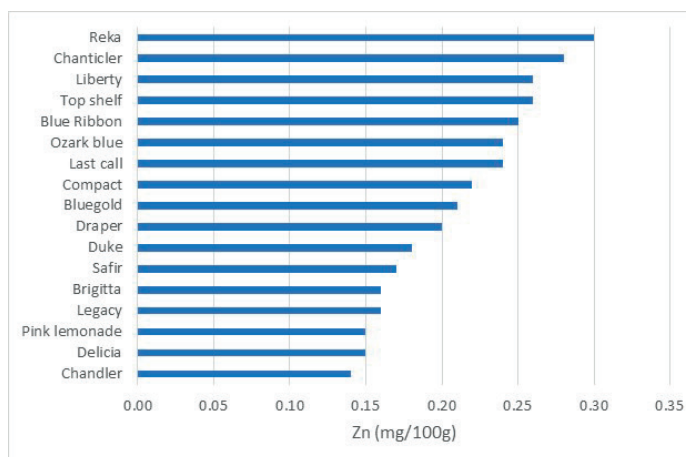


Figure 3. Variation of zinc (Zn) content in cultivated blueberry fruits

Aluminum (Al) content shows high variability, ranging between 0.25 and 1.06 mg/100 g (Figure 4). The ‘Brigitta’ (1.06 mg/100 g) and ‘Legacy’ (1.04 mg/100 g) varieties stand out with significantly higher values, while ‘Draper’ records the lowest concentration (0.25 mg/100

g), remaining above the minimum value of the reference range (0.1-1.5 mg/100 g) (Table 1). This wide dispersion suggests a strong influence of edaphic conditions, particularly soil acidity, as well as genetic differences in accumulation capacity.

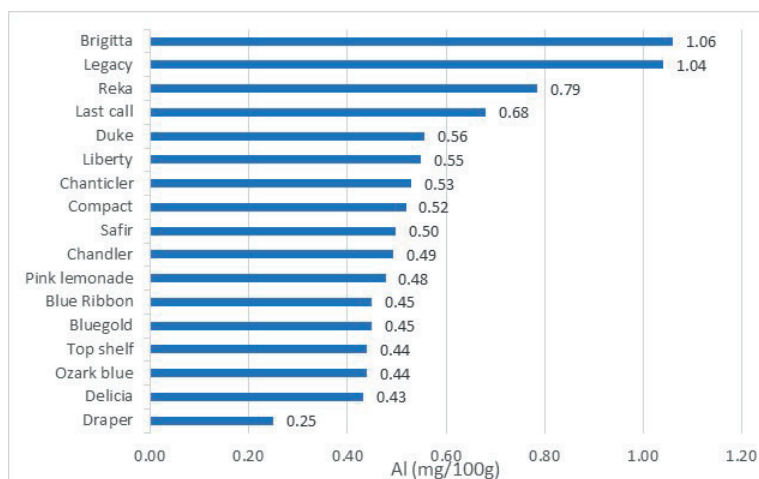


Figure 4. Variation of aluminum (Al) content in cultivated blueberry fruits

Copper (Cu) content shows relatively high variability (0.06-0.29 mg/100 g) (Figure 5) compared to the other microelements, being slightly higher in range. The ‘Duke’ variety stands out with a high value (0.29 mg/100 g), while ‘Ozark Blue’ records the lowest concentration (0.06 mg/100 g). Most varieties

are clustered around the reference value of 0.1 mg/100 g (0.05-0.20 mg/100 g) (Table 1). The observed differences reflect genetic particularities and possible soil influences, considering the role of copper in enzymatic reactions and oxidative processes.

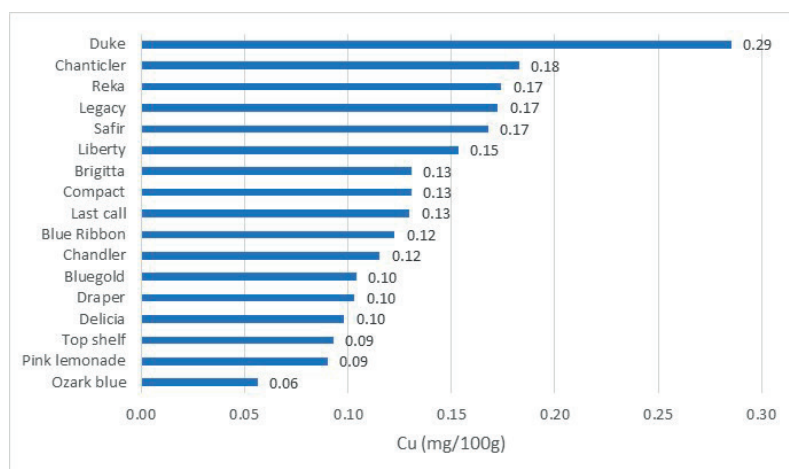


Figure 5. Variation of copper (Cu) content in cultivated blueberry fruits

Macronutrients

Magnesium (Mg) content ranges between 4.81 and 7.52 mg/100 g (Figure 6), showing relatively low variability among the varieties. Most values fall within the optimal range (5.00-7.00 mg/100 g), suggesting good physiological regulation of this essential macroelement (Table 1). The ‘Blue Ribbon’ (4.81 mg/100 g) and ‘Ozark Blue’ (4.99 mg/100 g) varieties

show values slightly below the lower limit, while ‘Duke’ (7.52 mg/100 g) slightly exceeds the upper limit.

The relatively uniform distribution indicates that magnesium, a central component of the chlorophyll molecule, is metabolically stable, and the observed differences are minor, likely determined by genetic traits or soil availability.

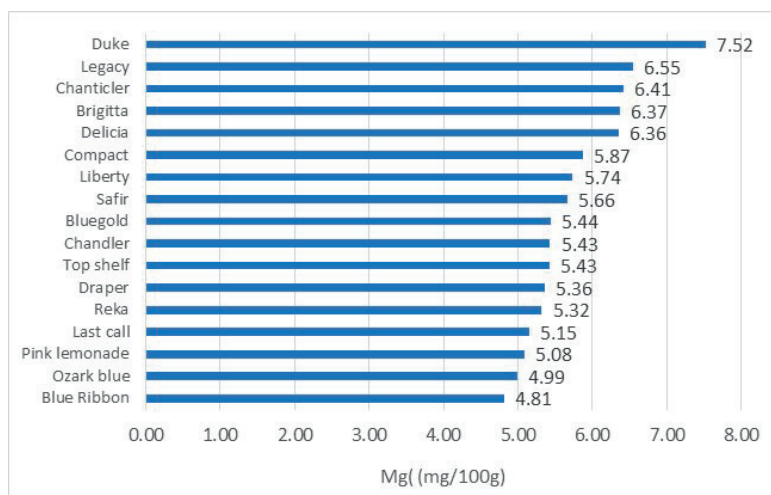


Figure 6. Variation of magnesium (Mg) content in cultivated blueberry fruits

The phosphorus (P) content in the 17 cultivated blueberry (*Vaccinium corymbosum* L.) varieties ranges between 6.66 and 17.22 mg/100 g (Figure 7), with most varieties falling within the optimal range reported in the scientific literature (7.00-20.00 mg/100 g) (Table 1). The

‘Duke’ (12.55 mg/100 g), ‘Legacy’ (14.66 mg/100 g), and ‘Safir’ (17.22 mg/100 g) varieties show high values, indicating strong metabolic potential and increased efficiency in energy-related processes. The ‘Bluegold’ variety is the only one slightly below the lower

limit. Overall, the distribution of values suggests an adequate phosphorus supply, essential for ATP synthesis and for fruit growth and maturation processes.

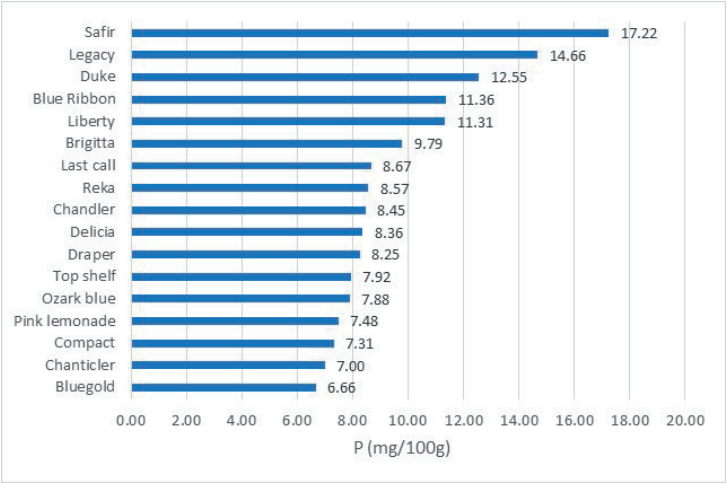


Figure 7. Variation of phosphorus (P) content in cultivated blueberry fruits

Potassium (K) content in the 17 cultivated blueberry (*Vaccinium corymbosum* L.) varieties ranges within a relatively wide interval (66.76-93.14 mg/100 g) (Figure 8), reflecting moderate to high variability among the varieties. Most values fall within the optimal range (70-90 mg/100 g) (Table 1); however, the ‘Blue Ribbon’, ‘Pink Lemonade’, and ‘Delicia’ varieties show values below the lower limit,

while ‘Ozark Blue’ (93.14 mg/ 100 g) exceeds the upper limit. Considering the role of potassium in regulating osmotic balance and sugar accumulation, these differences may directly influence the sensory quality of the fruits, with varieties showing higher values potentially being sweeter and juicier.

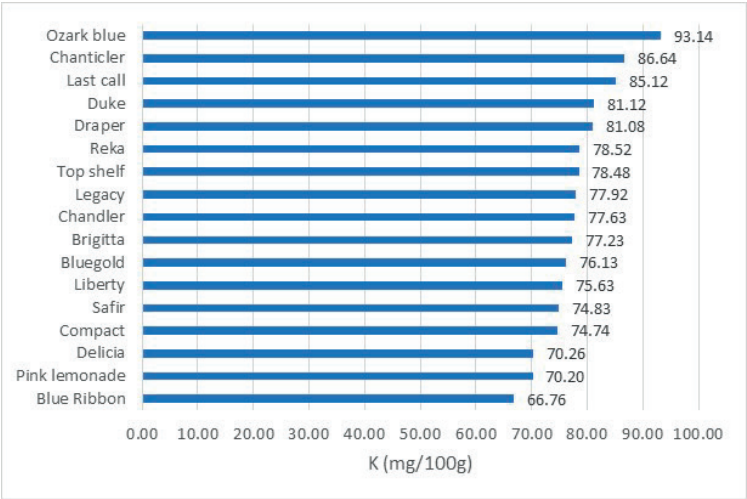


Figure 8. Variation of potassium (K) content in cultivated blueberry fruits

Calcium (Ca) content ranges between 6.22 and 10.02 mg/100 g (Figure 9), with most varieties falling within the optimal range (6.00-9.00 mg/100 g) (Table 1). The ‘Brigitta’, ‘Liberty’,

‘Chanticleer’, and ‘Ozark Blue’ varieties exceed the upper limit, indicating a higher accumulation of this element. Calcium is essential for the integrity of cell walls, and higher values may be associated with increased

fruit firmness and better resistance to handling and storage.

The distribution of values suggests important varietal differences in the capacity for calcium accumulation.

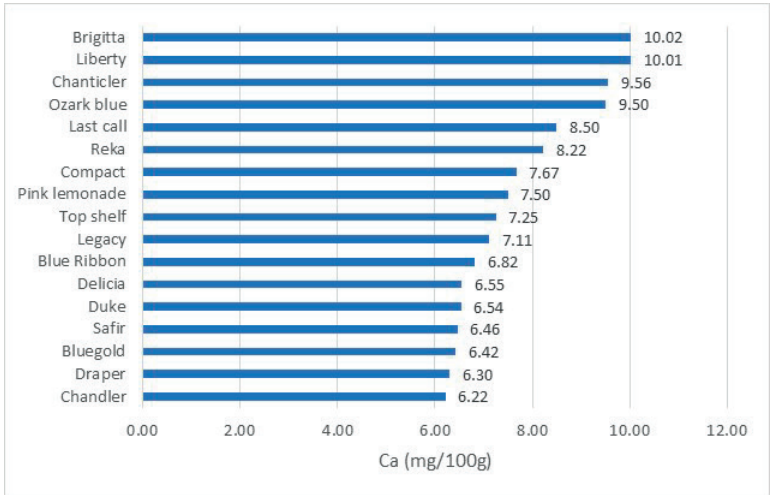


Figure 9. Variation of calcium (Ca) content in cultivated blueberry fruits

Protein content ranges between 0.38 and 0.91 g/100 g (Figure 10), with most varieties falling within the optimal range (0.4-0.7 g/100 g) (Table 1). The ‘Duke’ (0.65 g/100 g) and ‘Legacy’ (0.63 g/100 g) varieties are close to the optimal limits, while ‘Blue Ribbon’ (0.91

g/100 g) shows a significantly higher value compared to the others.

This distribution confirms that blueberries are not a major source of protein, and the observed variations are mainly of genetic origin.

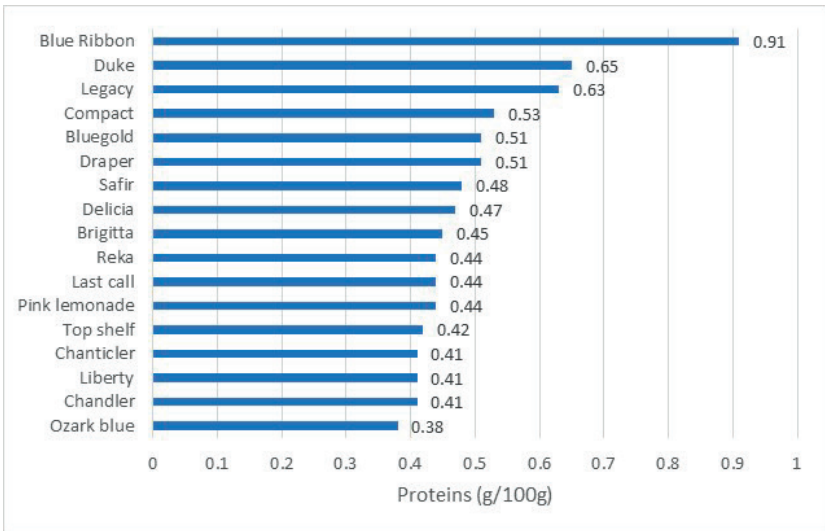


Figure 10. Variation of protein content in cultivated blueberry fruits

Lipid content ranges between 0.30 and 0.58 g/100 g (Figure 11), with most varieties falling within the optimal range (0.3-0.5 g/ 100 g) (Table 1). The ‘Duke’ variety (0.58 g/100 g)

slightly exceeds the upper limit, while other varieties such as ‘Top Shelf’ (0.50 g/100 g) and ‘Blue Ribbon’ (0.48 g/100 g) show values close to this limit.

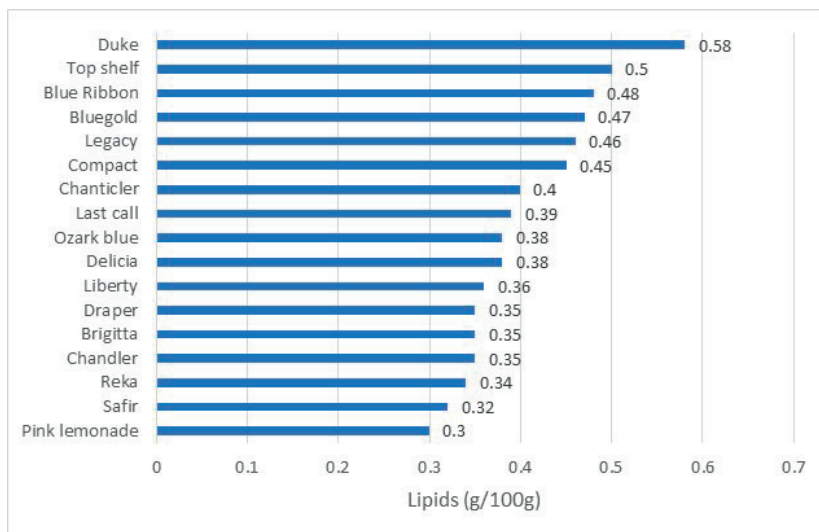


Figure 11. Variation of lipid content in cultivated blueberry fruits

CONCLUSIONS

The fruits of cultivated blueberry (*Vaccinium corymbosum* L.) from the 17 analyzed varieties, grown under the pedoclimatic conditions of Bucharest, are characterized by a variable chemical composition, influenced both by genetic factors and by the environmental conditions specific to the region.

The results confirm that the 17 analyzed varieties largely fall within the parameters specific to cultivated blueberry, demonstrating good agronomic adaptation and appropriate nutritional quality. The identified deviations are limited and can be explained by genetic variability and environmental conditions, without indicating major imbalances. Overall, the mineral and biochemical profile of the studied varieties is representative of the species *Vaccinium corymbosum* L. and confirms their value for both consumption and processing.

The ‘Duke’, ‘Legacy’, and ‘Blue Ribbon’ varieties stood out due to their superior mineral and biochemical profiles and are recommended for producing fruits with high nutritional value under the conditions of the Bucharest area.

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RealTimeFruit: AN AI AND IoT-BASED DECISION-SUPPORT SYSTEM FOR PHENOLOGICAL MONITORING AND GROWTH-STAGE FORECASTING IN FRUIT ORCHARDS

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Abstract

Accurate, real-time monitoring of fruit-tree phenology underpins timely decisions on irrigation, frost protection, pollination, thinning, spraying, and harvest, yet conventional manual observation is labor-intensive, subjective, and hard to scale across many trees and species. This paper describes RealTimeFruit, an integrated artificial intelligence (AI) and Internet of Things (IoT) decision-support system (DSS) developed at USAMV Bucharest for automated phenological monitoring and growth-stage forecasting in experimental orchards of apple, pear, peach, nectarine, apricot, paw-paw, and jujube at two sites (Bucharest and Istrița). The system couples fixed RGB field cameras and Pessl iMetos 3.3 weather stations with automated AI-based BBCH classification of daily images (Anthropic Claude models) and with phenological models based on the Dynamic Model (Chill Portions), Chilling Hours, and Growing Degree Hours (GDH). AI-derived stages are validated against expert manual observations using a structured, tiered protocol, and forecasts are generated by a chilling-to-forcing model calibrated via leave-one-year-out (LOYO) cross-validation. Across species, full-bloom dates were predicted with a mean absolute error of about 2 to 8 days, and harvest/maturity with about 4 to 8 days; the largest errors reflected biologically diffuse flowering rather than model structure. All components converge on an operational web dashboard that displays parallel manual and camera-derived BBCH stages across multi-year datasets (2023-2026). The DSS provides a reproducible, extensible foundation for stage-targeted orchard management.

Key words: artificial intelligence, BBCH classification, chilling portions, growing degree hours, orchard monitoring.

INTRODUCTION

Phenology - the timing of recurring biological events such as bud-break, flowering, fruit development, and leaf senescence - governs almost every decision in modern orchard management. The date of full bloom conditions the risk of frost damage and the scheduling of pollination and thinning, whereas the progression of fruit growth and ripening determines the timing of plant protection, irrigation, and harvest. Because these events shift from year to year with weather and are strongly cultivar-dependent, growers and researchers need accurate, spatially explicit,

and, ideally, forward-looking information on the developmental stage of each tree, all the more so as climate change is already shifting orchard phenology (Luedeling, 2012; Drepper et al., 2020; Reeves et al., 2022).

Traditionally, phenological stages are recorded by trained observers using the BBCH scale, a standardized two-digit code describing plant development from dormancy to senescence (Meier, 2001; Meier et al., 2009). Manual observation, however, is labor-intensive, inevitably subjective, difficult to scale across many trees, cultivars, and sites, and usually captures only a few dates per season. At the same time, forecasting the next stages requires

process-based models that translate temperature into physiological progress: chill accumulation during dormancy is commonly quantified with the Dynamic Model as Chill Portions (Fishman et al., 1987; Erez et al., 1990), while the subsequent heat requirement is expressed as Growing Degree Hours (Anderson et al., 1986). Combined chill-and-heat frameworks such as PhenoFlex (Luedeling et al., 2021) unify the two phases into a single calibrated predictor, building on earlier unified and sequential chilling-forcing formulations (Chaine, 2000; Cesaraccio et al., 2004); recent studies warn that unusually warm winters increasingly compromise the performance of models calibrated on historical data (Fernandez et al., 2022).

Advances in low-cost imaging and machine learning now make it feasible to automate stage recognition from field images and to integrate observation and prediction into a single operational tool (Kim et al., 2025). However, most reported systems address a single species or a single task, and few couple validated, camera-derived observations with weather-driven forecasting within a decision-support system (DSS) that a research team can actually operate day-to-day.

The present work describes RealTimeFruit, an integrated AI and IoT-based DSS developed at the University of Agronomic Sciences and Veterinary Medicine of Bucharest (USAMV). The system was conceived not as an algorithm for one crop but as a multi-species, multi-site platform that senses, ingests, classifies, validates, forecasts, and automates. It currently covers seven fruit species with contrasting phenological behavior - two pome fruits (apple, pear), three stone fruits (peach, nectarine, apricot), and two new species for Romania (paw-paw and jujube) - across two locations. The aims of this paper are: (i) to present the architecture and data flow of the DSS; (ii) to describe the AI-based BBCH classification and the structured validation protocol that make camera-derived stages trustworthy; and (iii) to report, across all seven species, the accuracy of the forecasting layer and the physiological findings that emerged from calibrating it. Species-specific analyses, treated only synthetically here, are the subject of companion papers.

MATERIALS AND METHODS

Experimental orchards and plant material

The study was carried out in the experimental orchards of USAMV Bucharest at two sites: Bucharest (the main site, 44.43° N, 26.07° E) and Istrița (45.13° N, 26.47° E), which experiences a slightly colder microclimate. Seven species were monitored: apple (*Malus domestica* Borkh.), pear (*Pyrus communis* L.), peach and nectarine (*Prunus persica* L. Batsch), apricot (*Prunus armeniaca* L.), paw-paw (*Asimina triloba* L. Dunal), and jujube (*Ziziphus jujuba* Mill.). Monitored cultivars, cultivation forms, rootstocks, and sites are summarised in Table 1. The two new species were included deliberately to test the AI classifier against atypical canopies and flowering habits, from the maroon, bell-shaped flowers of paw-paw to the small, inconspicuous, and very late flowers of jujube.

IoT sensing layer

Each monitored tree or plot was observed by a fixed Boly trail camera acquiring several time-stamped RGB images per day (with night infrared frames extending coverage), so that each image is a dated record of canopy state - bud, leaf, flower, and fruit development, color and density changes, and visible stress. Microclimatic data were provided by Pessl iMetos 3.3 stations installed within the orchards (Bucharest station 00215ABF; Istrița station 00215ADE), delivering high-frequency air temperature and humidity data via the FieldClimate API. Because the stations are co-located with the cameras, every image can be linked to the temperature history of the same tree.

Ingestion

Images are delivered automatically (by e-mail and cloud transfer) and organized by species, tree, and date into a machine-readable archive. One representative image per tree and day is selected and linked to the corresponding weather data and any manual records, yielding a dataset in which each observation includes a tree identity, a date, and its microclimate. A dedicated key (the message identifier rather than the recurring camera file name) is used to avoid

collapsing records, since camera file names are reused across years.

AI-based BBCH classification

Selected daily images are classified into BBCH stages by Anthropic Claude models, forming a tiered set that balances accuracy, speed, and cost across thousands of images: a high-capability model for demanding or ambiguous frames, a balanced model for routine daily classification, and a fast, lightweight model for high-volume clear cases. Each image yields a BBCH stage along with the model's stated reasoning, which is stored to ensure automated decisions remain traceable and reviewable. Two parallel tracks are scored per image - a floral track (BBCH_florifer) and a vegetative track (BBCH_vegetativ) - so that flowering and shoot growth are validated independently.

Validation protocol

Before automation, camera-derived stages were validated against expert manual observation using a structured, four-step protocol designed for demonstrable coverage rather than spot checks: (i) a logical and temporal pass cross-checking free-text notes and calendar consistency against the coded stage, which detects systematic errors regardless of the model's stated confidence; (ii) tiered visual re-verification of all flagged images through parallel image montages; (iii) a random control sample drawn from the unverified remainder to estimate the residual error rate with a Wilson confidence interval, followed by targeted sweeps where errors clustered into patterns; and (iv) a final developmental-monotonicity check, whereby a stage may not fall below a level already sustained in the season (the end-of-season reset excepted).

Forecasting models

Chill accumulation over winter was computed with the Dynamic Model as Chill Portions (CP) (Fishman et al., 1987; Erez et al., 1990) and, as a comparison, with Chilling Hours (0-7.2°C). Heat accumulation after dormancy was computed as Growing Degree Hours (GDH) following the Anderson et al. (1986) cosine formulation (base 4°C, optimum 25°C, critical 36°C). Rather than accumulating absolute GDH

from 1 January, the DSS anchors each transition on the difference in GDH between successive principal stages, which proved more stable. A key physiological result, obtained during calibration and reported below, is that the heat required to reach bud-break is not fixed but decreases as winter chill increases; this chilling-to-forcing coupling is expressed as a linear relationship between accumulated CP and the GDH at bud-break, and it is used to correct the forecast. The integrated PhenoFlex model (Luedeling et al., 2021), which unifies chill and heat with a flexible transition function, was implemented as an experimental benchmark. Fruit development and maturity stages were anchored on the predicted full-bloom date plus a cultivar-specific offset expressed either in GDH from bloom or, for stone fruit, in days from full bloom.

Calibration and evaluation

Thresholds and coupling parameters were calibrated on the multi-year datasets (2023-2026) and evaluated by leave-one-year-out (LOYO) cross-validation, reporting the mean absolute error (MAE) and the root mean square error (RMSE) in days between predicted and observed stage dates. All components converge into an operational web dashboard that displays manual and camera-derived BBCH stages in parallel, projects upcoming stages, and is refreshed daily for both sites.

RESULTS AND DISCUSSIONS

The Decision Support System

RealTimeFruit turns daily images and microclimate data into validated, self-updating phenology through an integrated six-stage chain: sense, ingest, classify, validate, forecast, and automate (Figure 1). In its current state, the platform manages multi-year datasets (2023-2026) for the seven species of Table 1 at two sites. It presents them in an operational dashboard with a per-species tab, a chilling tab, an experimental PhenoFlex tab, and a farm-level 60-day outlook. For every monitored tree, it shows the manual and camera-derived BBCH stages side by side, with accumulated chill and heat and the projected date of the next stage, so records and forecasts are visible in one place.

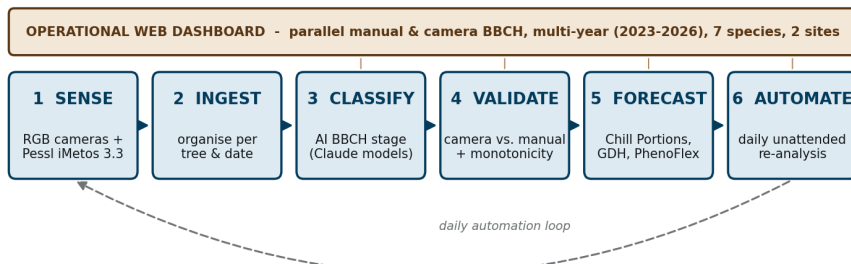


Figure 1. Architecture and six-stage data flow of the RealTimeFruit DSS, from sensing to daily automation, converging in the operational web dashboard

AI classification and the value of dual observation

Across all species, the camera reliably captured the phenological signal that matters most for decisions - the onset and progression of flowering and of vegetative growth - and the AI translated it into BBCH codes with reasoning stored for audit. Two limitations recurred and shaped the methodology. First, once fruit is set, it is frequently hidden by dense foliage, so the late fruit-growth and ripening stages (BBCH 71-89) are more reliably taken from manual records than from the camera in most species; the exception is jujube and the close-up cameras,

where green and coloring fruit remain visible. Second, color is not a maturity signal in several cultivars (red pears, red-skin peaches), whose color is determined by fruit set as a varietal trait, so maturity must be assessed by final size, softening, and disappearance at harvest rather than by color. Conversely, the camera consistently added value that manual observation misses: it recorded the early sequence from bud swell to bud break, which is rarely captured by periodic manual visits, and in 2026, the camera-derived full-bloom dates matched the manual dates to within one day.

Table 1. Species, cultivars, sites, and cameras monitored by the RealTimeFruit DSS

Species (scientific name)	Type	Monitored cultivars	Rootstock/canopy	Site(s)	Cameras
Apple (<i>Malus domestica</i>)	Pome	Generos, Goldrush + 15 manual-only cultivars	M29/SJA; Vertical Axe	Buc, Istrița	4
Pear (<i>Pyrus communis</i>)	Pome	Euras, Williams, Red Williams + other cultivars	Franc/Quince/Own roots; Trident, Bi-baum	Buc	2+
Peach (<i>Prunus persica</i>)	Stone	Royal Summer, Filip	GF677/SJA; Trident, Bi-baum, Vertical Ax	Buc	3
Nectarine (<i>Prunus persica</i> var. <i>nucipersica</i>)	Stone	Big Top	GF677; Trident, Vertical Ax	Buc	2
Apricot (<i>Prunus armeniaca</i>)	Stone	Farbali, Farclo, Primaya + 26 manual cultivars	M29C/SJA; Trident	Buc	5
Paw-paw (<i>Asimina triloba</i>)	Berry	Sunflower, Simina, Pianu, Allegheny, Wilson	-	Buc, Istrița	5
Jujube (<i>Ziziphus jujuba</i>)	Stone	Dong Zao, Hupingzao, Xuan Chen, Ziprim	-	Buc, Istrița	5

Forecasting accuracy

The forecasting layer was evaluated by LOYO cross-validation for every species and cultivar (Table 2), reporting both mean absolute error (MAE) and root mean square error (RMSE). Full-bloom (BBCH 65) was predicted with a mean absolute error of about 2 to 8 days for the

pome and stone fruits, and harvest or maturity with about 4 to 8 days. For pear, introducing the chilling-to-forcing correction reduced the LOYO error at bud-break from 16.6 to 3.5 days (RMSE 4.7), and the atypically cold winter of 2025-2026 - which the uncorrected model treated as an outlier - was predicted exactly once

winter chill was included as the missing factor. Anchoring fruit stages on an intermediate, robust reference (full bloom, or the half-size fruit stage BBCH 75 in stone fruit) rather than

chaining heat units from bud-break markedly improved the late-stage forecasts, because it prevents thermal errors from accumulating across the long fruit-growth period.

Table 2. Forecasting accuracy (LOYO cross-validation) of the DSS, as mean absolute error (MAE) and root mean square error (RMSE) in days

Species	Cultivar/site	Full bloom (BBCH 65) MAE / RMSE (d)	Harvest (BBCH 87) MAE / RMSE (d)	Anchor
Pear	Euras	2.8 / 3.0	11.0 / 11.4	GDH, chill→forcing
Pear	Red Williams / Williams	3.5 / 4.7	5.7 / 6.6	GDH (BBCH65)
Apple	Goldrush, Bucharest	4.0 / 4.5	4.7 / 6.1	chill→forcing
Apple	Generos, Istrița	6.3 / 7.8	10.5 / 10.5	forcing (GDH)
Peach	Royal Summer (Trident)	1.7 / 2.1	4.9 / –	days-from-BBCH75
Peach	Filip	2.3 / 2.9	8.4 / –	days-from-bloom
Nectarine	Big Top (Trident / Ax)	0.3-2.3 / 0.6-2.4	8.4 / –	days-from-bloom
Apricot	Farbali/Farclo/Primaya	5.2-6.7 / 5.6-8.3	3.0-11.0 / 3.6-13.1	GDH-from-bloom
Jujube	Buc + Istrița cultivars	11.8 / –	7.2 / –	GDH (BBCH65/87)
Paw-paw	Bucharest cultivars	7-10 / –	–	GDH (BBCH65)

A physiological finding: chilling that is largely non-limiting. Calibration revealed that at both sites, winter chill accumulates well beyond the reported requirements of most cultivars (Table 3). Peak Chill Portions of about 100-119 exceed the roughly 44-64 CP reported for pear and apple (Ferlito et al., 2021; Fadón et al., 2023) and the ~75 CP reported for apricot in a comparably cold climate (Guo et al., 2015), and both jujube (whose flowering is facultative with respect to chill) and paw-paw are far above their needs. The practical consequence is that heat, not chill, is the limiting factor for spring

development at Bucharest, which justifies anchoring the forecast on a forcing model. Chill nevertheless remains informative for the pome fruits, where the heat required for bud-break falls almost linearly as accumulated chill rises ($r = -0.95$ for pear and apple), whereas for apricot, peach, and the new species, the coupling is weak or absent, consistent with a non-limiting chill regime in which spring warmth dominates flowering time (Ruml et al., 2011; Fadón et al., 2020, 2023; Yang et al., 2025). This gradient across species is itself a useful output of the DSS.

Table 3. Winter chill accumulated at Bucharest versus the reported requirements, and the chill-forcing coupling

Species	Chill accumulated, Buc (CP)	Reported requirement (CP)	r (CP, GDH at bud-break/bloom)
Pear	~100-104 (peak)	~44-49	-0.95
Apple	~100-104 (peak)	50-64 (Golden Del.); 54 (Gold Rush)	-0.95 / -0.96
Apricot	~100-119	~75 (Beijing)	-0.05
Peach/Nectarine	~100-119	low (early-breaking)	-0.26
Jujube	~100-119	facultative	n.s.
Paw-paw	~100-124	~400 h	n.s.

Species contrasts

The pome and stone fruits behaved predictably and were forecast to within a few days. The two unconventional species stress-tested the system as intended. Jujube showed the largest bloom error (11.8 days), but this reflects biology rather

than model failure: its flowering is extended over weeks, its flowers are only 2- 3 mm across and hard to resolve in a wide field of view, and its floral induction is partly photoperiodic (Yao, 2015; Zhang et al., 2016). An explicit photoperiod predictor showed no improvement

because jujube blooms in a narrow mid-summer window when day length is near-constant between years; the residual error is therefore observational, and harvest for the same species is predicted to be 7.2 days. Paw-paw, with its atypical maroon flowers borne on old wood, was classified successfully and its bloom predicted to 7-10 days, extending the standardized BBCH approach to species for which the scale has only recently been described - paw-paw by Ferrer-Blanco et al. (2022) and jujube by Hernández et al. (2015).

Relation to established models

Taken together, the DSS behaves as an interpretable, 'artisanal' version of PhenoFlex: it reproduces the same coupling of Dynamic-Model chill and GDH forcing, but with a transparent linear transition rather than a twelve-parameter fit. On the short series available (three to four years), this simplicity is an advantage - the flexible model tended to over-fit, and for pear and the stone fruits our specialized models matched or beat it; for apple, PhenoFlex held a small edge and is retained as a benchmark. The bloom errors reported here are close to the roughly four-day accuracy cited for PhenoFlex in the literature (Luedeling et al., 2021), and the sensitivity of all models to warm or atypical winters (Fernandez et al., 2022; González-García et al., 2025) reinforces the value of the DSS's explicit chill term and of its extrapolation warnings.

CONCLUSIONS

RealTimeFruit integrates fixed RGB cameras, an in-orchard weather station, AI-based BBCH classification, and weather-driven forecasting into a single operational decision-support system covering seven fruit species at two sites. A structured, tiered validation protocol - logical screening, visual re-verification, a control sample with a confidence interval, and a developmental-monotonicity check - makes camera-derived stages demonstrably trustworthy and exposed correctable, systematic faults in the automated classifier. Calibrated by leave-one-year-out cross-validation, the forecasting layer predicted full bloom with a mean absolute error of about 2 to 8 days and harvest with about 4 to 8 days across

the pome and stone fruits; the larger jujube bloom error is biological, since harvest for the same species is predicted to be about seven days. Winter chill at Bucharest is largely non-limiting, so heat is the main driver of spring development; nevertheless, the heat needed for bud-break in pome fruits falls almost linearly with accumulated chill ($r = -0.95$), and including this coupling cut the pear bud-break error from 16.6 to 3.5 days and captured an atypically cold winter exactly.

The system functions as an interpretable, low-parameter counterpart to integrated chill-heat models, providing a reproducible and extensible foundation for stage-targeted orchard management and species-specific analyses reported in companion papers.

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EVALUATION OF AGRONOMIC PERFORMANCE OF ARONIA SHRUBS UNDER THE IMPACT OF DIGITAL TECHNOLOGY INTEGRATION IN BISTRITA AREA

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Abstract

This study evaluates the integration of digital technologies and their direct impact on the agronomic performance of Aronia shrubs, a crop increasingly valued for its nutraceutical properties. The real-time data analytics facilitated early detection of hydric stress, preventing productivity losses common in conventional management while maintaining an optimal fruit nutritional composition. The management of technological factors not only improves the physiological vigour of Aronia plants but also ensures their economic sustainability within the plantation. This optimization highlights a significant improvement in resource-use efficiency, crucial for adapting to climate variability. By quantifying the correlation between sensor-derived metrics and agronomic performance, the study validates digital tools as essential for risk mitigation. This research provides a model for transitioning from traditional aronia berry cultivation to a data-driven, high-performance horticultural system, offering a framework for producers aiming to maximize both environmental responsibility and commercial profitability in modern berry crops.

Key words: *Aronia melanocarpa*, precision agriculture, IoT sensors, agronomic yield, digital workflows.

INTRODUCTION

Precision agriculture is the fundamental element of the digital shift in modern horticulture, moving from basic field management to site-specific treatments based on real-time data analytics (Gebbers & Adamchuk, 2010). This development reflects a broader industrial revolution within the horticultural sector, characterized by the substitution of empirical intuition with data-driven management to optimize operational performance (Zambon et al., 2019). In the context of a global transition toward sustainable and nutrition-oriented fruiting shrub growth, the cultivation of *Aronia melanocarpa* has gained remarkable popularity among Romanian producers, even in Bistrita County. Due to its extraordinary profile of bioactive compounds, including antioxidants, polyphenols, and vitamins, aronia berries have transitioned into a robust and economically competitive alternative to conventional fruit species (Konecka & Jablonska-Rys, 2021). The increasing market demand for these health-promoting properties has consolidated the crop

position as a high-value functional food source (Sidor & Gramza-Michałowska, 2019).

This expansion is supported by the shrub's natural resistance to low temperatures and pests, characteristics that align perfectly with the specific pedoclimatic conditions of the Transylvanian plateau.

The agronomic performance of these plantations no longer depends solely on natural factors, but increasingly on the farmers' ability to integrate digital technologies (Segarra et al., 2020). The modernization of the fruit growing sector is undergoing a critical transformation, from intuition-based management to one grounded in data-driven precision (Mulla, 2013; Bacco et al., 2019).

While small farms maintain conventional methods, an increasing number of medium and large farms in established fruit-growing basins have begun adopting Smart Farming solutions such as IoT monitoring systems for water resource management; satellite remote sensing for evaluating vegetation indices; and digital weather stations capable of issuing frost alerts and droughts, a phenomenon with a major

impact on the local microclimate (Balafoutis et al., 2017; Ferrández-Pastor et al., 2018).

The integration of these technologies represents not only a competitive advantage but a necessity to optimize yield per unit area and ensure consistent fruit quality under conditions of increasing climatic variability (Chira et al., 2020). The present study's aim is to perform a comparative evaluation of the 2024 and 2025 growing seasons and how these digital tools influence the agronomic performance of aronia crop in the Bistrița area, providing a perspective on technological efficiency in modern horticulture.

MATERIALS AND METHODS

The research was conducted in an aronia plantation located in Bistrita-Nasaud County, latitude 47°08'20"N longitude 24°30'01"E. The study was carried out on plot T26/2L at the Fruit Research and Development Station Bistrița. The region is characterized by a moderate continental climate, with an average annual temperature of 9.91°C. This represents an increase of nearly 1.6°C above the historical annual average recorded in the last 25 years. Additionally, the mean annual precipitation stands at 650 mm. The experimental shrubs plot covers in total of 1.024 ha, with the aronia crop situated on a 0.18 ha sub-plot, representing 18.08% of the total area (Figure 1).



Figure 1. The experimental plot of shrubs from FRDS Bistrita

The biological material consisted of four chokeberry cultivars: 'Nero', 'Melrom', 'Viking', and 'Galicjanka'. The first two were established in 2020, while the remaining two were added in 2022 to complete the collection using 2-year-old potted plants. Randomized block design with three replicates was used, with plant spacing of 4 m x 1.5 m (1,667 plants/ha),

each cultivar included 63 plants. Data interpretation was based on a comparative assessment of plant and fruit biometric parameters (canopy volume, total yield, and plant height) integrated with fruit measurements, to evaluate performance over the study period.

Environmental monitoring was performed using a nMetos 80 SM autonomous mini weather station (Pessl Instruments, Austria). The device recorded soil parameters (moisture and temperature at 40 cm) and microclimate data (air temperature, relative humidity, dew point, vapor pressure deficit VPD) in 15-minute increments. Real-time data telemetry was achieved through NB-IoT connectivity, with all parameters stored and processed via the Field Climate cloud.

RESULTS AND DISCUSSIONS

The data collected by the nMetos 80 SM station revealed significant inter-annual climatic variability in the Bistrita area regarding meteorological parameters that has been monitored during the first nine months of 2024 and 2025. While both years align with the global warming trend, the analysis highlights a transition from an extreme thermal regime (2024) to a precipitation regime characterized by severe instability, featuring extreme drought followed by hydric excess (2025) (Figure 2).

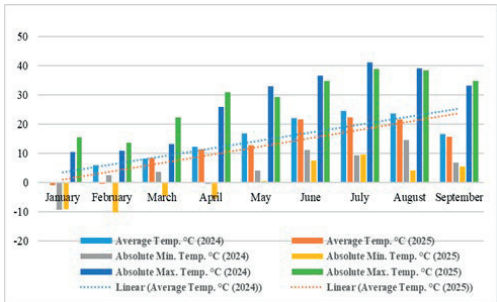


Figure 2. Monthly average of air parameters in 2024-2025 season

The year 2024 was marked by critical thermal stress in July, reaching an absolute maximum temperature of 41.2°C. In contrast, 2025 exhibited early thermal aggression. Although the monthly averages were relatively similar, the thermal amplitude in April 2025 was remarkable; an absolute maximum of 30.8°C

(typical of a summer day) was followed by a severe frost of -5.5°C .

This rapid alternation in 2025 indicates a high physiological risk for crops. The early onset of vegetation induced by high temperatures in March and early April was abruptly interrupted by frost; a phenomenon that was much milder in 2024 (-0.64°C).

The most significant difference between the two years lies in the rainfall distribution. Although the cumulative total in 2025 through September (647.4 mm) is higher than that of 2024 (552.6 mm), water-use efficiency was likely lower in 2025 due to non-uniform distribution. Critical deficit (June) recorded in 2024 provided optimal hydration with 107.8 mm. In 2025, the same period recorded a historical minimum of only 5.6 mm, triggering extreme pedological drought during the critical development phase of aronia berries. August 2025, brought a massive influx of 167.4 mm, compared with only 40.6 mm in 2024. This volume, while high, often stems from convective events (storms), which can cause erosion without fully compensating for the drought stress accumulated in June (Figure 3).

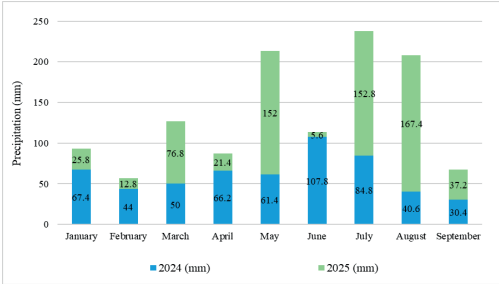


Figure 3. Monthly average of rainfall in 2024-2025 season

The data comparison reveals that 2024 was a year dominated by extreme summer thermal stress, whereas 2025 was defined by hydric instability and spring temperature shocks. For agricultural management, the data suggests that in 2025, irrigation systems were vital in June, whereas in 2024, the primary need was heat protection during the July peaks. These fluctuations underscore the necessity of adopting resilience strategies based on real-time monitoring of Vapor Pressure Deficit (VPD) and soil moisture.

The monitoring period (2024-2025) based on soil and air sensor data, revealed significant

inter-annual variations in atmospheric moisture. In 2024, the mean relative humidity during the growing season (May-September) remained below 60%, with a minimum of 56.11% recorded in May. This correlated with a high VPD who reached a maximum of 1.64 kPa in July 2024 (Table 1).

In contrast, the 2025 season exhibited higher atmospheric humidity, reaching a maximum monthly average of 78.00% in May. Consequently, the VPD values in 2025 were substantially lower, with the lowest recorded mean of 0.43 kPa in May and a seasonal peak of only 1.30 kPa in June.

Soil water tension, measured via Watermark sensors (kPa), showed divergent trends between the two years. The 2024 season was characterized by progressive, severe drying of the soil profile. Values increased from 66.88 kPa in May to a seasonal maximum of 178.86 kPa in August, indicating extreme matric suction. Conversely, the 2025 season started with near-saturation levels in March (0.01 kPa), while the soil tension increased during the summer months, reaching a maximum of 84.76 kPa in July.

Table 1. Digital footprint of climate resilience of aronia plants

Parameter	2024	2025	Sensor Utility
Maximum soil tension (kPa)	178.86	84.76	Irrigation needs/Soil moisture
Critical		Optimal	
Minimum air humidity (%)	56%	78%	Air moisture stress
Maximum VPD	1.64 kPa	1.30 kPa	Transpiration Control
2024 vs 2025	Critical stress	Favorable Growth	Irrigation necessity

Growth indicators for 2025 reveal distinct adaptive responses to improved environmental conditions (reduced water stress). All cultivars increased in height. ‘Nero’ showed the highest growth rate (+22%, reaching 111 cm), while ‘Melrom’ remained the tallest (113 cm). ‘Viking’ surpassed 100 cm, whereas ‘Galicjanka’ maintained a compact habit (75 cm).

In terms of canopy volume, ‘Melrom’ (0.795 m^3) and ‘Nero’ (0.775 m^3) exhibited the highest soil coverage and branching density. In contrast, ‘Galicjanka’ experienced a slight volume reduction following pruning intended to

stimulate compaction, while ‘Viking’ recorded the lowest volume in the plot (0.103 m³). ‘Nero’ and ‘Melrom’ dominated with >20 shoots/plant. ‘Galicjanka’ remained stable (10 shoots), while ‘Viking’ improved but stayed below average (8 shoots) (Figure 4).

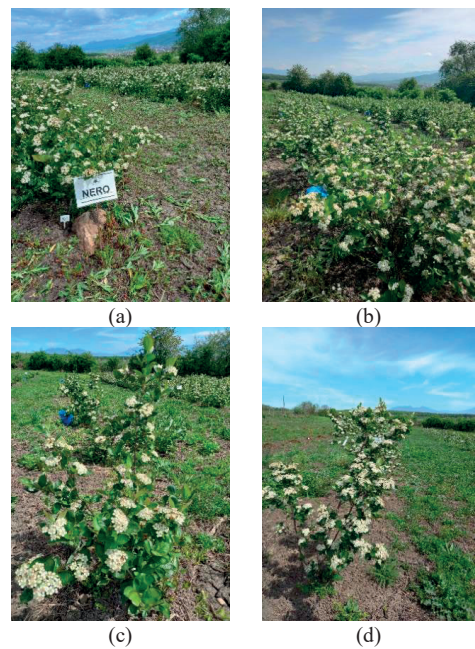


Figure 4. The four aronia cultivars’ bush shape: (a) ‘Nero’; (b) ‘Melrom’; (c) Galicjanka; (d) ‘Viking’

The distinct shift in pedoclimatic conditions between the two years (2024-2025) directly dictated the vegetative performance and adaptability of the cultivars (Table 2).

Table 2. Vegetative growth dynamics by cultivar

Cultivar	Height dynamics	Volume dynamics	Branching status (2025)
‘Nero’	Sharp increase (+20 cm)	Stable/ Increasing	Very high (>20)
‘Melrom’	Consistently high	Maximum (0.795 m ³)	Very high (>20)
‘Viking’	Moderate growth	Low (0.103 m ³)	Medium (8)
‘Galicjanka’	Consistently low	Stable/ Increasing	Medium (10)
Top cultivar vigor	Moderate growth	Max (>20 shoots)	Performance tracking

The correlation between biometric data, yield performance, and environmental monitoring confirms that atmospheric and soil moisture levels, alongside extreme weather events, were

the primary drivers of growth and fruit quality throughout the study. In 2024, the cultivars faced a highly restrictive environment defined by low relative humidity, high VPD (Vapor Pressure Deficit, kPa) and extreme soil suction, while emergency manual water tank applications were used to mitigate the drought in the absence of a permanent irrigation system, a severe hail event in 2024 significantly compromised season output.

This mechanical damage, combined with environmental stress, resulted in minimal yields of approximately 1 kg for ‘Nero’ and ‘Melrom’ and only 0.370 kg and 0.230 kg for the ‘Viking’ and ‘Galicjanka’ cultivars. Furthermore, the surviving fruits in 2024 were visibly dehydrated and compromised in quality as the plants struggled to maintain turgor pressure under high atmospheric demand (Figure 5).



Figure 5. Impact of hail (2024) on fruit quality

The favorable conditions in 2025 marked by higher humidity, lower VPD, and near-saturated soil, triggered a substantial recovery. This environmental shift, coupled with the absence of extreme weather, allowed for optimal fruit hydration and quality in 2025 (Figure 6).



Figure 6. Aronia fruits in 2025 season

‘Melrom’ and ‘Nero’ demonstrated exceptional phenotypic plasticity, capitalising on the improved moisture levels to reach canopy volumes and a dominant productivity of 4.49 t/ha (56.62 kg) and 3.33 t/ha (42.31 kg), respectively. In contrast, ‘Galicjanka’ and ‘Viking’ maintained stable, compact habits with significantly lower 2025 yields, averaging 0.61 t/ha. Evaluating the agronomic performance of aronia crops requires a rigorous comparative analysis of biometric and physico-chemical characteristics across multiple vegetation cycles. In this context, the present study introduces comparative data obtained during the 2024 and 2025 seasons for four representative varieties: ‘Nero’, ‘Melrom’, ‘Viking’, and ‘Galicjanka’. The analysed parameters, such as fruit diameter, average weight, and soluble solids content (SSC), highlight not only the productive potential of each genotype but also their adaptability to annual climatic variations, providing essential indicators for optimising harvest timing and production quality. The following tables present the evolution of these indicators, providing a baseline for evaluating the productivity and sensory quality of each cultivar (Table 3).

Table 3. Morphological and quality characteristics of aronia fruits in 2024 vs. 2025

Cultivars	Year	Fruit weight (g)	Weight of 100 fruits (g)	Fruit Ø (mm)	SSC °Brix
Nero	2024	0.60	51.00	6.40	27.00
	2025	0.90	85.00	10.07	20.15
Melrom	2024	0.75	54.00	6.72	26.50
	2025	0.95	90.00	10.14	21.10
Viking	2024	0.85	69.50	7.83	26.50
	2025	0.86	80.00	10.14	21.10
Galicjanka	2024	0.85	60.00	7.25	26.50
	2025	0.85	80.00	10.11	21.12

The 2024-2025 comparative study of aronia cultivars reveals a significant morphological shift, with 2025 characterised by a "size explosion" where all varieties exceeded the 10 mm diameter threshold. ‘Nero’ exhibited the most dramatic evolution, increasing its mean diameter from 6.4 mm to 10.07 mm and its average weight by 50% (0.60 g to 0.90 g). While ‘Melrom’ emerged as the 2025 leader in mass (0.95 g/fruit and 90 g/100 fruits), the ‘Viking’ cultivar maintained a consistent mass (0.85 g to 0.86 g) despite a significant increase in diameter, indicating a reduction in fruit density

during the 2025 season. A clear inverse correlation was identified between fruit volume and quality, as the average Soluble Solids Content (SSC) decreased from 26.6° Brix in 2024 to 20.8° Brix in 2025, indicating a dilution effect likely caused by higher hydration. Ultimately, the substantial increase in the mass of 100 fruits reaching up to 90 g in 2025 compared to the 51- 69.5 g range in 2024 confirms that 2025 was a superior year for gross biomass yield, whereas 2024 favored high-concentration processing. This inter-annual variability underscores the strong influence of environmental factors on aronia productivity and fruit chemical composition. The integration of soil and air sensors proved essential in quantifying how the transition from the restrictive, hail-damaged 2024 season to a favorable 2025 environment not only drove a 22% growth surge but also restored commercial viability, shifting from the dehydrated, minimal yields of the first year to the high-quality production of the second. These results highlight that integrating soil and air sensors via a weather station is a critical tool for precision agricultural management, particularly in the absence of automated irrigation systems. The sensors provided the necessary data to quantify this stress, while the manual watering reduced the drought’s impact; the high VPD still forced plants to prioritize survival over expansion. The integration of these sensors allows for a direct correlation between environmental dynamics and biometric output, proving that even with manual interventions, shrub vigor remains a measurable indicator of environmental resource efficiency and the limitations of non-automated water management.

CONCLUSIONS

The integration of these digital tools allowed for a precise environmental monitoring of the aronia experimental plot regarding drought and provided the needed technological management through precise irrigation. The sensors proved essential for real-time stress detection, providing a robust technological framework for optimizing horticultural management in the Bistrița region. The study highlights a climatic evolution from extreme heat in 2024 (41.2°C) to severe hydric

instability in 2025, where June precipitation recorded a historical minimum of 5.6 mm. Digital sensors were vital for monitoring soil tension, which reached a critical 178.86 kPa in 2024, and VPD levels that peaked at 1.64 kPa.

'Melrom' and 'Nero' proved to be the most resilient cultivars, achieving dominant 2025 yields of 4.49t/ha and 3.33t/ha respectively, while showing superior growth with over 20 shoots per plant. In contrast, 'Viking' and 'Galicjanka' showed slower adaptability, averaging yields of only 0.61t/ha.

This study demonstrates the efficiency of real-time data acquisition in identifying critical water stress thresholds and validates the role of sensor-based monitoring as a strategic tool for optimizing irrigation management and crop resilience under fluctuating climatic conditions.

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MORPHOMETRIC CHARACTERISTICS AND VALORIZATION OF *PRUNUS DOMESTICA* L. STONES

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Abstract

European plum (*Prunus domestica* L.) is one of the most important fruit species in the Balkan Peninsula, where it is predominantly used for fruit brandy production. Destoning during processing generates large quantities of stones and seeds that are mostly discarded as waste. This study aimed to evaluate the morphometric characteristics of plum stones and seeds of four cultivars ('Čačanska Lepotica', 'Stanley', 'Empress', and 'Požegača') to assess varietal differences relevant for further processing. For valorisation studies, seeds were subjected to cold-press oil extraction to determine the fatty acid composition and oxidative stability of the obtained oils. Oleic acid was the dominant fatty acid in plum seed oil, reaching 72.49% in Stanley and 58.55% in Empress, followed by linoleic acid. Oxidative stability ranged from 35.17 (Empress) to 73.51 h (Stanley), indicating high oil stability. Oil cakes were further evaluated for techno-functional properties and for their potential as a source of bioactive compounds using eutectic solvent-based extraction. These results highlight plum stones as a valuable raw material for sustainable processing.

Key words: plum stones, seed, seed oil, oil cake.

INTRODUCTION

European plum (*Prunus domestica* L.) is one of the most economically important fruit species in many European countries, particularly in the Balkan region, where it represents a traditional and widely cultivated crop. Its fruits are used for fresh consumption as well as for various processed products, including dried fruits, jams, juices, and distilled beverages, which significantly increases its economic importance in the food industry (Dimkova et al., 2018). Plum is the most widely cultivated fruit species in Bosnia-Herzegovina. Although it is predominantly grown under traditional production systems, increasing demand on the domestic market (distilled spirit production) and on international markets for fresh consumption highlights the need for more intensive cultivation and improved production efficiency (Cvetković and Mičić, 2018). Successful plum production requires rootstocks and cultivars

well adapted to specific environmental conditions (Milošević et al., 2022). Given the interaction between genotype and environment, it is necessary to evaluate the morphological and agronomic performance of cultivars under local growing conditions to determine their suitability for production and processing (Vitanova et al., 2004; Nesheva et al., 2021). Beyond modernizing cultivation technologies, maximizing the use of all components of the production process offers additional opportunities to enhance the sustainability of plum cultivation. In recent years, our research has been devoted to the valorization of agro-industrial by-products as sustainable sources of bioactive compounds and functional ingredients (Ćirić et al., 2024; Dabić Zagorac et al., 2024). After industrial processing of plums into various food products, a large number of by-products remains, such as plum pits, skins, and similar materials. Plum pits are the source of plum seeds, which have great potential as an

unconventional oil source, with oil content up to 50% (Vladić et al., 2020). The dominant fatty acid in this oil is the monounsaturated oleic acid, at over 60%, which significantly contributes to the oil's good oxidative stability (Rabrenović et al., 2021). In addition to food, this oil can be used for biodiesel production and in the cosmetics and pharmaceutical industries. After cold pressing, in addition to plum seed oil, a significant amount of oil cake with a high protein content remains. Plum seed oil cake can be considered an inexpensive and readily available source of protein (up to 40%) (Čakarević et al., 2019). The aim of this study was to evaluate the morphological characteristics of plum stones and seeds of four European plum cultivars, as well as to assess the techno-functional properties of the obtained oilcakes and their valorisation potential as a source of bioactive compounds. Particular attention was given to the morphometric traits of stones and seeds, since these parameters may influence the yield and technological potential of plum seed oil and its by-products. Considering the intended industrial use of functional ingredients in bakery products, the research plan was designed in accordance with green chemistry principles, using eutectic solvent systems for extraction. Additionally, determining the total polyphenol content and antioxidant activity of the extracts provides valuable information on the potential utilization of the studied cultivars and their processing by-products.

MATERIALS AND METHODS

Stone and seed analysis. Uniform plum fruits were harvested during the 2024 growing season at optimal technological maturity. Fruits of the cultivars 'Čačanska Lepotica', 'Stanley', and 'Empress' were collected from the Experimental and Educational Center of the Faculty of Agriculture, University of Banja Luka, while fruits of 'Požegača' were obtained from a nearby commercial orchard (44.948174, 17.209729). Stones were separated by mechanical crushing and air-dried in the shade for 40 days, then labeled and stored under ambient conditions. At the end of 2025, stones and seeds were subjected to morphometric analyses. Measurements were performed on 15 stones and 15 seeds per

cultivar, in two replications. Stone and seed mass were determined using a precision balance, while linear dimensions (height, width, and width across the suture) were measured with a digital caliper. The seed-to-stone ratio was subsequently calculated. Morphometric analyses were conducted at the Laboratory of Pomology and Biotechnology, Faculty of Agriculture, University of Banja Luka. For further valorization studies, 500 ± 20 g of seeds per cultivar were prepared, labeled, and delivered to the Faculty of Chemistry at the University of Belgrade.

Cold-pressing. Plum seeds were dried at room temperature for 24 hours immediately before pressing, after which the cold-pressing procedure was performed using a laboratory screw press (Gorenje OP650V, Slovenia). During pressing, the temperature of the extracted oil was monitored by an infrared thermometer (TFI 260, Ebro-Xylem, Germany) and did not exceed 45°C. After 24 hours of sedimentation, the oils were filtered through filter paper and stored in 10 mL plastic cuvettes at -20°C until analysis. Oil cakes were ground, sieved, and further evaluated for techno-functional properties.

Fatty acid composition. Fatty acid methyl esters were prepared according to the standard method (SRPS EN ISO 12966-2:2017). Separation and detection of methyl esters were performed by gas chromatography, following the standard (SRPS EN ISO 12966-1:2015), using a gas chromatograph (Agilent Technologies 6890, USA) equipped with a split-splitless injector, a flame ionisation detector (FID), and a Supelco SP-2560 capillary column (100 m length $\times$ 0.25 mm inner diameter $\times$ 0.20 μ m film thickness, Supelco, Bellefonte, USA). Helium at a flow rate of 5 mL/min was used as the mobile phase. Injector and detector temperatures were 250°C and 260°C, respectively. The injected volume was 1 μ L, and the injector split ratio was set to 20:1. The column temperature was programmed from an initial 50°C (held for 5 minutes) to 240°C (held for 20 minutes) at a rate of 4°C/min. Chromatographic peaks in the sample were identified by comparing the relative retention times of the fatty acid methyl esters in the samples with those of the Supelco 37 Component FAME Mix standard. The fatty acid content was calculated in mg/g of lipids and

expressed, as a relative quantity, as a mass percentage of total fatty acids.

Oxidative stability. The oxidative stability of the oil samples was tested using an OXITEST device (Velp® Scientifica, Usmate, Italy). Ten grams of each oil sample were weighed into two titanium thermostatic chambers of the device. The chambers were hermetically sealed. The temperature was set to 90°C, and the initial O2 pressure to 0.6 MPa. Using dedicated software (OXISoft, Velp, Italy), the change in pressure in the chambers was monitored, thereby indirectly measuring the oxygen consumed by individual components. For each chamber, the software calculated the induction period (IP) at the end of the test, in minutes or hours. A longer IP indicates better oxidative stability of the sample.

Techno-functional characteristics of oil cakes.

Moisture content. The moisture content of the POC was determined in accordance with the SRPS EN ISO 665:2020 standard method. The moisture content of a portion of the test sample was determined by drying at 103°C ± 2°C in an oven at atmospheric pressure until a constant mass was reached.

Swelling Capacity (SC). The swelling capacity was determined as follows: 1.0 g of each oil cake was placed in a measuring vessel, and 30 mL of distilled water was added. The mixture was left to stand for 18 hours without mixing, and the volume of the plum oil cake (POC) layer was recorded at the end of this period. Results are expressed as the volume bound by the POC relative to the original weight of the POC (mL/g) (Zlatanovic et al., 2019).

Water Holding Capacity (WHC). POC samples (1.0 g) were dispersed in 30 mL of distilled water and left to stand at room temperature for 24 hours. After centrifugation at 3600 rpm for 20 minutes, the aqueous supernatant was removed, and the residue was measured. Results are expressed as the weight of bound water per gram of POC (g/g) (Rana et al., 2015).

Oil Holding Capacity (OHC). A POC sample (1.0 g) was dispersed in 10 mL of sunflower oil in a pre-weighed plastic centrifuge cuvette and left undisturbed at room temperature for 24 hours. The contents were then centrifuged at 3600 rpm for 25 minutes. The supernatant was decanted and dried, and the precipitate was measured as the residue after centrifugation. The

result is expressed as the weight of bound oil per gram of POC (g/g) (Zlatanovic et al., 2019).

Solvent Retention Capacity (SRC). The SRC is defined as the weight of solvent (g) retained by the POC after centrifugation, expressed as a percentage. Four solvents were used: water, a 50% sucrose solution, a 5% sodium carbonate solution, and a 5% lactic acid solution. A POC sample with known moisture content (5.0 ± 0.05 g) was weighed into a pre-measured cuvette (with lid) of 50 mL volume and conical bottom. 25 ± 0.05 g of the selected solvent was added, the cuvette was sealed, and the mixture was shaken vigorously to disperse the flour in the solvent without lumps. The sample was hydrated for 20 minutes, with vigorous shaking five times during this period. After hydration, the POC suspension in the solvent was immediately centrifuged at 3000 rpm for 15 minutes. The supernatant was decanted, the pellet was drained for 15 minutes, and then weighed. SRC values were calculated as described by Haynes, Bettge, and Slade (2009):

$$SRC\% = \left(\frac{\text{wet pellet (g)}}{\text{oil cake (g)}} - 1 \right) \cdot \left(\frac{86}{100 - \text{oil cake moisture } \left(\frac{\text{g}}{100\text{g}} \right)} \right) \cdot 100$$

Determination of total phenolic content (TPC) and radical-scavenging activity (RSA).

Three different mixtures (malic acid and sucrose in a molar ratio of 1:1, glycerol and urea in a molar ratio of 1:1, and glycerol and citric acid in a molar ratio of 4:1) were used to prepare NADES. Detailed conditions for preparing NADES were presented in our previous research (Ćirić et al., 2024). Freshly prepared NADES were used to extract polyphenols from plum seed oil cakes. The solid-to-liquid ratio used was 2 g to 30 mL of solvent. Extraction was performed in an ultrasonic bath for 30 minutes. After centrifugation at 8000 rpm for 7 min, the supernatant was separated and used for the determination of TPC and RSA. TPC and RSA were determined following the procedures described in our previous paper (Fotirić Akšić et al., 2022). TPC amounts were expressed as gram gallic acid equivalents (GAE) per kg of plum oil cake. Results of RSA were presented as mmol trolox equivalents (TE) per kg of plum oil cake. Data were analyzed by one-way ANOVA using Statgraphics Centurion software, and Tukey's

HSD test was used to separate means at $p \leq 0.05$. Results are presented as mean $\pm$ SD.

RESULTS AND DISCUSSIONS

Stone morphology. The results of this study revealed significant differences among the examined plum cultivars in stone and seed morphometric characteristics. These results confirm that stone morphology is strongly influenced by cultivar genotype. Highly significant differences were observed among the four plum cultivars for all analyzed stone morphometric traits (Table 1), confirming a strong cultivar-dependent effect on stone size and shape.

Table 1. Morphometric characteristics of plum stones of four plum cultivars

	Stone mass (g)	Stone height (mm)	Stone width (mm)	Stone width across the suture (mm)
'Č.lepotica'	1.37 $\pm$ 0.10 ^a	24.99 $\pm$ 1.52 ^a	8.45 $\pm$ 0.46 ^b	13.17 $\pm$ 0.72 ^b
'Stanley'	1.24 $\pm$ 0.10 ^b	22.11 $\pm$ 1.46 ^b	9.19 $\pm$ 0.66 ^a	13.71 $\pm$ 0.73 ^a
'Požegača'	0.59 $\pm$ 0.06 ^c	19.75 $\pm$ 0.98 ^c	6.52 $\pm$ 0.35 ^c	11.21 $\pm$ 0.55 ^c
'Empress'	1.26 $\pm$ 0.18 ^b	24.04 $\pm$ 2.52 ^a	8.75 $\pm$ 0.53 ^b	13.28 $\pm$ 0.80 ^{ab}
F	260.36***	54.55***	160.51***	74.45***
p	< 0.001	< 0.001	< 0.001	< 0.001

Values are presented as mean $\pm$ standard deviation (SD). Different superscript letters within the same column indicate significant differences according to Tukey's HSD test ($p \leq 0.05$).

Stone mass ranged from 0.59 g in 'Požegača' to 1.37 g in 'Čačanska Lepotica', with 'Stanley' and 'Empress' showing intermediate and statistically similar values. A nearly identical stone mass for the cultivar 'Čačanska Lepotica' (1.35 g) was reported by Milošević et al. (2022). In contrast, higher stone mass values for the cultivar 'Stanley' were reported by Dimkova et al. (2018), ranging from 1.34 to 1.86 g, as well as by Nesheva et al. (2021), who recorded a value of 1.89 g, and Vitanova et al. (2004), who reported a stone mass of approximately 1.6 g, indicating considerable variability of this trait among different studies and agro-ecological conditions. Stone height followed a comparable pattern, with the highest values recorded in 'Čačanska Lepotica' and 'Empress', intermediate values in 'Stanley', and the lowest in 'Požegača'. Cultivar-dependent differences were also evident for stone width and stone width across the suture. 'Stanley' exhibited the widest stones, while 'Požegača' consistently showed the smallest values for both parameters. 'Čačanska

'Lepotica' and 'Empress' formed a homogeneous group with intermediate measurements. Overall, the high F-values emphasize the dominant influence of cultivar on stone morphology. These results demonstrate that stone size and shape in European plum are strongly cultivar-specific traits, with potential relevance for processing systems.

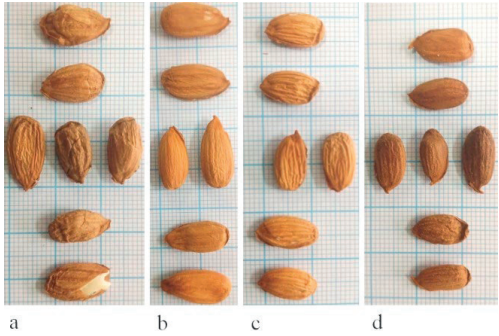


Figure 1. Morphological appearance of plum seeds of the studied cultivars: (a) 'Empress', (b) 'Čačanska Lepotica', (c) 'Stanley', and (d) 'Požegača'

Seed morphology. All analyzed seed morphometric traits differed significantly among the four plum cultivars (Table 2), confirming a strong cultivar effect on seed size and shape. Seed mass ranged from 0.13 g in 'Požegača' to 0.27 g in 'Čačanska Lepotica', with 'Empress' showing comparable values and forming a homogeneous group with 'Čačanska Lepotica', while 'Stanley' and 'Požegača' exhibited significantly lower values. A similar cultivar-dependent pattern was observed for seed height, with the highest values recorded in 'Čačanska Lepotica' and 'Empress', and significantly lower values in 'Stanley' and 'Požegača'. Clear differences were also evident for seed width and seed width across the suture. 'Empress' and 'Čačanska Lepotica' exhibited wider seeds, whereas 'Stanley' and 'Požegača' consistently showed lower values for both parameters. Overall, the high F-values highlight the dominant influence of cultivar on seed morphology. These results demonstrate that seed size and shape in European plum are strongly cultivar-specific traits and should be considered when comparing cultivars intended for different production and processing systems.

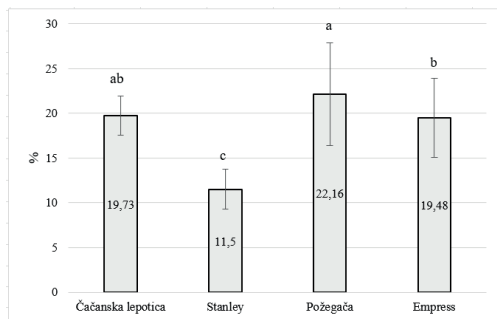
Table 2. Morphometric characteristics of plum seeds of four plum cultivars

	Seed mass (g)	Seed height (mm)	Seed width (mm)	Seed width across the suture (mm)
'Č. lepotica'	0.27±0.03 ^a	15.76±0.87 ^a	3.95±0.34 ^a	7.33±0.43 ^a
'Stanley'	0.14±0.02 ^b	11.69±1.05 ^b	3.45±0.63 ^b	6.50±0.70 ^b
'Požegača'	0.13±0.03 ^b	12.23±0.73 ^b	3.53±0.42 ^b	6.13±0.45 ^b
'Empress'	0.25±0.06 ^a	15.15±1.14 ^a	4.06±0.61 ^a	7.39±0.56 ^a
F	93.49***	135.21***	10.44***	36.96***
p	< 0.001	< 0.001	< 0.001	< 0.001

Values are presented as mean ± standard deviation (SD).

Different superscript letters within the same column indicate significant differences according to Tukey's HSD test ($p \leq 0.05$).

The seed-to-stone ratio differed significantly (Graph 1), indicating a cultivar-dependent allocation of biomass between seed and stone. The highest ratio was recorded in 'Požegača' (22.16%), followed by 'Čačanska Lepotica' (19.73%) and 'Empress' (19.48%), which were statistically comparable, while 'Stanley' exhibited the lowest ratio (11.50%).



Bars represent mean values ± SD.
Different letters above the bars indicate significant differences according to Tukey's HSD test ($p \leq 0.05$).

Graph 1. Seed-to-stone ratio (%) of four European plum cultivars

The higher seed proportion in 'Požegača' corresponds to its smaller stone size, whereas the lower ratio observed in 'Stanley' reflects its wider, more robust stone structure. Intermediate values in 'Čačanska Lepotica' and 'Empress' indicate a more balanced relationship between stone and seed size. Overall, these results confirm that the seed-to-stone ratio is a strongly cultivar-specific trait closely linked to differences in stone and seed morphology.

Fatty acid composition. As shown in Table 3, the dominant fatty acid in the examined oil samples was monounsaturated oleic acid, with a content

ranging from 58.55% ('Empress') to 72.49% ('Stanley'). The second most abundant fatty acid was linoleic acid, a polyunsaturated fatty acid, with a content ranging from 19.68% ('Stanley') to 32.11% ('Empress'). Among the saturated fatty acids, palmitic acid was present in significant amounts, ranging from 5.19% to 6.40%. The results obtained are consistent with the findings of Rabrenović et al. (2021) regarding the content of oleic fatty acid (56.5-61.8%), linoleic acid (21.9-29.9%), and palmitic fatty acid (5.3-5.6%) in plum seed oil, which are a by-product of plum brandy production.

Oxidative stability - The OXITEST method. Given the high proportion of monounsaturated fatty acids, the tested samples exhibited excellent oxidative stability, as indicated by the induction period (h), which ranged from 35.17 h ('Empress') to 73.51 h ('Stanley') (Table 3). Tsao et al. (2021) examined the oxidative stability of 10 cold-pressed oil samples using the OXITEST method under identical experimental conditions. The induction period ranged from 4.60 h (linseed oil) to 81.52 h (peanut oil).

Table 3. Fatty acid composition and induction period of the examined samples

Fatty acid (%) ^a	'Č. Lepotica'	'Stanley'	'Požegača'	'Empress'
C14:0	0.03±0.005 ^c	0.06±0.006 ^b	0.02±0.001 ^c	0.09±0.001 ^a
C16:0	5.19±0.33 ^b	5.47±0.38 ^{ab}	5.62±0.33 ^{ab}	6.40±0.40 ^a
C16:1	1.07±0.02 ^{bc}	0.95±0.05 ^c	1.10±0.06 ^a	1.58±0.06 ^a
C18:0	0.62±0.04 ^b	1.02±0.05 ^a	0.61±0.06 ^b	1.02±0.08 ^a
C18:1	72.16±2.03 ^a	72.49±1.61 ^a	71.53±2.08 ^a	58.55±2.35 ^b
C18:2	20.74±2.18 ^b	19.68±1.95 ^b	20.95±1.28 ^b	32.11±1.19 ^a
C18:3	0.03±0.002 ^c	0.08±0.01 ^a	0.03±0.003 ^c	0.05±0.004 ^b
C20:0	0.10±0.01 ^c	0.16±0.01 ^a	0.09±0.01 ^c	0.13±0.01 ^b
C20:1	0.04±0.004 ^{bc}	0.07±0.01 ^a	0.03±0.003 ^c	0.05±0.003 ^b
IP** (h)	56.23±1.29 ^b	73.51±1.51 ^a	51.54±1.51 ^c	35.17±0.90 ^d

C14:0 - Myristic acid; C16:0 - Palmitic acid; C16:1 - Palmitoleic acid; C18:0 - Stearic acid; C18:1 - Oleic acid; C18:2 - Linoleic acid; C18:3 - Linolenic acid; C20:0 - Arachidic acid; C20:1 - Gondoic acid; **IP - induction period

Techno-functional characteristics of oil cakes.

The techno-functional characteristics of the tested oil cakes are presented in Table 4. The SRC of water is the reference SRC value because this parameter is influenced by all functional components of the analyzed food matrix (Kweon et al., 2011). SRC values for water varied among the analyzed POCs, with the highest in the 'Empress' variety and the lowest in the 'Požegača' variety.

Table 4. Techno-functional characteristics of plum oil cakes

Parameter	'Č. Lepotica'	'Stanley'	'Požegača'	'Empress'
Moisture (%)	6.45±0.56 ^a	6.90±0.41 ^a	6.71±0.50 ^a	6.61±0.44 ^a
Water holding capacity (g/g)	2.93±0.27 ^b	4.02±0.38 ^a	2.85±0.18 ^b	2.84±0.16 ^b
Oil holding capacity (g/g)	1.00±0.06 ^b	1.33±0.10 ^a	1.22±0.12 ^{ab}	1.24±0.13 ^{ab}
Swelling capacity (mL/g)	5.58±0.48 ^c	9.83±0.78 ^a	7.37±0.73 ^b	6.33±0.53 ^{bc}
Solvent retention capacity (%)				
Distilled water	223.85±3.52 ^b	217.72±2.61 ^b	182.41±3.30 ^c	234.04±3.89 ^a
5% Lactic acid	285.68±5.58 ^b	380.44±4.92 ^a	287.27±6.15 ^b	268.00±3.24 ^c
5% Sodium carbonate	245.81±3.90 ^c	374.87±3.65 ^a	276.76±3.81 ^b	254.77±4.36 ^c
50% Sucrose solution	303.38±2.37 ^d	477.81±3.71 ^a	405.15±4.04 ^b	371.15±2.88 ^c

Differences in this parameter among POCs could be explained by variations in the contents of fibers, proteins, and hydrophilic polysaccharides (Rosell et al., 2010), as well as by genotypic differences among the analyzed varieties (Ram and Singh, 2004). The POC of the 'Stanley' variety was superior to other varieties in terms of SRC for lactic acid, sodium carbonate, and sucrose. This suggests that this POC has the highest content of functional proteins, or proteins with greater hydration capacity, as confirmed by the highest swelling capacity of this variety (Kweon et al., 2011; Xue et al., 2018). It is also the POC with the highest proportion of pectin, a soluble fiber, and the greatest ability to form viscous dispersions, indicating potential for use in the production of functional food products (Rosell et al., 2010). In addition to these parameters, the POC of the Stanley variety exhibited the highest water-holding capacity (WHC), oil-holding capacity (OHC), and swelling capacity among the samples. The WHC depends on the content of hydrophilic compounds, particularly fibers and proteins, as it is a seed. Higher WHC values indicate greater water-holding capacity (Elleuch et al., 2011). Xue et al. (2018) reported similar values for this parameter in plum seed proteins. Meanwhile, OHC, according to Deng et al. (2019), reflects the ability of components to interact with lipids, with protein type being the

most influential factor. This is consistent with the SRC sucrose results, which indicate the highest content of hemicellulose and other polysaccharides, thereby enhancing lipid-holding efficiency. Variations in the functional and technological properties of the tested POC across different plum varieties can be attributed to differences in genotype, chemical composition, seed structure, grinding conditions, particle size after grinding, degree of cellular damage, moisture content, ecological growing conditions, and other factors.

Results of TPC and RSA of the investigated plum oil cakes. TPC values of the investigated plum oil cakes ranged from 2.74 to 4.09 g GAE kg⁻¹ (Table 5), depending on both the plum cultivar and the NADES extraction system used. Three different eutectic mixtures (malic acid/sucrose, glycerol/urea, and glycerol/citric acid) were used to extract bioactive compounds from plum oil cakes.

Table 5. TPC (g GAE kg⁻¹) and RSA (mmol TE kg⁻¹) were determined in the investigated plum oil cakes

Samples	NADES	TPC	RSA
'Č. Lepotica'	malic acid: sucrose (1:1)	3.65 ± 0.01 ^f	1.01 ± 0.04 ^g
'Stanley'		3.93 ± 0.01 ^d	1.26 ± 0.04 ^e
'Požegača'		3.87 ± 0.00 ^e	1.13 ± 0.02 ^f
'Empress'		2.89 ± 0.01 ^j	0.74 ± 0.00 ⁱ
'Č. Lepotica'	glycerol:urea (1:1)	3.43 ± 0.02 ^h	0.86 ± 0.02 ^h
'Stanley'		3.65 ± 0.00 ^f	1.12 ± 0.00 ^f
'Požegača'		3.57 ± 0.00 ^g	0.88 ± 0.04 ^h
'Empress'		2.74 ± 0.00 ^k	0.62 ± 0.06 ^j
'Č. Lepotica'	glycerol: citric acid (4:1)	3.99 ± 0.01 ^c	2.02 ± 0.04 ^c
'Stanley'		4.09 ± 0.01 ^a	3.26 ± 0.06 ^a
'Požegača'		4.03 ± 0.00 ^b	2.46 ± 0.00 ^b
'Empress'		3.03 ± 0.00 ⁱ	1.91 ± 0.04 ^d

Values are presented as mean ± standard deviation (SD). Different superscript letters within the same column indicate significant differences according to Tukey's HSD test ($p \leq 0.05$).

Among the tested NADES, the glycerol/citric acid (4:1) system provided the highest extraction efficiency for all cultivars, with the maximum TPC observed in 'Stanley' (4.09 g GAE kg⁻¹) and 'Požegača' (4.03 g GAE kg⁻¹) samples. In contrast, slightly lower TPC values were obtained with the malic acid/sucrose (1:1) and glycerol/urea (1:1) systems. Differences among the investigated cultivars were also observed. Generally, lower TPC values were observed in Empress plum oil cakes than in the 'Stanley', 'Požegača', and 'Čačanska Lepotica' samples. The obtained TPC values are in agreement with the literature data. For instance, Savić and Savić-Gajić (2022) reported TPC values for defatted plum seed cakes ranging from 1.8 to 2.7

g GAE kg⁻¹, depending on the extraction conditions. The slightly higher values obtained in our study may be attributed to differences in the extraction solvents, as NADES systems are known to enhance the extraction efficiency of phenolic compounds compared with some conventional extraction methods.

RSA values of the extracts ranged from 0.62 to 3.26 mmol TE kg⁻¹. The highest antioxidant capacity was also observed in extracts obtained using the glycerol/citric acid (4:1) mixture, particularly for the Stanley cultivar (3.26 mmol TE kg⁻¹). Other cultivars extracted with this solvent also showed relatively high RSA values (Table 5), indicating a strong antioxidant potential of these extracts. On the other hand, the lowest RSA values were observed for the glycerol/urea system, with the minimum value recorded for Empress (0.62 mmol TE kg⁻¹).

CONCLUSIONS

The results of this study confirmed that the morphometric characteristics of plum stones and seeds are cultivar-dependent. Significant differences were observed among the examined cultivars for all analyzed parameters, indicating that stone and seed size and shape are largely determined by genotype. Larger stones and seeds were characterized by 'Čačanska Lepotica' and 'Empress', whereas 'Požegača' showed the smallest stone dimensions but the highest seed-to-stone ratio. In contrast, 'Stanley' exhibited relatively large and robust stones with the lowest proportion of seed mass. The observed differences in the seed-to-stone ratio indicate variations in biomass allocation between seed and stone among cultivars. Such differences may be relevant when considering cultivars for processing and the utilization of plum pits as a potential source of valuable raw materials. The morphometric characteristics of stones and seeds, therefore, represent useful indicators for assessing the technological and processing potential of different plum cultivars. Finally, the results presented indicate that plum seed oil represents a vegetable oil, with a favourable fatty acid profile and high oxidative stability. The high oleic acid content and exceptional oxidative stability of plum seed oil, particularly from the seeds of the Stanley cultivar, provide a promising basis for further

research into its application as a functional edible oil. In addition, considering the techno-functional and nutritional properties of plum seed oil cakes, their potential remains largely unexplored, opening opportunities for further studies and possible industrial-scale applications.

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INFLUENCE OF TEMPERATURE DURING THE SCIONS GROWTH ON THE PERCENTAGE OF GRAFT TAKE SUCCESS IN WALNUT PROPAGATION

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Abstract

*The experiment was conducted at the Fruit Growing Institute-Plovdiv during the period 2022-2025. The local walnut cultivar Izvor 10 was used, grafted onto a rootstock (*Juglans regia* L.). Scions were collected during winter dormancy, immediately before grafting. The percentage of graft take success was evaluated under two propagation methods: Variant I - plants propagated in a stratification room and Variant II - plants propagated in a heated tunnel with a water-based nutritional solution. Average monthly and maximal outdoor temperatures for the experimental region were recorded for the entire observation period. Statistical analysis revealed a clear tendency toward decreasing graft take success in winter grafting of walnut. The study examined the potential relationship between temperature conditions in the preceding year and the percentage of graft take success under controlled callus-formation conditions (air temperature 26°C and relative humidity 80-90%). The results suggest that elevated summer temperatures may negatively affect scion quality and subsequent grafting success.*

Key words: walnut, propagation, temperature, scions, influence.

INTRODUCTION

Walnut (*Juglans regia* L.) is considered one of the most difficult fruit species to propagate by grafting, and achieving high graft take success remains a major challenge for nurseries worldwide. Increasing the efficiency of propagation and reducing production costs are therefore of key importance for industrial walnut nurseries (Hassankhah et al., 2017). Successful grafting depends strongly on environmental conditions, particularly temperature, which plays a critical role in callus formation and graft union development. According to Hartmann et al. (1997), optimal callusing temperatures vary among species, but maintaining stable conditions is essential for proper tissue differentiation. For walnut, maintaining a temperature of approximately 26°C and relative humidity of 80-95% for 3-4 weeks is considered optimal for callus formation (Sadeghi-Majd et al., 2022).

A wide range of grafting techniques and propagation systems have been developed for walnut. Classical studies by Lagerstedt (1979, 1982) established the principles of hot-callus

grafting and emphasized the importance of performing grafting during winter dormancy. In Bulgaria, extensive work has been conducted at the Fruit Growing Institute - Plovdiv, where various propagation approaches, including hot-callus and epicotyl grafting, have been tested and optimized (Gandev, 2007, 2014, 2016). The use of stratification rooms for indoor walnut propagation has long been practiced in the region (Nedev et al., 1983). Under controlled callusing conditions, high grafting success rates ranging from 65% to 90% have been reported (Paunović et al., 2012).

The quality and physiological state of the scions are among the most important factors determining grafting success. Scions are typically collected during winter dormancy and stored at 1-4°C until grafting (Achim & Botu, 2001; Lagerstedt, 1982; Soleimani et al., 2010; Paunović et al., 2011; Raufi et al., 2017). Other authors recommend collecting scions immediately before grafting (Kasmi et al., 2013; Mostakhdemi et al., 2022). Scion age and thickness also influence grafting success; for example, Sharma et al. (2022) reported that

pencil-thick scions aged 6-12 months are most suitable.

Temperature plays a central role in walnut phenology. Buds require sufficient chilling during winter dormancy to break rest, followed by adequate heat accumulation to initiate budburst (Charrier et al., 2011). Dehghan et al. (2010) demonstrated that heat accumulation may be more influential than chilling in determining the timing of budburst. High summer temperatures can accelerate phenological development, potentially leading to insufficient chilling fulfillment and reduced bud quality (Hassankhah et al., 2017). Since walnut bud differentiation occurs over a long period - from late spring through the following winter - temperature anomalies during the growing season may negatively affect the physiological condition of the buds that later serve as scions.

Given the increasing frequency of temperature extremes in many regions, understanding how climatic conditions influence scion quality and grafting success has become increasingly important. The present study examines the hypothesis that elevated summer temperatures negatively affect the vegetative growth and physiological quality of shoots used as scions for winter grafting. The aim of the study was to establish the relationship between temperature conditions in the preceding year and the percentage of graft take in walnut propagated under controlled callus - formation conditions.

MATERIALS AND METHODS

The study was conducted at the Fruit Growing Institute - Plovdiv during the period 2022-2025. The local walnut cultivar Izvor 10 grafted onto seedling rootstocks of *Juglans regia* L. was used. Two propagation methods were evaluated:

- Variant I: Plants propagated in a stratification chamber;
- Variant II: Plants propagated in a heated tunnel with a water-based nutritional solution.

Grafting was performed in March of each experimental year. During the callus formation period, the grafted plants were maintained for 30 days at a temperature of 26°C and relative humidity of 80-90%, conditions considered optimal for walnut callusing.

Propagation in a Stratification Chamber (Variant I)

Grafted plants were placed in wooden box pallets measuring 75 × 40 × 40 cm. The root systems were covered with moist sand, and the pallets were wrapped with polyethylene film to maintain humidity. The boxes were then placed in a controlled-environment room with stable temperature and humidity suitable for callus formation.

Propagation in a Heated Tunnel with Nutrient Solution (Variant II)

Plants were grafted on a bench using the epicotyl grafting method. After grafting, they were planted in polystyrene trays containing eight cavities, each with a volume of 3 L. A substrate mixture of peat and perlite (2:1, v/v) was used. The trays were placed in a heated tunnel equipped with a water-based nutritional solution maintained at the same temperature and humidity as in Variant I. A combined fertilizer (N:P:K 20:5:10, MgO 2%, SO₃ 25%, plus trace elements: Fe 0.07%, Mn 0.04%, Mo 0.004%, Zn 0.025%, B 0.025%, Cu 0.01%) was applied at a concentration of 1 g L⁻¹. For each propagation method, 100 grafted plants were used, each representing a separate replication.

Vegetative Growth Assessment (2023-2024)

The vegetative performance of successfully grafted plants was evaluated during 2023 and 2024. Plants were grown in containers filled with a peat-perlite mixture under 50% shading. Three fertilization treatments were applied:

- Control: No fertilization
- Variant I: N 1.3 – P 0.3 – K 0.7 – Mg 0.1
- Variant II: N 2.7 – P 0.7 – K 1.3 – Mg 0.3

Each treatment included 10 plants; each considered an independent replication. The following parameters were recorded:

- plant height (cm);
- annual shoot thickness (mm);
- root system volume (cm³);
- number of compound leaves.

Temperature Data

Average monthly and maximal outdoor temperatures for the experimental region were recorded for the entire study period (2021-2025) to assess potential relationships between climatic conditions and graft-take success.

Statistical Analysis

A one-way analysis of variance (ANOVA) was performed to evaluate the significance of differences among treatments and years. When ANOVA indicated significant effects ($p \leq 0.05$), the Duncan Multiple Range Test (DMRT) was applied to separate the means (Steele & Torrie, 1980). Statistical analyses were conducted using RStudio (R Core Team, 2020) with the packages **agricolae** (Mendiburu, 2021), **readxl** (Wickham, 2019), **Rcpp** (Eddelbuettel, 2018), and **rstatix** (Kassambara, 2021).

RESULTS AND DISCUSSIONS

Graft Take Success (2022-2025)

The percentage of graft take showed a clear decreasing trend during the four-year study period (Table 1). In plants propagated in a stratification chamber, graft take ranged from 84% in 2022 to 20% in 2025, while in the heated tunnel with a water-based nutritional solution, the values decreased from 60% to 15.6%. Although the absolute percentages differed between the two propagation methods, ANOVA revealed no statistically significant effect of the propagation method, indicating that the observed variation is primarily determined by year-to-year environmental conditions rather than by the technology used.

Table 1. Graft take rate for different walnut propagation methods and statistically significant differences between groups (Duncan Multiple Range Test)

Method	Percentage of graft take, %	
	Stratification chamber	Heated tunnel with water based nutritional solution
Year		
2022	84% a	60% a
2023	80% a	47% b
2024	50% b	34.4% c
2025	20% c	15.6% d
Average value	58.5 % a	39.25 % a



Picture 1. Walnut plants propagated by different methods

The Duncan Multiple Range Test confirmed that 2025 formed a statistically distinct group, significantly different from 2022, 2023 and 2024. The latter three years did not differ significantly from each other. This pattern demonstrates that the sharp decline in graft take is concentrated in the final year of the study, suggesting the influence of an external factor that changed markedly in 2025.

Temperature Dynamics and Their Relationship to Graft Take

To explore whether temperature conditions in the preceding growing season may explain the observed decline in grafting success, average monthly and maximal temperatures for the period 2021-2025 were analyzed (Figures 1 and 2). The data show substantial interannual variability, particularly during the summer months (June-August), which are critical for the differentiation and maturation of walnut buds.

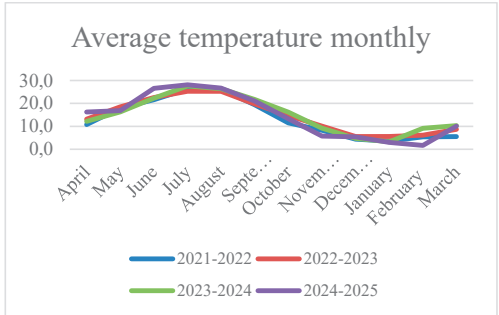


Figure 1. Dynamics of average monthly temperatures for the period 2021-2025

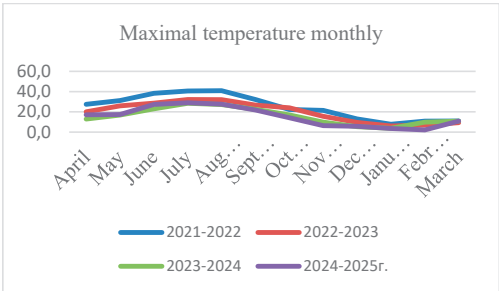


Figure 2. Dynamics of maximal monthly temperatures for the period 2021-2025

The 2025 growing season was characterized by the highest average summer temperatures, with mean values of 26.6°C in June, 28.1°C in July, and 26.7°C in August. Although maximal

temperatures in 2025 did not reach the extreme peaks recorded in 2022 (up to 40.9°C), the prolonged exposure to elevated average temperatures suggests a sustained heat load on developing shoots. This prolonged heat stress is likely more physiologically damaging than short-term temperature extremes. According to Mitov et al. (2008), optimal temperatures for walnut shoot development range between 15-25°C, while temperatures above 35°C can cause tissue damage. Walnut bud differentiation occurs over a 10-month period beginning in late spring; therefore, persistent summer heat may impair bud vitality, reduce carbohydrate accumulation, and increase the chilling requirement needed for proper dormancy release. These physiological disruptions may explain the markedly lower graft take observed in 2025. The results align with the findings of Hassankhah et al. (2017), who reported that increased temperatures accelerate phenological development and may lead to insufficient fulfillment of chilling requirements. Similarly, Charrier et al. (2011) emphasize that budburst depends on the balance between accumulated cold and subsequent heat units. In the present study, the combination of warmer summers and relatively mild autumn-winter periods (October-January) likely contributed to incomplete dormancy progression and reduced scion quality. Additional supporting evidence from the literature further confirms the sensitivity of walnut propagation to temperature anomalies. Karadeniz (2005) and Erdogan (2006) report that walnut grafting success often declines sharply when environmental conditions are suboptimal, highlighting the importance of scion physiological status. Rotondo Walnuts (2007) also observed inconsistent results in patch budding under warm climatic conditions, reinforcing the idea that high summer temperatures can negatively affect bud development. These findings support the hypothesis that scions harvested from heat-stressed mother plants may exhibit reduced vitality, leading to lower graft take under controlled callusing conditions.

Vegetative Growth of Grafted Plants (2023-2024)

The vegetative performance of grafted walnut plants grown in containers during 2023 and

2024 is presented in Tables 2 and 3. Plant height, stem diameter, and root system volume were significantly influenced by mineral nutrition. In 2023, plants receiving the higher fertilizer dose (Variant II) reached a height of 36.3 cm, compared with 31.6 cm in Variant I and 25.4 cm in the control. Stem diameter ranged from 6.3 mm in the control to 7.8 mm in Variant II, while root system volume varied between 76.7 cm³ (Variant I) and 126.7 cm³ (Variant II).

Table 2. Vegetative behaviour of grafted walnut plants during winter dormancy in 2023

Variant	Height, cm	Thickness, mm	Root system volume, cm ³	Number of leaves
Control	25.4 b	6.3 b	111.7 ab	10.7 a
Variant I	31.6 ab	7 ab	76.7 b	9.7 a
Variant II	36.3 a	7.8 a	126.7 a	11.7 a

The same trend was observed in 2024, where Variant II again produced the most vigorous plants, with a height of 35.1 cm, stem diameter of 7.1 mm, and root system volume of 121.7 cm³. Control plants exhibited the lowest values for all measured parameters, confirming the positive effect of mineral nutrition on vegetative development.

Table 3. Vegetative behavior of grafted walnut plants during winter dormancy in 2024

Variant	Height, cm	Thickness, mm	Root system's volume, cm ³	Number of leaves
Control	24.2 b	5.6 b	106.7 ab	10.7 a
Variant I	30.4 ab	6.3 ab	71.7 b	9.7 a
Variant II	35.1 a	7.1 a	121.7 a	11.7 a



Picture 2. Container growing of walnut planting material

The number of compound leaves did not differ significantly among treatments in either year, indicating that leaf formation is less responsive to fertilization than stem and root growth. Overall, the results demonstrate that increasing fertilizer rates enhances plant vigor, particularly in terms of height, stem thickness, and root system development. These findings are consistent with previous studies on container-grown walnut seedlings, which also report improved vegetative performance under balanced mineral nutrition.

Importantly, this experiment is independent of the graft take analysis and is not influenced by the temperature - related factors discussed earlier. Instead, it provides complementary information on the post - grafting development of successfully propagated plants. Therefore, the vegetative growth results should be interpreted separately from the environmental effects affecting scion quality and grafting success.

The combined analysis of graft take dynamics, temperature patterns, and vegetative growth provides a comprehensive understanding of the factors influencing walnut propagation under controlled callusing conditions. The most striking result is the sharp decline in graft take in 2025, which coincides with the highest average summer temperatures recorded during the study period. This supports the hypothesis that prolonged heat stress during the growing season negatively affects scion physiological quality, reducing their ability to form callus and successfully unite with the rootstock.

The literature strongly reinforces this interpretation. Hassankhah et al. (2017) and Charrier et al. (2011) demonstrated that temperature-driven shifts in phenology can disrupt the balance between chilling and heat requirements, leading to premature or incomplete dormancy transitions. Karadeniz (2005), Erdogan (2006) and Rotondo Walnuts (2007) further show that walnut grafting is highly sensitive to environmental anomalies, with success rates declining sharply under suboptimal climatic conditions.

In contrast, the vegetative growth experiment (2023-2024) revealed that mineral nutrition strongly influences post-grafting development, but these effects are independent of the environmental factors affecting graft take. This distinction highlights the importance of

separating scion physiological quality (determined by the previous year's climate) from nursery management practices (such as fertilization), which influence plant performance after successful grafting.

Overall, the study demonstrates that temperature conditions in the preceding growing season are a major determinant of grafting success in walnut, while fertilization primarily affects subsequent vegetative growth. These findings underscore the need for careful selection of scion sources, monitoring of climatic conditions, and adaptation of propagation practices in regions experiencing increasing temperature variability.

CONCLUSIONS

A decrease in the percentage of graft take was found in walnut propagation by both methods (Stratification Room and Heated Tunnel) under controlled conditions for callus formation after grafting during the period 2022-2025. Regarding the propagation method, no statistically significant differences were found between the average graft take values, indicating that the observed variations are determined by the annual temperature conditions and not by the technology used. Probably, the cool autumn and early winter (October-January period) are the main prerequisite for successful propagation in walnut. We assume that the warm summer has a negative effect by reducing the vitality of the buds, affecting the development of tissues, and increasing the need for cooling in winter. The spring months (March-April) also create a prerequisite for a negative impact, as they can provoke premature activation of the cambium. This study assumes that high summer temperatures may have a major impact on the grafts used for walnut propagation under controlled conditions. Further studies are needed to establish stronger relationships and dependencies between the individual factors influencing callus formation and attachment.

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GENETIC DIVERSITY ANALYSIS OF *CORYLUS AVELLANA* L. GENOTYPES USED FOR NEW VARIETY DEVELOPMENT

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Abstract

A study compared nine hazelnut genotypes from Transylvania's spontaneous flora with five Corylus avellana cultivars, using 10 microsatellite markers to assess genetic diversity and identify valuable genotypes for developing new varieties. Among the primer sets tested, cavSSR14418 was the most informative, with Na = 5, Ne = 3.88, I = 1.56, PIC = 0.71, and He = 0.74. The observed heterozygosity (Ho) ranged from 0.64 (cavSSR 2135; cavSSR 3909; cavSSR 4217; cavSSR 14875) to 1.0 (cavSSR 2704), with an overall mean of 0.72. The NJ dendrogram grouped the genotypes into two main clusters. The clear separation between clusters grouping the established cultivars and those including the new selections demonstrates the latter's potential to enrich the current genetic pool and confirms that wild genotypes exhibit high genetic variability, making them valuable biological resources for biodiversity conservation and future breeding programs.

Key words: hazelnuts, wild accessions, cultivars, morphological characteristics, SSR markers.

INTRODUCTION

Corylus avellana L., The European hazelnut belongs to the Betulaceae family and is a diploid ($2n = 22$), monoecious, wind-pollinated plant with sporophytic incompatibility that requires cross-pollination (Bocacci et al., 2013; Martins et al., 2015). It can be cultivated in commercially intensive orchards as a shrub or as a single-trunk tree because of its importance in nut production (Brown et al., 2016; Oztolan-Erol et al., 2021). In Romania, investments in fruit growing have increased the number of hazelnut orchards, with many using organic methods (Stănică et al., 2023). Hazelnut trees also occur in spontaneous or semi-spontaneous stands at the edges of deciduous forests, along watercourses and roads, and in almost all hilly and mountainous areas, reaching altitudes of 900-1200 m (Botu et al., 2018). As a result, many Romanian hazelnut accessions from *in situ* collections maintained by research and development stations have wild ancestry, and natural selection has strongly influenced their genetic structure (Pop et al., 2010).

For example, morphological characterization can reveal wide phenotypic variation in fruit traits and in the environmental adaptation of selected individuals, which can be exploited in breeding programs. However, there is limited information on the relationship between cultivated and wild hazelnut forms in Romania, as assessed using SSR (Simple Sequence Repeats) molecular markers. In this context, exploiting the genetic diversity of wild hazelnut individuals can be crucial for developing new varieties that are more resilient to climate change.

Examining the relationship between wild and cultivated forms within a geographic area using SSR markers can provide valuable insights into possible domestication events, evolutionary links, or gene flow between them (Campa et al. 2011).

Previous research has highlighted the importance of microsatellite (SSR) markers in hazelnuts. Several studies, including those conducted by Bassil et al. (2004), Gökirmak et al. (2009), Gürcan et al. (2010), Leinemann et al. (2013), Tanhuanpää et al. (2019), Kavas et al. (2020), and Farrokhi and Ershadi (2022), have

underscored the value of these DNA-based molecular markers in research related to *C. avellana*.

From this perspective, the present study aims to evaluate the genetic relationships between wild hazelnuts and cultivated varieties to identify locally valuable germplasm for developing new hazelnut varieties.

MATERIALS AND METHODS

The plant material used in this study for SSR analysis consisted of young leaves provided by a private commercial orchard for the varieties: ‘Tonda di Giffoni’, ‘Tonda Gentile delle Langhe’, and ‘Tonda Gentile Romana’. The leaves for ‘Uriășe de Vâlcea’ and ‘Cozia’ used for DNA isolation were provided by SCDP Vâlcea. Additionally, leaves from nine wild hazelnut genotypes were collected from the experimental field of the Cluj-Napoca Horticultural Research Station of UASVMCN (see Table 1).

Table 1. The codification and provenance of local wild hazelnut genotypes used for SSR analysis

No.	Sample Code	Provenance
1	al 2	Făget forest, Cluj county
2	al 3	Plăiești forest, Cluj county
3	al 4	Gârbova forest, Alba county
4	al 5	Făget forest, Cluj county
5	al 6	Făget forest, Cluj county
6	al 9	Gheorgheni forest, Cluj county
7	al 10	Făget forest, Cluj county
8	al 14	Făget forest, Cluj county
9	al 20	Făget forest, Cluj county

Genomic DNA was extracted using a DNeasy Plant Pro kit (Qiagen, Germany) according to the manufacturer's protocol. The concentration and purity of the extracted DNA were measured using a Nanodrop ND-1000 spectrophotometer (Thermo Fisher Scientific, USA).

A set of 10 SSR primers was used in this study. The characteristics of the primers are presented in Table 2.

The PCR amplification profile was set on a thermocycler (SuperCycler Trinity, Kyratec, Australia), as described by Öztürk et al. (2018). Thermal cycling conditions included an initial denaturation step of 10 minutes at 94°C, followed by 30 cycles each consisting of 30 seconds at 94°C, 30 seconds at 55°C, and 30 seconds at 72°C, ending with a final extension of 10 minutes at 72°C.

Table 2. SSR primer names and sequences

Primer name	Forward primer sequences (5'-3')	Reverse primer sequences (5'-3')	SSR motifs
cavSSR 1361	GATATCACTCACG TCTACCG	GGTCTCTTGGTC TTGATGTT	(AG/TC) 14
cavSSR 2135	ATGTAGCGAGCCT TGATAAG	GTTGTCAGGTA GCTTGAAGA	(TTAA/AATT) 6
cavSSR 2704	GCGGAGTTGGTA GTGATAAT	ATATAGGTATA AGGGGGCCC	(TAG/ATC) 10
cavSSR 3909	AGATGAAGCTGA AGAAAGGG	TATCGCCATCA CACCATTAG	(GGA/CCT) 8
cavSSR 4217	GACAGTTGGCAT GAAAGATG	GCACTCATCAG AGAGTCAAA	(ATT/TAA) 10
cavSSR 12862	TAAATTTGGGCT ACACTTCC	CCAGTACAGGA AGATACGAA	(CT/GA) 13
cavSSR 14219	TATATGGACAGCT GACTCCA	GAGGGAGTTTG TCTGCTTT	(AAT/TTA) 9
cavSSR 14418	GACTGCAAGAAT GACAACAG	GTCTCTCTCTT TTTCGTAG	(TTGG/AACC) 6
cavSSR 14875	CACAAGATGATA CCCATGCT	TATCAGCTCCT AAAACGACG	(TACA/ATGT) 6
cavSSR 14937	TGAGCTCTCTGGT TTCTTTC	ACTGGATCTGC TTTTATGGG	(CT/GA) 12

In this study, we adjusted the master mix reaction volume to 12 µL per sample, and its composition: 5x GoTaq Flexi Green buffer, nuclease-free water, 1.5 mM MgCl₂, 0.2 mM dNTP mix (Promega, USA), 1 µM Cy5-labeled forward SSR primer, 1 µM reverse SSR primer (GeneriBiotech, Czech Republic), and 0.8 U GoTaq polymerase (Promega, USA). A volume of 3 µL of each DNA sample (20 ng/µL) was used for each PCR reaction. Amplification products were verified by running them on 1.6% agarose gels (Promega, USA) stained with RedSafe™ nucleic acid stain (iNtRON Biotech, South Korea) in 1x TBE buffer at 0.29 V/cm for 2.5 hours. The PCR fragments were visualized using the UVP Biospectrum AC Imaging System (UVP BioImaging Systems, USA). Prior to migration and separation within the CEQ 8800™ capillary DNA analysis system (Beckman Coulter, USA), each PCR sample was mixed with 30 µL of sample-loading solution and 0.25 µL of the Genome DNA Standard Kit-400 (Beckman Coulter, USA). Then, allele sizes for each SSR locus were determined using the internal CEQ fragment analysis software.

Genetic diversity among hazelnut genotypes was assessed using GenAlEx version 6.503 (Peakall & Smouse, 2012). The analysis included parameters such as the number of alleles per locus (Na), effective number of alleles (Ne), observed heterozygosity (Ho), expected heterozygosity (He), unbiased expected heterozygosity (uHe) and Shannon

information index (I). Allele frequencies for each SSR locus were calculated based on allele size (bp). The polymorphism information content (PIC) and the null allele frequency (F null) were estimated with Cervus 3.0.7 (Kalinowski et al., 2007). Multivariate analysis was also performed. Clustering was carried out using MEGA7 (Kumar et al., 2016) which created a pairwise distance-based tree via the Neighbor-joining (N-J) method in Tree Explorer, with scaling based on codominant genotypic distances computed in GenAlEx 6.503.

RESULTS AND DISCUSSIONS

Assessment of genetic diversity among wild and cultivated hazelnut genotypes shows that the spontaneous flora of the Transylvanian region contains hazelnut genetic resources that can be used for selecting and developing new genotypes. The results of molecular descriptive statistics are presented in Table 3.

Table 3. Descriptive statistics and genetic diversity of the 14 hazelnut genotypes at 10 microsatellite loci

Locus	Na	Ne	Ho	He	uHe	I	PIC	F (null)
cavSSR 1361	5	2.72	0.71	0.63	0.66	1.21	0.58	-0.049
cavSSR 2135	3	2.65	0.64	0.62	0.65	1.03	0.55	-0.036
cavSSR 2704	5	3.47	1.00	0.71	0.74	1.39	0.67	-0.194
cavSSR 3909	3	2.21	0.64	0.55	0.57	0.93	0.49	-0.063
cavSSR 4217	3	2.65	0.64	0.62	0.65	1.03	0.55	-0.002
cavSSR 12862	5	3.47	0.71	0.71	0.74	1.39	0.67	0.007
cavSSR 14219	4	3.81	0.71	0.74	0.76	1.36	0.69	0.008
cavSSR 14418	6	3.88	0.79	0.74	0.77	1.56	0.71	-0.037
cavSSR 14875	3	2.80	0.64	0.64	0.67	1.06	0.57	-0.009
cavSSR 14937	5	3.84	0.71	0.74	0.77	1.47	0.70	0.011
Mean	4.2	3.15	0.72	0.67	0.69	1.24	0.61	-

All ten SSR primer pairs used in this study were polymorphic, generating 42 distinct alleles across 14 genotypes analysed (n = 14); the number of alleles per locus ranged from 3 to 6, with an average of 4.2 alleles per locus. This mean value indicates moderate allelic richness as compared with others previously reported studies (Bocacci et al., 2008; Fiore et al., 2022). It is important to note that comparisons with other studies are only approximate, since factors like the number of SSRs used and the sample

sizes analysed can significantly affect the research outcomes. The effective number of alleles, accounting for alleles at relevant frequencies in the hazelnut samples, ranged from 2.21 at locus cavSSR 3909 to 3.88 at locus cavSSR 14418, with a mean of 3.15. This result aligns with those reported by Erfatpour et al. (2011) in a study of genetic diversity among some Iranian 90 hazelnut genotypes.

In the present study, the al_4 hazelnut genotype exhibited private alleles at three loci (cavSSR1361, cavSSR14418, cavSSR14937), similar to Tonda Gentile delle Langhe and Tonda Gentile Romana (Table 4).

Table 4. List of the genotypes with one or more private alleles

Genotype name	No. of loci	Loci with private alleles
al_2	1	cavSSR14418
al_3	1	cavSSR2704
al_4	3	cavSSR1361; cavSSR14418; cavSSR14937
al_5	2	cavSSR2704; cavSSR14937
al_6	2	cavSSR14418; cavSSR14937
al_9	1	cavSSR12862
al_10	1	cavSSR14937
al_14	2	cavSSR14418; cavSSR14937
al_20	2	cavSSR12862; cavSSR14418
Tonda di Giffoni	3	cavSSR1361; cavSSR12862; cavSSR14418
Tonda Gentile delle Langhe	3	cavSSR1286; cavSSR14418; cavSSR14937
Tonda Gentile Romana	2	cavSSR14418; cavSSR14937
Urișe de Vâlcea	1	cavSSR14937

This suggests that the al_4 genotype, collected from wild flora, may possess valuable traits that can be exploited in hazelnut breeding and conservation strategies Table 4 lists the hazelnut genotypes with one or more private alleles identified by GenAlEx analysis.

In this study, SSR markers were employed to evaluate genetic variability among the analysed varieties, using observed and expected heterozygosity (Ho and He). The observed heterozygosity (Ho) ranged from 0.64 (cavSSR 2135; cavSSR 3909; cavSSR 4217 and cavSSR 14875) to 1.0 (cavSSR 2704), with an overall mean of 0.72. The expected heterozygosity (He) ranged from 0.55 (cavSSR 3909) to 0.74 (cavSSR 14219; cavSSR 14418 and cavSSR 14937), with an average of 0.67. The overall mean observed heterozygosity was higher than expected (0.72 versus 0.67) due to presumed admixture of individuals from distant populations and different origins, indicating that

the analysed hazelnut samples do not originate from a single isolated population but are a mix of genetically distinct groups (for example, due to recent migration or gene flow) (Allendorf et al., 2013). Furthermore, this excess of heterozygosity signifies a high level of outbreeding, where reproduction occurs between unrelated individuals from different source populations. This pattern aligns with recent genomic studies on hazelnut diversity, which show that human-mediated transport and the absence of strict reproductive barriers often lead to increased genetic variation and the preservation of high heterozygosity levels (Öztürk et al., 2021; Wrigley et al., 2020). The cavSSR 3909 primer pair produced the lowest PIC (0.49), He (0.55), and I (0.93) values. The highest values for PIC (0.71), He (0.74), and I (1.56) were recorded with the cavSSR 14418 primer pair, as shown in Table 3. The recorded values for the null allele frequencies (F null) were negative for almost all of the user SSR primer pairs, except cavSSR 12862, cavSSR 14219, and cavSSR 14937, where F (null) values were positive but less than 0.02. A null allele frequency below 0.02 (2%) in SSR (Simple Sequence Repeat) markers is generally considered very low and acceptable in most genetic studies. Although null alleles (alleles that fail to amplify in PCR due to mutations at primer-binding sites) are common (Jahnke et al., 2022), the low frequency observed in this study indicates that the SSR markers successfully amplified all DNA samples.

The NJ dendrogram grouped the analyzed genotypes into two main clusters, designated I and II. The first main cluster comprises two subclusters (A and B) that group all wild hazelnut genotypes, as well as one international reference genotype (Tonda di Giffoni). Subcluster B includes two genotypes: al_4 and al_5, both wild samples, which are separated from the other genotypes in the first main cluster, suggesting greater genetic diversity than the genotypes grouped in the A subcluster. The second main cluster (II) comprises two subclusters, each containing only the cultivars considered reference varieties, namely Tonda Gentile delle Langhe and Tonda Gentile Romana, and Cozia and Urișe de Vâlcea, respectively, as shown in Figure 1.

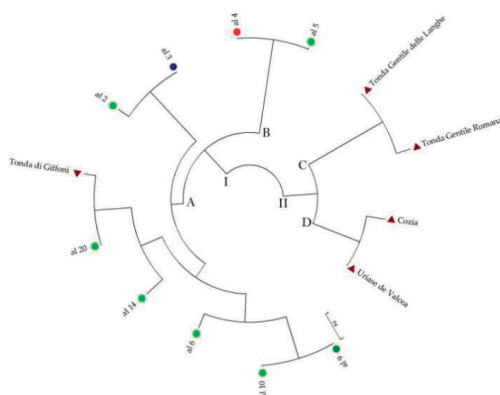


Figure 1. NJ dendrogram grouping the wild hazelnut genotypes (marked with red, blue, and green circles) and reference varieties (marked with red triangles); I, II - main clusters; A, B, C, D - subclusters

The Neighbor-Joining (NJ) analysis revealed genetic diversity among the hazelnut genotypes studied, confirming genetic variability within the analysed germplasm that can be used in future breeding programs. The clear separation between clusters grouping established cultivars (C and D) and those including the new selections (A and B) demonstrates the latter's potential to enrich the current genetic pool. In particular, the genetic isolation of selections al_4 and al_5 from the first main genotype group (cluster I) highlights their possible divergent origins and their value as novel genetic resources.

CONCLUSIONS

Based on SSR data analysis, this study confirms that local genotypes exhibit high genetic variability, making them valuable biological resources for biodiversity conservation and for future breeding programs to develop new varieties that enhance genetic resilience.

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IMPACT OF FERTILIZER TYPES ON VEGETATIVE GROWTH AND FRUIT QUALITY IN BLUEBERRY

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Abstract

*This study evaluated the influence of different fertilizer types on vegetative growth, productivity, and fruit quality in four highbush blueberry (*Vaccinium corymbosum* L.) cultivars - 'Simultan', 'Lax', 'Pastel', and 'Blueray'- grown under Romanian conditions. Research was conducted during the 2024 and 2025 seasons in a young plantation established on acidic soil. Fertilization treatments were compared with respect to their effects on plant vigor, yield, and fruit quality attributes, including pH and total soluble solids (TSS). Results demonstrated that fertilizer type significantly affected both vegetative development and fruit quality parameters, though responses varied among cultivars. Certain fertilizer treatments enhanced productivity and improved fruit biochemical composition, while others had neutral or cultivar-specific effects. Overall, findings highlight the importance of fertilizer selection in optimizing growth and fruit quality in blueberry plantations cultivated on acidic soils.*

Key words: different growing condition, fertilization, highbush, Romanian conditions, vegetative growth.

INTRODUCTION

Highbush blueberry is a high-value small fruit crop that is increasingly cultivated because of its attractive color, desirable flavor, and nutritional benefits. Highbush blueberries originated in North America, and cultivated varieties are classified as either northern or southern types based on their chilling requirements for breaking winter dormancy. Blueberry fertilization has long been a subject of debate. Because blueberries evolved in areas with low nutrient availability, they can survive with surprisingly low levels of fertilization (Banados et al., 2012). However, experience over time has clearly shown that a well-managed fertilization program is essential for rapid growth of young plants and high yields in mature plantings. This is especially true for southern highbush blueberries (Godoy et al., 2008). Blueberry plants are characterized by relatively low nutrient demands compared with many other horticultural crops and are well suited to acidic soils with a pH ranging from 4.5 to 5.5, where the availability of nutrients such as nitrate-nitrogen (NO₃-N), phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg) is naturally limited (Korcak, 1988). Although

blueberries are capable of surviving under low-fertility conditions, the application of a well-designed fertilization program is essential to ensure vigorous vegetative growth and the production of high-quality fruit (Hanson & Hancock, 1996; Hart et al., 2006). Nitrogen is the most important nutrient supplied to blueberries and must be applied annually. In contrast to most crops, blueberry plants preferentially take up nitrogen in the ammonium (NH₄⁺) form rather than nitrate, largely because of their low nitrate reductase activity in both roots and leaves (Merhaut & Darnell, 1995; Peterson et al., 1988). Nitrogen deficiency typically results in reduced shoot development, pale green to yellow leaf coloration, and in some cases a reddish tint. In addition to nitrogen, blueberries often require supplemental applications of phosphorus, potassium, calcium, magnesium, elemental sulfur (to maintain or lower soil pH), iron, boron, copper, and zinc (Hanson & Hancock, 1996; Hart et al., 2006). Manganese is also essential for plant growth (Larco, 2010) but is generally abundant in acidic soils and therefore rarely limiting (Korcak, 1988). Visual examples of nutrient deficiencies and toxicities in northern highbush blueberry were provided by Ramsdell (1995) and Chen

(2004). Vargas and Bryla (2015) evaluated fertigation versus granular fertilizer applications using different nitrogen sources during the first five years of fruiting in 'Bluecrop' northern highbush blueberry. They observed that granular fertilizers slightly lowered soil pH compared with fertigation; however, plants receiving granular fertilizer also had lower leaf nitrogen concentrations. The highest yields were achieved when nitrogen was supplied through fertigation, particularly using ammonium sulfate or urea sulfuric acid. Similar findings were reported by Ehret et al. (2014) in 'Duke' northern highbush blueberry grown in British Columbia, where fertigation resulted in greater yields while requiring less nitrogen than broadcast fertilizer applications. Together, these studies demonstrate that northern highbush blueberry responds well to fertigation. Although increasing nitrogen rates enhanced vegetative growth in both experiments, higher rates did not increase yield in any year and led to reduced berry size during the early years of production. Overall, annual nitrogen applications of 67-93 kg·ha⁻¹ or less were sufficient to achieve optimal fruit production, regardless of whether nitrogen was applied through fertigation or as granular fertilizer.

MATERIALS AND METHODS

Plant material

The study was carried out at the Research Institute for Fruit Growing in Pitesti - Maracineni (RIFG) in southern Romania, located at 44°54'12" north latitude and 24°52'18" east longitude, with an altitude of 284 meters. The study included the following genotypes: -Three Romanians cultivars: 'Simultan', 'Lax' and 'Pastel' cv., obtained from open pollination of 'Spartan' cv. -And 'Bluecrop' - an American-developed northern highbush blueberry (*Vaccinium corymbosum*) cultivar that has been widely cultivated since its introduction in the 1950s. The comparative technological study of different blueberry cultivars, factor A, with the following levels:

- a1 – cultivar *Simultan*,
- a2 – cultivar *Pastel*,
- a3 – cultivar *Lax*,
- a4 – cultivar *Bluecrop*.

The replication plot consists of 10 plants with 3 replications. The experimental layout is arranged in blocks and positioned in rows. Experimental factor B is the type of certified organic fertilizer, which will be tested on blueberry, as follows: b1 = completely natural organic fertilizer at a dose of 150 g/plant – soil application (plants 1-10); b2 = fertilization with vegetable amino acids, an organic fertilizer with nitrogen and phosphorus at doses of 0.6 mL/plant (0.2%) + 1.5 mL/plant (0.5%) + 300 g/plant – foliar and soil application (plants 11–20); b3 = fertilization with vegetable amino acids and fully natural organic fertilizer at doses of 1.2 mL/plant (0.3%) + 150 g/plant, foliar and soil application (plants 21-30); b4 = unfertilized control (plants 31-40).

Determination of yield: By weighing the total yield/plant (kg/plant).

Determination of average weight: By weighing a sample of 50 fruits for each genotype (15 plants/genotype), the average weight of fruits was determined, and the results were expressed in g/fruit.

Determination of length, diameter, and shape index: The length and diameter of the fruit were determined by measuring this using digital calliper. The length and diameter of the fruit were determined by measuring the fruit using a digital caliper. The shape index (SI) of the fruit was calculated as the ratio of these two dimensions (Tudor et al., 2014; Jamieson, 2016).

Determination of fruit firmness: By measuring each fruit sample with a Bareis HPE II Fff non-destructive testing, with a measuring surface of 0.25 cm².

Determination of fruit pH: The pH values were measured in blueberry fresh juice (Song et al., 2018) using a pH meter (ISFET pH Meter, IQ 125, Japan).

Determination of total titratable acidity: TTA was determined by titrable method (Ermakov et al., 1987). The principle of the method is to neutralize a volume of aqueous fruit extract with a solution of NaOH 0.1N in the presence of phenolphthalein as an indicator.

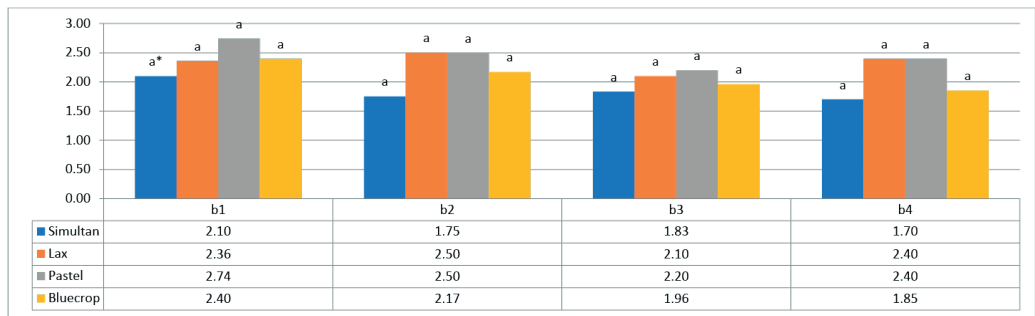
Determination of Total Soluble Solid Content: Total soluble solids (TSS) were determined using a Kruss DR201-95 refractometer, and the results were reported as Brix at 20 °C.

Statistical Analysis: All analyses were performed in triplicate, and data were reported as mean standard deviation (SD). Microsoft Excel 2021 (XLSTAT) was used for data statistical analysis. One-way analysis of variance (ANOVA) and two-way ANOVA and Duncan's multiple range tests, were performed.

RESULTS AND DISCUSSIONS

The obtained results highlight a positive effect of fertilization on fruit yield (Figure 1) per plant for all four blueberry cultivars compared to the unfertilized control (b4). For the 'Simultan' cultivar, the highest yield was recorded in treatment b3 (2.54 kg/plant), indicating a good response to the combined fertilization with b3. Treatments b1 and b2 showed similar values, both clearly higher than the control (1.95 kg/plant). In the case of 'Lax' cv., fertilization

led to a noticeable increase in yield, with the maximum value also observed in b3 (2.82 kg/plant). The differences among fertilized treatments suggest a higher efficiency of combined foliar and soil applications compared to soil-only fertilization. The 'Pastel' cultivar exhibited high yield levels under all fertilized treatments, reaching the highest value in b3 (2.93 kg/plant). Compared with the control (2.16 kg/plant), the yield increase confirms the positive responsiveness of this cultivar to organic fertilizers and biostimulants (Bryla et al., 2015). For 'Bluecrop', which recorded the highest absolute yield values among the studied cultivars, treatments b1 (3.04 kg/plant) produced superior results, demonstrating a high productive potential and good adaptability to different fertilization strategies. Meanwhile, the unfertilized control showed a considerably lower yield (2.25 kg/plant).

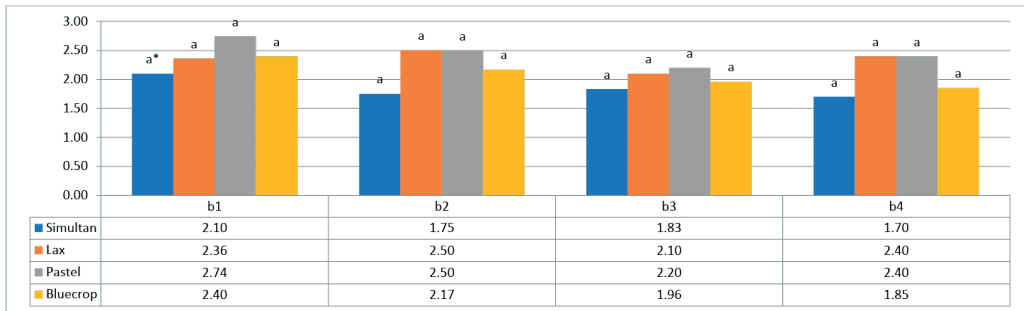


Note: *Different letters between cultivars denote significant differences (Duncan test, $p < 0.05$)

Figure 1. The influence of fertilizer on the production of blueberry cultivars

The data indicate that fertilization treatments influenced the average fruit weight (Figure 2) in all analyzed blueberry cultivars, although the response varied depending on the cultivar and fertilization strategy (Jiang et al., 2019). For the 'Simultan' cultivar, the highest average fruit weight was recorded in treatment b1 (2.10 g/fruit), while the lowest value occurred in the unfertilized control (b4 - 1.70 g/fruit). Treatments b2 and b3 showed intermediate

values, suggesting that the soil application of b1 alone had a more pronounced effect on fruit size for this cultivar. In the case of 'Lax', the greatest average fruit weight was observed in treatment b2 (2.50 g), followed closely by the unfertilized control (2.40 g). This indicates a favorable response to the combined foliar and soil application of b2, while treatment b3 resulted in comparatively smaller fruits.

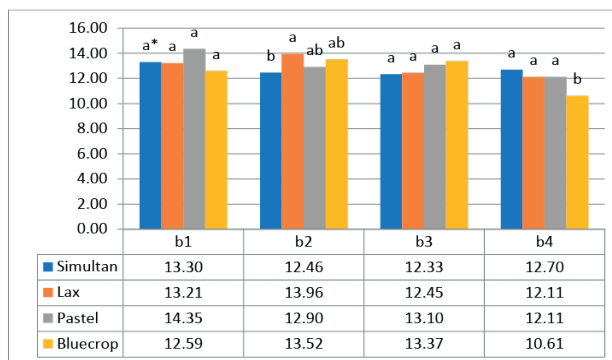


Note: *Different letters between cultivars denote significant differences (Duncan test, $p < 0.05$)

Figure 2. The influence of fertilizer on the berry weight of the blueberry cultivar

The 'Pastel' cultivar exhibited the highest fruit weight across all cultivars, with a maximum value in treatment b1 (2.74 g). All fertilized treatments produced larger fruits than the control, demonstrating the positive effect of fertilization on fruit size for this cultivar. For 'Bluecrop', average fruit weight decreased progressively from b1 (2.40 g) to the unfertilized control (1.85 g). Fertilization treatments, particularly b1, clearly enhanced fruit weight (Figure 2), indicating a strong dependence of this cultivar on an adequate nutrient supply for optimal fruit development. The results show that fertilization treatments affected fruit height in all blueberry cultivars, with distinct responses depending on the cultivar and fertilization scheme applied. For the 'Simultan' cultivar, the highest fruit height was recorded in treatment b1 (13.30 mm), while slightly lower but comparable values were observed in the fertilized treatments b2 and b3. The unfertilized control (b4) showed intermediate values, indicating a moderate response of fruit height to fertilization in this cultivar. In the case of 'Lax',

the maximum fruit height was observed in treatment b2 (13.96 mm), followed by b1 (13.21 mm). In the case of 'Lax', the maximum fruit height was observed in treatment b2 (13.96 mm), followed by b1 (13.21 mm). Treatments b3 and the control resulted in smaller fruits, suggesting that the combined foliar and soil application of b2 had a stronger influence on fruit elongation. The 'Pastel' cultivar exhibited the highest fruit height among all cultivars, reaching a maximum value in treatment b1 (14.35 mm). All fertilization treatments resulted in larger fruits compared to the unfertilized control, confirming the positive effect of nutrient application on fruit size for this cultivar (Jayasinghe et al., 2024). For 'Bluecrop', fertilization markedly increased fruit height compared with the control. The highest values were obtained in treatments b2 (13.52 mm) and b3 (13.37 mm), while the unfertilized control showed the lowest value (10.61 mm), indicating a strong dependency of fruit height on fertilization in this cultivar (Figure 3).

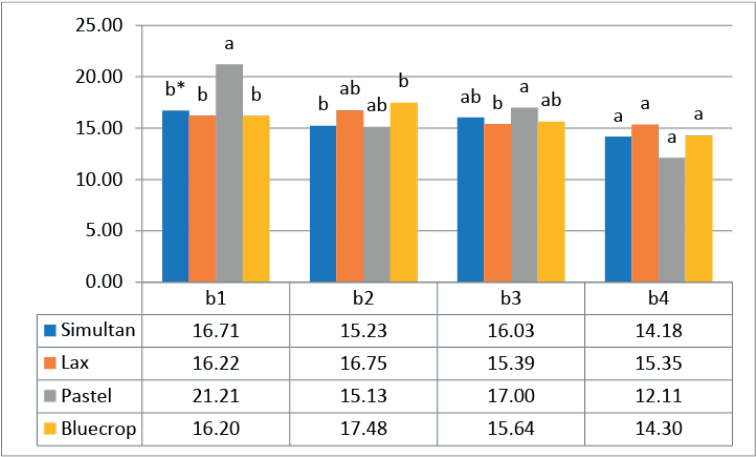


Note: *Different letters between cultivars denote significant differences (Duncan test, $p < 0.05$)

Figure 3. The influence of fertilizer on the berry height of blueberry cultivars

The results indicate that fertilization treatments significantly influenced fruit diameter (Figure 4) across all blueberry cultivars (Coates et al., 2012), with notable differences depending on the cultivar and fertilization strategy (Zhang et al., 2023). For the 'Simultan' cultivar, the largest fruit diameter was recorded in treatment b1 (16.71 mm), followed by b3 (16.03 mm). All fertilized treatments resulted in larger fruit diameters compared to the unfertilized control (14.18 mm), highlighting the positive effect of fertilization on fruit size. In the case of 'Lax', the highest fruit diameter was observed in treatment b2 (16.75 mm), slightly exceeding b1 (16.22 mm). Treatments b3 and the control showed similar and lower values, indicating that the

combined foliar and soil application of b2 was most effective in increasing fruit diameter for this cultivar. The 'Pastel' cultivar exhibited the greatest fruit diameter among all cultivars, reaching 21.21 mm in treatment b1, which represents a substantial increase compared with b2 (15.13 mm). Fertilization markedly improved fruit diameter in this cultivar Jeyabaskaran et al. (2021) particularly under soil application of b1 (Mazzoni et al., 2020). For 'Bluecrop', the maximum fruit diameter was recorded in treatment b2 (17.48 mm), followed by b1 (16.20 mm). All fertilized treatments outperformed the control (14.30 mm), confirming the strong response of this cultivar to fertilization in terms of fruit diameter.



Note: *Different letters between cultivars denote significant differences (Duncan test, $p < 0.05$)

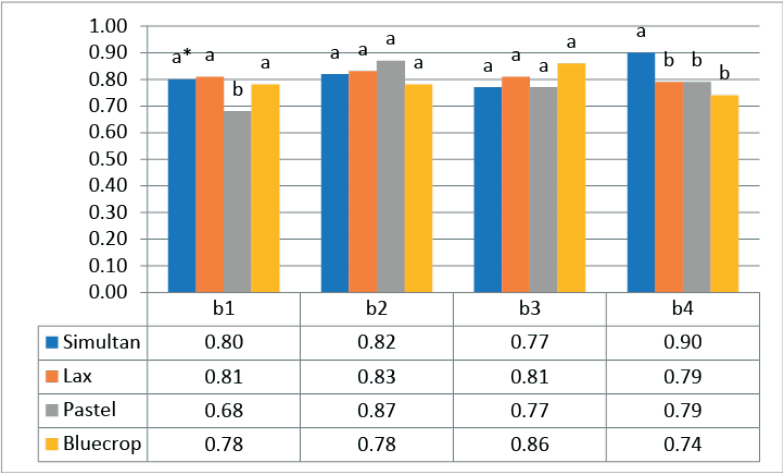
Figure 4. The influence of fertilizer on the berry diameter of blueberry cultivars

The fruit shape index values indicate that fertilization treatments influenced fruit shape, although the magnitude of variation was generally moderate across cultivars (Mota et al., 2022). For the 'Simultan' cultivar, the highest shape index was recorded in the unfertilized control (b4 - 0.90), while fertilized treatments showed slightly lower values, particularly in b3 (0.77). This suggests that fertilization tended to produce more rounded fruits, whereas the control resulted in slightly more elongated fruits. In the case of 'Lax', fruit shape index values were relatively uniform across treatments, ranging from 0.79 to 0.83, indicating a stable fruit shape that was only marginally affected by fertilization strategy. The 'Pastel' cultivar exhibited greater variability in fruit

shape index (Figure 5), with the highest value observed in b2 (0.87). This result suggests that the combined foliar and soil application of b2 influenced fruit elongation, while the lowest value was recorded in b1 (0.68). For 'Bluecrop', the maximum fruit shape index was obtained in treatment b3 (0.86), while the lowest value occurred in the unfertilized control (0.74). These findings indicate that fertilization, particularly treatment b3, contributed to a more elongated fruit shape in this cultivar. The results demonstrate that fertilization treatments had a pronounced effect on fruit firmness in all analyzed blueberry cultivars, with clear differences among treatments. For the 'Simultan' cultivar, fruit firmness was highest in treatment b1 (25.87 N) and decreased progressively

through b2 and b3, reaching the lowest value in the unfertilized control (13.05 N). These results indicate that fertilization, particularly soil application of b1, significantly enhanced fruit firmness. In the case of 'Lax', the highest firmness was observed in treatment b2 (20.52

N), followed by b1 (18.70 N) and b3 (17.53 N). The unfertilized control again showed the lowest firmness (14.22 N), suggesting a positive effect of combined foliar and soil fertilization on fruit texture.

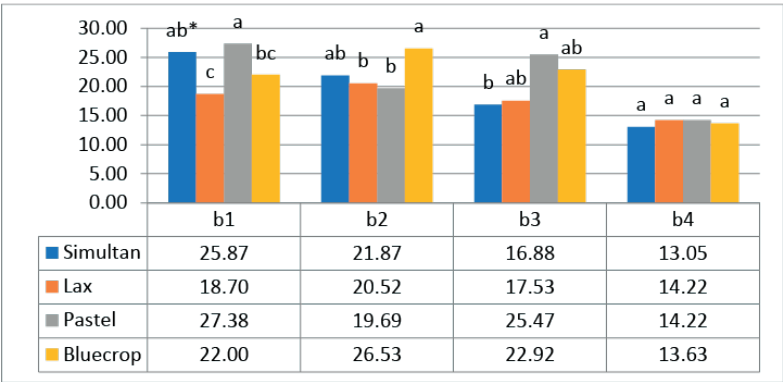


Note: *Different letters between cultivars denote significant differences (Duncan test, $p < 0.05$)

Figure 5. The influence of fertilizer on the berry shape index of blueberry cultivars

The 'Pastel' cultivar exhibited the highest firmness values overall, with maximum firmness recorded in b1 (27.38 N) and b3 (25.47 N). In contrast, the control treatment resulted in markedly lower firmness (14.22 N), emphasizing the strong influence of fertilization on fruit structural integrity in this cultivar. For

'Blueray', the greatest fruit firmness was achieved in treatment b2 (26.53 N), followed by b3 (22.92 N) and b1 (22.00 N). The unfertilized control displayed the lowest firmness (13.63 N), highlighting the importance of fertilization for maintaining fruit firmness in this cultivar (Figure 6).

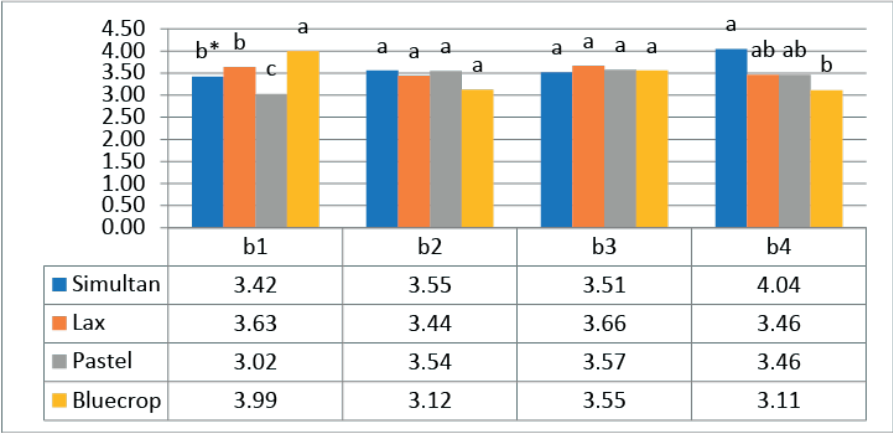


Note: *Different letters between cultivars denote significant differences (Duncan test, $p < 0.05$)

Figure 6. The influence of fertilizer on the berry firmness of blueberry cultivars

The results show variable effects of fertilization on the pH of the cellular juice across different blueberry cultivars (Figure 7). For the 'Simultan' cultivar, the highest pH of the cellular juice was recorded in the unfertilized control (4.04), while fertilized treatments showed slightly lower values, ranging between 3.42 and 3.55. This suggests that fertilization may contribute to a modest acidification of fruit juice in this cultivar. In the case of 'Lax', the pH of the cellular juice values was relatively stable across treatments, ranging from 3.44 to 3.66, with no clear trend associated with fertilization,

indicating a cultivar-specific pH regulation. The 'Pastel' cultivar exhibited slightly higher pH values in fertilized treatments (3.54 to 3.57) compared to the control (3.46), which may suggest a mild alkalizing effect of fertilization on fruit juice in this cultivar. For the 'Bluecrop' cultivar, the lowest pH was observed in the unfertilized control (3.11), with fertilized treatments showing increased pH values, particularly treatment b1 (3.99). This indicates that fertilization, especially the soil application of b1, can raise the cellular juice pH in this cultivar.

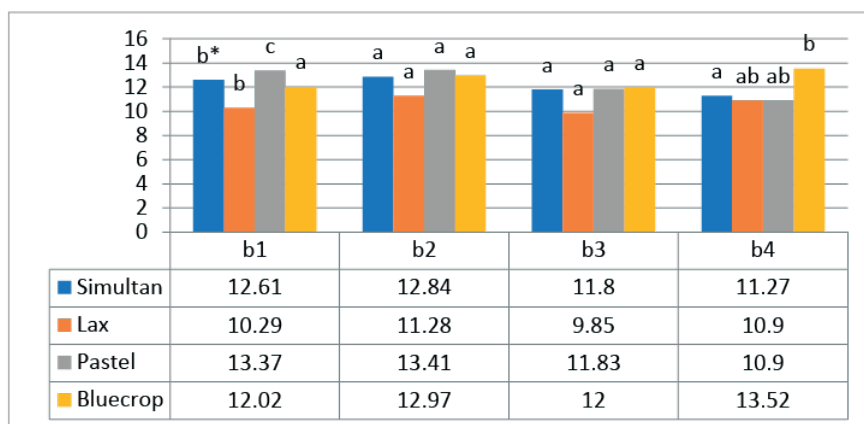


Note: *Different letters between cultivars denote significant differences (Duncan test, $p < 0.05$)

Figure 7. The influence of fertilizer on the berry pH of blueberry cultivars

The data indicate that fertilization treatments influenced the soluble solids content (SSC) of blueberry fruits, although the response varied among cultivars (Figure 8). For the 'Simultan' cultivar, the highest SSC was recorded in treatment b2 (12.84°Brix), followed closely by b1 (12.61°Brix). The lowest value was observed in the unfertilized control (11.27 °Brix), suggesting that fertilization contributed to higher sugar accumulation in the fruits. In the case of 'Lax', SSC values were moderately influenced by fertilization. The highest content was observed in b2 (11.28%), while treatment b3 resulted in the lowest SSC (9.85°Brix). The control showed intermediate values

(10.90°Brix), indicating a cultivar-specific response. The 'Pastel' cultivar exhibited the highest SSC among fertilized treatments, with maximum values in b2 (13.41°Brix) and b1 (13.37°Brix), while the control showed lower content (10.90°Brix). These results demonstrate the positive effect of fertilization, particularly b1, on fruit sugar accumulation. For 'Bluecrop', the highest SSC was recorded in the unfertilized control (13.52°Brix), while fertilized treatments ranged between 12.00°Brix and 12.97°Brix. This suggests that fertilization had a minor effect on sugar content for this cultivar, possibly due to intrinsic genetic factors (Koort, 2023).

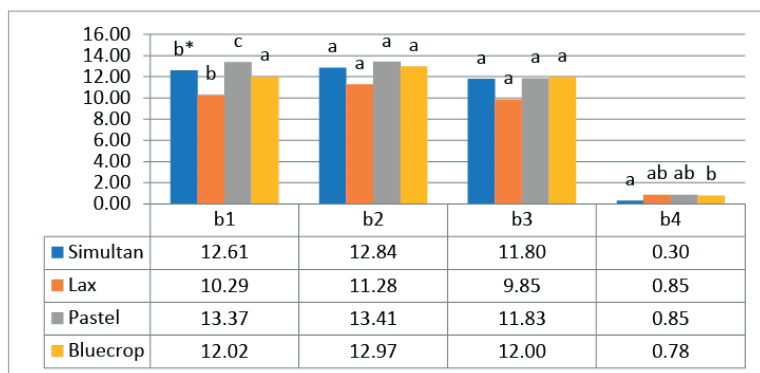


Note: *Different letters between cultivars denote significant differences (Duncan test, $p < 0.05$)

Figure 8. The influence of fertilizer on the berry total soluble solids content of blueberry cultivars

The results show that fertilization treatments had a substantial effect on fruit acidity across all blueberry cultivars. For the 'Simultan' cultivar, the highest acidity was recorded in fertilized treatments b1 (12.61%) and b2 (12.84%), while the unfertilized control showed an extremely low acidity (0.30%), suggesting that fertilization is critical for maintaining normal organic acid levels in fruits. In the case of 'Lax', fruit acidity followed a similar trend, with the highest values observed in b2 (11.28%) and b1 (10.29%), while the control (Figure 9) had very low acidity (0.85%). Fertilization clearly supports the

accumulation of organic acids in this cultivar. The 'Pastel' cultivar also exhibited the highest acidity in fertilized treatments (b2 - 13.41%, b1 - 13.37%), whereas the control showed a significant decrease (0.85%), indicating a strong influence of nutrient management on fruit acidity. For 'Bluecrop', fertilized treatments produced higher acidity levels (b2 - 12.97%, b1 - 12.02%) compared with the control (0.78%), highlighting the critical role of fertilization in maintaining optimal acid content for fruit quality.



Note: *Different letters between cultivars denote significant differences (Duncan test, $p < 0.05$)

Figure 9. The influence of fertilizer on the berry total acidity content of blueberry cultivars

CONCLUSIONS

The results of this study demonstrate that fertilizer type has a significant influence on vegeta-

tive growth yield and fruit quality of highbush blueberry (*Vaccinium corymbosum* L.), with cultivar-specific responses. The application of organic fertilizers and biostimulants signifi-

cantly increased yield per plant in all studied cultivars compared with the unfertilized control. Fertilization positively affected fruit size parameters, including average fruit weight, height, and diameter. In most cases, the highest values were obtained under treatments involving soil-applied organic fertilizers. The 'Pastel' cultivar consistently produced the largest fruits across treatments, indicating strong genetic stability for fruit size. Fruit firmness was significantly improved by fertilization in all cultivars, which is an important quality attribute for storage, transport, and marketability. In general, fertilized treatments contributed to a more balanced sugar–acid profile, although the magnitude and direction of the response varied among cultivars. While 'Simultan' and 'Pastel' showed increased soluble solids and acidity following fertilization, 'Bluecrop' exhibited smaller changes, suggesting stronger genetic control over these traits. Overall, the use of organic fertilizers and biostimulants represents an effective strategy for improving yield and fruit quality of highbush blueberry under Romanian pedoclimatic conditions. Fertilization programs should be tailored to individual cultivars in order to maximize their biological and productive potential.

ACKNOWLEDGEMENTS

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THE INFLUENCE OF PARENTS ON PULP FIRMNESS AND KEEPING THE CAPACITY IN APPLE GENOTYPES

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Abstract

The aim of the study was to evaluate the influence of parents on the expression of pulp firmness, a morpho-physiological trait essential for the quality and keeping capacity of apple fruits. The research also sought to highlight the genetic contribution of different parental combinations to both the level and stability of this trait in order to identify valuable parents for breeding programs. The results revealed a significant phenotypic variability in pulp firmness among the 40 genotypes analyzed, as well as interannual differences determined by the genotype–environment interaction. Certain parental combinations, particularly those including the parent ‘Prima’, were frequently associated with high and stable firmness values, both at harvest and after storage. Moreover, the concordance between non-destructive and destructive evaluation methods confirms the efficiency of rapid firmness assessment in genotype selection. Overall, the findings underline the determining role of genetic background in firmness expression and the importance of careful parent selection for developing varieties with stable texture and superior storage capacity.

Key words: apple, fruit firmness, parental influence, genotype-environment interaction.

INTRODUCTION

The quality of apple fruit (*Malus domestica* Borkh.) is determined by a complex of genetic, physiological, and environmental factors; among which pulp firmness, skin thickness, and the presence of the waxy layer play an essential role in mechanical resistance, tolerance to pathogens, and storage behaviour (Iancu et al., 2023). The importance of firmness as the main indicator of commercial quality and storage capacity has been emphasized in numerous specialized works. Firmness is the main technological criterion for differentiating batches intended for long-term storage from those for rapid consumption (Goncalves et al., 2017). Firmness was included as an essential parameter in the overall quality index (OQi) model, along with acidity, demonstrating a direct correlation with sensory scores for overall quality (Ahmad et al., 2021). The results of a study conducted by Djekic et al. (2019) show that the notion of quality evolves along the value chain, from production parameters to internal sensory characteristics, confirming the

importance of firmness and texture as essential elements in the consumer's perception of quality. Also, the results obtained by Hardner et al. (2016) on genetic variation and correlations between instrumental and sensory firmness support the importance of firmness as a relevant heritable character for selection and for predicting the performance of new cultivars in breeding programs. The structure and composition of the superficial tissues of the fruit, especially the cuticle, significantly influence the postharvest storage potential, acting as a barrier against water loss, chemical aggression, mechanical damage, and microbial infections (Lara et al., 2014). Existing literature indicates that cuticle properties are correlated with loss of firmness and susceptibility to physical and biological stress, highlighting its role as a determinant of quality and storage behaviour. Although the physiological mechanisms of softening have been intensively studied, the variation in firmness observed in the market remains insufficiently explained, which makes it difficult to effectively manage fruit texture in the commercial chain (Johnston et al.,

2002). In the context of climate change and increasingly stringent market requirements regarding quality and shelf life, the identification of parents capable of transmitting these traits at high frequency is becoming a priority direction in apple breeding (ECPGR, 2022; Militaru et al., 2021; Gavrilă et al., 2025). Modern breeding strategies aim to integrate quality traits with resistance to biotic and abiotic factors in order to obtain varieties adapted to new pedoclimatic conditions and the requirements of the storage and marketing chain. The study by Morariu et al. (2025) shows that genotype and growing conditions significantly influence apple production and quality, highlighting the genotype-environment interaction and the importance of integrating genetic and agronomic factors in breeding and management. Studies have shown that firmness is closely related to cell wall structure, pectin composition, and degradation, as well as the activity of enzymes involved in the ripening process (Brummell & Harpster, 2001; Johnston et al., 2002; Li et al., 2026). At the same time, the thickness of the epidermis and the characteristics of the waxy layer contribute to reducing water loss, limiting microcracks, and mechanically protecting the fruit during handling and storage (Lara et al., 2014). However, the relationships between these traits, their correlations, and genetic determinism remain insufficiently clarified, especially in the specific pedoclimatic conditions of Romania, where the genotype $\times$ environment interaction can significantly influence the phenotypic expression of quality traits. The aim of the work was to evaluate the influence of parents on the expression of pulp firmness, as a morpho-physiological character essential for the quality and keeping capacity of apple fruits. The study aimed to highlight the genetic contribution of different parental combinations to the stability and level of this character, in order to identify valuable parents for breeding programs.

MATERIALS AND METHODS

Biologic materials

The biological material studied was represented by 40 apple genotypes from the collections of

the Pitesti-Maracineni Fruit Research and Development Institute and the Voinesti Fruit Research and Development Station. The analysed varieties have diverse genetic origins, including controlled hybridization, open pollination, and induced mutagenesis, which allowed a relevant assessment of the influence of the parents on the analysed characters.

Methods

Firmness was determined using the non-destructive manual penetrometer HPE II Fff Qualitest (Qualitest Inc., Canada), equipped with a measuring device with a surface area of 0.50 cm² for apples and expressed in HPE units, but also with the destructible penetrometer and expressed in kgf/cm². The instrumental assessment was carried out after harvesting, before the apples were placed in storage, and after 30 days of storage, on apples without any skin defects. Two determinations were made for each fruit, one on the sunny side and the other on the shaded side; the chosen measurement points were contained in the same plane. The penetration resistance was read from the screen after fixing the penetrometer on each measurement point for 3 s. The values obtained for HPE and FD were classified into firmness classes (Lateur et al., 2022) (Table 1). All measurements were performed under standardized laboratory conditions to ensure comparability of values and to evaluate the influence of storage on the firmness and external quality of the fruits.

Table 1. Fruit firmness classification classes

Level of firmness	HPE (non-destructible index)	FD (kgf/cm ² , destructible)
Soft	0-60	(3-5)
Moderate	61-70	(5-6-7)
Firm	71-80	(7-8)
Very firm	> 80	(8-9)

Statistical analysis

The data obtained has been processed using software Minitab 18. The paired t-test was applied assuming a normal distribution of differences and repeated measurements on the same cultivars across years. Differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSIONS

Variability of fruit firmness

The results indicate significant differences in fruit firmness between cultivars and between years (Table 2), both for non-destructive firmness (HPE) and destructive firmness (FD), reflecting the combined effect of genotype and ripening conditions. In 2022, HPE ranged from 60 ('Estival') to 81.63 ('Dany'), and FD from 6.11 to 8.32 kgf/cm². Cultivars with lineages based on 'Prima' or 'Starkrimson' ('Starkprim', 'Dany', 'Ciprian') showed high firmness values, suggesting favourable genetic transmission of

this trait. In 2024, most varieties recorded an increase in firmness (HPE and FD), especially 'Remus', 'Colmar', 'Colonade', 'Romus 5', 'Pomona', 'Luca', 'Bistrițean', 'Voinicel', 'Inedit', 'Doina' and 'Dacian', indicating a good capacity to maintain texture after storage. Genotypes with *Malus floribunda* germplasm (e.g. 'Remus') showed marked increases in firmness, confirming the role of this genetic material in improving pulp resistance.

In contrast, some variants obtained by gamma irradiation ('Iris', 'Real', 'Remar') showed more unstable values, suggesting a greater variability of the character in the case of induced mutations.

Table 2. Morphological characteristics of the apple varieties analysed on firmness*

No.	Cultivar	Parents	HPE			FD (kgf/cm ²)		
			at harvest		postharvest (30 days)	at harvest		postharvest (30 days)
			2022	2024	2024	2022	2024	2024
1	Romus 3	interspecific hybrid F4	72.25	72.25	69.07	7.36	7.36	0.32
2	Romus 4	Romus 3 x Prima	72	72	68.82	7.34	7.34	0.32
3	Remus	Camuzat x sel. PCF 9-100 (hybrid interspecific din <i>M. floribunda</i> 821)	61	87.2	85.30	6.22	8.89	0.19
4	Rebra	Prima x Florina	74.9	74.9	73.70	7.63	7.63	0.12
5	Romus 5	Romus 3 x Prima	71.03	80.4	78.31	7.24	8.19	0.21
6	Nicol	Mc Intosh Wijcik x Pionier	64.78	64.78	62.03	6.60	6.60	0.28
7	Colmar	Mc Intosh Wijcik x Florina	64.20	76.71	75.61	6.54	7.82	0.11
8	Colonade	Florina x Mc Intosh Wijcik	65.22	75.55	74.28	6.65	7.70	0.12
9	Rustic	Florina x Pionier	74.4	74.4	72.30	7.58	7.58	0.21
10	Frumos de Voinești	Jonathan x Belle de Boskoop	75.3	73.48	56.21	7.67	7.49	0.84
11	Delicios de Voinești	Golden Delicious x Crețesc	74.76	74.71	73.23	7.62	7.61	0.15
12	Generos	(Parmain d'or x M. kaido) x (Jonathan x V53-39-2) x (Frumos de Voinești x V60-6-51)	77.77	76.08	71.81	7.93	7.75	0.43
13	Voinea	Frumos de Voinești x Prima	73.5	72.75	71.01	6.8	7.41	0.17
14	Ciprian	Prima x Starkrimson	78.21	78.48	76.7	7.49	8.00	0.18
15	Redix	Goldspur x Prima seeds (o.p.)	74.4	76.91	76.03	7.58	7.84	0.09
16	Voinicel	gamma radiation of Prima (o.p.)	69.4	75.65	71.98	7.07	7.71	0.37
17	Iris	gamma radiation of Prima seeds (o.p.)	68.67	55.76	53.54	7.00	5.68	0.25
18	Irisem	Starkrimson x Prima	67.88	70.1	58.55	6.92	7.14	1.17
19	Luca	Champion x Prima	66.57	78.81	70.1	6.78	8.03	0.88
20	Real	gamma radiation of Prima seeds (o.p.)	68.22	61.95	59.63	6.95	6.31	0.24
21	Pomona	Starkrimson x Prima	69.12	81.36	79.5	7.04	8.29	0.19
22	Discoprim	Prima x Discovery	69.50	70.83	67.13	7.08	7.22	0.37
23	Remar	gamma radiation of Prima (o.p.)	68.70	67.83	65.71	7.00	6.91	0.22
24	Inedit	gamma radiation of Prima seeds (o.p.)	68.58	77.81	74.86	6.99	7.93	0.30
25	Dacian	Unknown	68.25	79.98	75.75	6.95	8.04	0.43
26	Valery	Goldspur x Florina	72.6	76.73	74.61	7.4	7.82	0.21
27	Cezar	Prima x Starkrimson	68	77.75	77.45	6.93	7.92	0.03
28	Revidar	gamma radiation of Prima seeds (o.p.)	69.6	75.53	71.50	7.09	7.70	0.41
29	Aura	Prima x BN 33/39	74.1	75.16	66.64	7.55	7.66	0.86
30	Starkprim	Starkrimson x Prima	79.21	80.7	75.75	8.07	8.22	0.50
31	Jonaprim	Jonathan x Prima	74.76	75.65	71.74	7.62	7.71	0.39
32	Salva	Golden Delicious x Prima	74.02	77.08	71.58	7.54	7.85	0.56
33	Bistrițean	Starkrimson x Prima	71.22	81.48	70.08	7.26	8.30	1.16
34	Alex	Golden Delicious x BN33/39a	72.58	72.58	71.28	7.4	7.4	0.13
35	Dany	Jonathan x Prima	81.63	79.35	73.61	8.32	8.09	0.58
36	Doina	Jonathan x Prima	62	80.7	74.89	6.32	8.22	0.59
37	Estival	NJR55 X Sir Prize	60	60	55.8	6.11	6.11	0.43
38	Productiv de Cluj	Parmen d'or (o.p.) x NJ 46	62.3	76.6	73.90	6.35	7.81	0.27
39	Precoce de Ardeal	Feleac x Sir Prize x Prima	64.5	70.95	65.01	6.57	7.23	0.60
40	Auriu de Cluj	CJ X-5-52 a x Mutsu	67.25	73.85	70.25	6.85	7.53	0.36

*Cv=Variety/Cultivar; HPE = Non-destructible firmness; FD (kgf/cm²) = Destructible firmness

The agreement between HPE and FD supports the usefulness of non-destructive methods for rapid firmness assessment, a fact also confirmed by Harker et al. (2002), and the application of non-destructive techniques such as DA-meter (IAD index) strengthens this approach by allowing objective estimation of maturity and post-harvest behaviour (Butcaru et al., 2021); at the same time, differences between cultivars highlight the importance of genetic selection for textural stability and keeping ability. The differences between the average fruit firmness values recorded in 2022 and 2024 were statistically significant, both for the non-destructible method (HPE) and for the destructible method (FD). Higher firmness values in 2024 indicate a significant increase in fruit firmness, confirmed by both assessment methods ($p < 0.001$) (Table 3).

Table 3. Statistical analysis of fruit firmness (2022 vs. 2024)

Parameter	Mean 2022	Mean 2024	T	df	p
HPE	70.31	74.62	-3.81	39	0.00048
FD (kgf cm ⁻²)	7.14	7.60	-4.10	39	0.00020

The distribution of varieties by firmness classes shows a clear shift towards the upper classes in 2024 (Table 4), for both the non-destructive (HPE) and destructive (FD) methods. For HPE, the share of varieties in the "firm" class increased from 45% (2022) to 70% (2024), and the "moderate" class decreased from 50% to 10%; The "soft" and "very firm" classes remained poorly represented. In the case of FD, the trend is more pronounced: the "firm" varieties increased from 52.5% to 65%, and the "very firm" ones from 5% to 22.5%, while the "moderate" class decreased from 42.5% to 12.5%. Although the destructive method more clearly highlights high firmness, the overall concordance between HPE and FD confirms the usefulness of HPE as a fast and effective tool for firmness classification in quality improvement and evaluation programs.

Table 4. Percentage of varieties by firmness classes (%)

Firmness class	2022 HPE	2024 HPE	2022 FD	2024 FD
Soft (0-60)	2.5%	5%	0%	0%
Moderate (61-70)	50%	10%	42.5%	12.5%
Firm (71-80)	45%	70%	52.5%	65%
Very firm (>80)	2.5%	15%	5%	22.5%

The increase in firmness in 2024 indicates a favourable effect of climatic conditions and/or cultivation technology on fruit quality. The significant differences between years confirm that firmness is strongly influenced by the environment - temperature, water regime, thermal amplitude, and harvest time - factors that affect cell wall structure, insoluble pectin content, and cell turgor. Firmness, ripening, and dry matter content in apple are controlled by numerous QTLs, some stable in different environments (e.g. on LG10 and LG16), and others dependent on cultivation conditions, demonstrating the complex genotype-environment interaction and its impact on selection for fruit quality (Chagné et al., 2014). Recent climate change has caused long-term changes in apple quality, with decreases in firmness, acidity, and the appearance of watercore, and sometimes increases in soluble sugars, probably due to early flowering and high temperatures during ripening, and these changes affect the taste and texture of the fruit available on the market (Sugiura et al., 2013). Also, high temperatures and increased CO₂ concentrations can reduce the firmness and commercial quality of the fruit, as demonstrated in the cultivar 'Fuji' (Lee et al., 2023). However, the maintenance of consistently high values in certain cultivars highlights the existence of a favourable genetic background. Costa et al. (2010) consider that the firmness and softening of apple fruits are genetically controlled by genes involved in cell wall metabolism and ethylene biosynthesis (e.g. Md-PG1, Md-ACO1), located on chromosome 10 in QTL regions associated with postharvest firmness, highlighting the polygenic basis of the trait and the utility of marker-assisted selection in improving texture. Several studies have shown that ethylene activates the pectinase enzymes responsible for firmness degradation (Ding et al. 2024; Gwanpua et al. 2012). Ethylene plays an important role in the formation and maintenance of fruit firmness and crispness (Liu et al., 2022). The two-way ANOVA analysis for HPE firmness shows that the variability in indestructible firmness is mainly determined by genetic differences between varieties, as evidenced by the very high F value for the Variety factor ($p < 0.001$), and that the effect of year is significant ($p < 0.001$), confirming the influence of environment and/or

storage on fruit firmness. The Variety $\times$ Year interaction is also significant ($p < 0.001$), indicating that certain varieties respond differently to the conditions of the crop year, an important aspect for the selection of stable varieties (Table 5).

Table 5. Bifactorial ANOVA Analysis (Variety $\times$ Year) for HPE Firmness

Source of variation	df	SS	MS	F	p
Variety	39	4378	112.26	245.3	<0.001
Year	1	84	84	183.7	<0.001
Variety $\times$ Year	39	220	5.64	12.3	<0.001
Error	79	36	0.46	—	—
Total	158	4718	—	—	—

Bifactorial ANOVA analysis for FD firmness shows that differences between varieties are again the main determinants of destructible firmness ($p < 0.001$), and the year of study significantly influences FD ($p < 0.001$), which shows the effect of storage and climatic conditions. The interaction Variety $\times$ Year is not significant for FD ($p = 0.12$), which suggests that most varieties show a similar response between years in terms of destructible firmness (Table 6).

Table 6. Bifactorial ANOVA analysis (Variety $\times$ An) for FD firmness

Source of variation	df	SS	MS	F	p
Variety	39	150.2	3.85	210.7	<0.001
Year	1	12.5	12.5	68.4	<0.001
Variety $\times$ Year	39	9.8	0.25	1.36	0.12
Error	79	1.44	0.018	—	—
Total	158	173.0	—	—	—

For both types of firmness, ANOVA analysis indicates that genetics dominate firmness, with differences between cultivars explaining most of the variation for both HPE and FD, and the interaction Cultivar $\times$ Year is significant for HPE, highlighting the need to select cultivars that are stable over time and under different conditions. This analysis supports the use of non-destructive firmness (HPE) as a rapid indicator in breeding programs and the identification of cultivars with high and stable firmness with increased commercial value. In 2024, firmness showed a wide phenotypic variability at harvest for both HPE and FD. HPE values ranged from 55.76 (‘Iris’) to 87.20 (‘Remus’), with most genotypes falling between

70-80 units, indicating a high level of firmness. For FD, the range was 5.68 kgf/cm² (‘Iris’) - 8.89 kgf/cm² (‘Remus’). Varieties that exceeded 8 kgf/cm² (‘Remus’, ‘Pomona’, ‘Starkprim’, ‘Doina’, ‘Bistrițean’, ‘Dacian’, ‘Luca’, ‘Dany’, ‘Ciprian’) are distinguished by good resistance to handling and transportation. After 30 days of storage, HPE registered moderate decreases (generally 1-4 units), maintaining high values, especially in ‘Remus’, ‘Starkprim’ and ‘Pomona’, which suggests a favourable genetic background. In contrast, FD decreased drastically in all genotypes (0.03-1.17 kgf/cm²), with reductions of over 85-95%, reflecting the intensification of the processes of maturation and degradation of the cell wall. Cultivars such as ‘Frumos de Voinești’, ‘Irisem’, ‘Luca’ and ‘Bistrițean’ showed a higher sensitivity to storage. The results confirm the existence of significant genetic variability at harvest, but also the major influence of postharvest conditions on the maintenance of firmness, a fact also supported by other authors (Hasan et al., 2024; Argenta et al., 2024). Postharvest softening of apples is influenced by pre-, at- and postharvest factors and involves anatomical and biochemical changes, but the mechanisms that determine the variation of firmness on the market remain incompletely understood, which complicates the constant maintenance of textural quality (Johnston et al., 2002; Iancu et al., 2025a, 2025b; Istrate et al., 2025). The study conducted by Saei et al. (2011) showed that the timing of thinning does not influence firmness at harvest or after storage, but a reduced fruit load on the tree determines higher firmness; in addition, fruit size was positively correlated with firmness, and dry matter concentration was shown to be a major factor influencing firmness both at harvest and during storage. Therefore, in breeding, it is necessary to evaluate not only the initial firmness but also the postharvest behaviour to identify genotypes with high phenotypic stability.

Analysis of frequently associated parents with high firmness

The genealogical analysis of varieties consistently classified in the "firm" and "very firm" classes highlights a clear association between high firmness and the repeated presence of some parents (Table 7). Among

these, 'Prima' appears most frequently in the pedigree of varieties with high HPE and FD values (e.g. 'Ciprian', 'Starkprim', 'Pomona', 'Bistrițean', 'Luca', 'Dacian'), confirming its major role as a donor of firmness and good keeping quality, including in progeny derived from open pollination or gamma irradiation. Combinations such as 'Romus 3 × Prima', 'Golden Delicious × Prima', 'Prima × Starkrimson', 'Starkrimson × Prima', 'Goldspur × Prima (o.p.)' or 'Florina × Pionier' were associated with high firmness values (>71 HPE) in both years of study, in varieties such as 'Romus 4', 'Romus 5', 'Redix', 'Valery', 'Salva', 'Alex', 'Jonaprim', 'Rustic', 'Dany', 'Aura', 'Starkprim', 'Ciprian', 'Frumos de Voinești', 'Romus 3', 'Delicios de Voinești', 'Generos' and 'Bistrițean'. In 2024, combinations such as 'Starkrimson × Prima', 'McIntosh Wijcik × Florina', 'Prima × Florina' or 'Golden Delicious × Crețesc' maintained high values even after storage, suggesting a favourable additive effect of the parents. The presence of the variety 'Florina' (with genetic input from *Malus floribunda*) is associated with

the maintenance of firmness in varieties such as 'Rebra', 'Colmar', 'Valery' and 'Rustic'. In contrast, varieties obtained exclusively by gamma irradiation or without parents established for pulp quality ('Iris', 'Real', 'Voinicel', 'Inedit', 'Revidar') show greater variability and softening tendencies. The analysis of the frequency of the parents (for the consistently firm varieties in 2022 and 2024) shows that 'Prima' appears in approximately 30% of the combinations, followed by 'Starkprim' and 'Jonaprim' (~10%), 'Florina' and 'Golden Delicious' (~7.5%) and 'Goldspur' (~5%). These results support the polygenic nature of firmness and the importance of the choice of parents in breeding (Iancu et al., 2022), highlighting the superior value of the 'Prima' variety as a donor of stable firmness, in line with the specialized literature demonstrating the complex genetic control (QTLs on LG10 and LG16) and the role of genes involved in cell wall metabolism and ethylene biosynthesis (e.g. Md-PG1, Md-ACO1) in determining firmness and postharvest behaviour (Costa et al., 2010; Kumar et al., 2012; Su et al., 2022).

Table 7. Parents associated with high fruit firmness (varieties classified as "firm" and "very firm", based on HPE and FD)

Parent	Frequency in firm variety (%)*	Observations
Prima	~30%	Present in 12 hybrid combinations; the most frequent parent in the pedigree of varieties constantly "firm" and "very firm"; it confirms a major role in transmission of stable firmness.
Starkprim	~10%	Identified in 4 hybrid combinations; significant contribution to expressing high firmness in both years of study.
Jonaprim	~10%	Present in 4 hybrid combinations; associated with maintaining firmness both at harvest and after storage.
Florina	~7.5%	Found in 3 hybrid combinations; participates in transmitting firmness in validated parental combinations.
Golden Delicious	~7.5%	Present in 3 hybrid combinations; constant contribution to the expression of the firmness character in the offspring.
Goldspur	~5%	Identified in 2 hybrid combinations; moderate involvement in the pedigree of constantly firm varieties.

*Frequency calculated only for varieties that showed the expression "firm" and "very firm" in both study years (2022 and 2024).

Also, the parental combinations 'Prima × Starkrimson' and 'Prima × Jonathan' proved to be particularly effective, suggesting a favourable additive effect on the mechanical structure of the pulp. Therefore, the repeated use of validated parents - in particular 'Prima', but also 'Starkprim', 'Jonaprim', 'Florina' and 'Golden Delicious' - represents an effective strategy in breeding programs aimed at obtaining varieties with high firmness and good

keeping ability, adapted to current market requirements and long-term storage.

CONCLUSIONS

The data analysis indicates the existence of some varieties that, when used as parents, transmit the characteristic of pulp firmness with higher frequency. Of these, 'Prima' stands out the most, being associated with high and stable

values of firmness in the progeny. In contrast, other parents show a more variable or insignificant influence on firmness, which underlines the important role of the interaction. The differences between years highlight the major influence of environmental conditions on pulp firmness, a character known to be polygenic and strongly dependent on the genotype–environment interaction. However, the maintenance of high values of firmness in certain varieties, despite climatic variability, indicates the existence of a favourable genetic background and superior phenotypic stability.

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STATISTICAL ASSESSMENT OF ESSENTIAL TRACE ELEMENTS IN BILBERRY AND ROSEHIP FRUITS

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Abstract

*The total mineral concentration in foods does not always reflect the actual nutritional value, as only the fraction released from the food matrix during digestion becomes available for intestinal absorption. The study aimed to evaluate the total concentration, bioaccessible concentration, and bioaccessible fraction of four essential microelements - iron (Fe), manganese (Mn), zinc (Zn), and copper (Cu) - in bilberry (*Vaccinium myrtillus* L.) and rosehip fruits (*Rosa canina* L.) harvested from hilly and submontane areas of western Romania. Total concentrations were determined by atomic absorption spectrometry, and bioaccessibility was assessed using a standardized in vitro gastrointestinal digestion model. The results revealed significant differences ($p < 0.05$) between the harvesting sites for the total and bioaccessible concentrations of the analyzed elements. Rosehip fruits showed higher levels of Fe, Mn, and Zn, while bilberries showed comparable or higher levels of Cu. Iron had the lowest bioavailability and copper the highest, while Mn and Zn had intermediate values. The results suggest that rosehip fruits are a more important source of Fe, Mn, and Zn, while bilberries can contribute significantly to dietary Cu intake.*

Key words: *in vitro* digestion, bioaccessibility, *Rosa canina*, *Vaccinium myrtillus*, Duncan test for multiple comparisons.

INTRODUCTION

Blueberries and raspberries are fruits rich in antioxidants, especially vitamin C and flavonoids, which help protect the body from oxidative stress (Varzaru et al., 2024; Drózdź et al., 2018; de Souza et al., 2014). Both fruits are a good source of fiber, vitamins (B, C, and K), folic acid, fatty acids, polyphenols (anthocyanins), and minerals such as Cu, Fe, I, K, Mg, which support digestion and contribute to maintaining a healthy cardiovascular system (Pereira et al., 2018). Bilberries and blueberries (fruits of the species *Vaccinium myrtillus* and *Vaccinium corymbosum*) are rich in bioactive compounds (polyphenols, anthocyanins, vitamins, minerals, and fiber) that give them a remarkable nutritional and functional value, which is why these fruits are intensively studied for their potential benefits on human health (Wang et al., 2024). In terms of mineral composition, blueberries contain potassium,

calcium, magnesium, phosphorus, iron, manganese, and zinc - essential elements for optimal body functioning (Cabrera et al., 2021). The mineral profile of blueberries (genus *Vaccinium*), both cultivated (especially *Vaccinium corymbosum*) and wild (*Vaccinium myrtillus*) species, highlights a complex composition of essential macro- and microelements, influenced by genetic, pedoclimatic, and technological factors. For cultivated blueberries (*V. corymbosum*), Cabrera et al. (2021) reported, in eight varieties in northeastern Argentina, high concentrations of K (417,650-1073,058 mg/100 g), Ca (54,738-206,104 mg/100 g) and Mg (31,527-63,025 mg/100 g), as well as relevant levels of Fe (3,274-7,518 mg/100 g), Zn (1,245-2,761 mg/100 g), Cu (0.470-1.137 mg/100 g) and Mn (0.656-5.163 mg/100 g), concluding that blueberries are an excellent source of Mn, Fe and Cu and a valuable source of Zn, K, Mg and Ca. Pereira et al. (2025) confirmed the

predominance of potassium in the Bluecrisp variety (801 mg/kg K), along with notable contributions of Mg and Mn.

Comparisons between wild and cultivated blueberries generally indicate a higher mineral density in fruits from spontaneous flora. Drózdź et al. (2018) showed higher concentrations in wild blueberries for Ca (164-203 vs. 112-162 mg/kg), Mg (75.1-96.9 vs. 48.1-51.4 mg/kg), Mn (5.82-29.0 vs. 2.94-4.47 mg/kg) and K (880-1105 vs. 825-1031 mg/kg), as well as trace amounts of Cd, Pb, Ni and Cr, reflecting the influence of the environment. Similarly, Karlsons et al. (2018) reported higher values in *V. myrtillus* compared to *V. corymbosum* for K, Ca, Mg, Mn, and Cu, while Fe and S were slightly higher in cultivated blueberries. Gentscheva et al. (2014) and Donut et al. (2014) confirmed considerable contents of Mg, Fe, Mn, Zn, and Cu in wild blueberries, along with trace elements (Se, Mo, Cr, Ni) in low concentrations. The data provided by the USDA database for raw blueberries indicate mean values of 86 mg/100 g K, 12 mg/100 g Ca, 6.2 mg/100 g Mg, 0.34 mg/100 g Fe, 0.09 mg/100 g Zn, 0.046 mg/100 g Cu, 0.423 mg/100 g Mn, and 13 mg/100 g P, confirming the relevant contribution to K intake, Mn and Mg.

Overall, the literature supports the idea that bilberries are an important source of macroelements (especially K, Ca, Mg) and essential microelements (Mn, Fe, Zn, Cu), with a general tendency to accumulate more in wild blueberries. The variability in mineral content depends on species, variety, and environmental conditions, underscoring the need to standardize analytical methodologies and reporting units for adequate inter-study comparability. From a nutritional perspective, bilberries can be considered foods with a high mineral density, helping maintain metabolic homeostasis.

Rosehips, the fruits of the *Rosa canina* shrub (Rosaceae family), have a complex chemical composition that gives them high nutritional and functional value (Paunović et al., 2018; Kulaitiene et al., 2021). Through the diversity of classes of nutritional compounds (vitamins, carbohydrates, minerals, fiber) and bioactive compounds (carotenoids, polyphenols, terpenoids, glycosides), rosehip fruits from *Rosa canina* are distinguished as a plant raw material with high functional value, with applications in

nutrition, phytotherapy, and the food industry (Paunović et al., 2019).

The fruits of wild rosehips (*Rosa canina*) are an important source of macro- and microelements essential for human health, and their mineral content varies significantly depending on the region of origin and environmental conditions. Recent studies have highlighted a varied mineral profile of rosehips, with particular concentrations of calcium (Ca), potassium (K), magnesium (Mg), phosphorus (P), manganese (Mn), zinc (Zn), and copper (Cu). According to research by Zlateva et al. (2024), rosehip flour contains the following minerals per 100 g: 169 mg Ca, 1.06 mg Fe, 69 mg Mg, 61 mg P, 429 mg K, 4 mg Na, 0.25 mg Zn, 0.113 mg Cu, and 1.02 mg Mn. These data are supported by Milenković et al. (2024), who report significant concentrations of K (4710-9850 mg/kg), Ca (2650-5140 mg/kg), and P (532-1610 mg/kg) in *Rosa* fruits. Also, the predominant microelements in these fruits are manganese (Mn), silicon (Si), and iron (Fe), with values of 6.8-64 mg/kg, 15.2-51.6 mg/kg, and 9.9-30 mg/kg, respectively.

Studies by Paunović et al. (2019) on rosehip fruits harvested from Serbia confirmed high mineral contents in both fresh and dried fruits. In dried fruits, 348.12 mg/100 g of Ca, 566.75 mg/100 g of K, and 135.61 mg/100 g of Mg were identified. Also, the comparative analysis carried out by Ivanova et al. (2023) between wild and those cultivated in Bulgaria highlighted the significant differences in the concentrations of P, K, Na, and Mg, with wild rosehips having values higher than P (0.415%), K (1039.56 mg/kg), and Mg (2331.7 mg/kg). The mineral composition of rosehips also varies across genotypes. For example, research by Benković-Lacić et al. (2022) on different genotypes of wild rosehips in Croatia showed variable values of essential minerals such as P (1284-1848 mg/kg), Na (22.9-91.1 mg/kg), Mg (1410-2587 mg/kg), K (9386-18383 mg/kg), and Ca (2856-8934 mg/kg). Similarly, recent studies by (Kulaitiene et al., 2021) and Igual et al. (2022) on freeze-dried rosehip powders reported significant mineral contents such as K (2630.46 mg/100 g), Ca (590.33 mg/100 g), P (690.81 mg/100 g), Mg (340.66 mg/100 g), Fe (7.52 mg/100 g), Zn (2.55 mg/100 g) and Mn (3.19 mg/100 g), thus strengthening their nutritional

value. Peña et al. (2023) also pointed out that, compared to other berries, rosehips have a higher content of essential minerals, such as Ca (1.69 mg/g), P (0.61 mg/g), K (4.29 mg/g), Mg (0.69 mg/g), Zn (0.25 mg/g), Cu (0.113 mg/g) and Mn (1.02 mg/g), thus emphasizing their importance in the daily diet.

In conclusion, wild rosehips are an important source of minerals, with their mineral content influenced by geographical and genetic factors. These fruits can significantly contribute to meeting daily requirements for macro- and microelements, with a higher nutritional potential than other berries (Pereira et al., 2018). Further studies on the mineral profile of rosehips from different regions of the world are essential for understanding their variability and for their application in human nutrition.

From the above, it can be stated that wild blueberries and rosehips contain important amounts of mineral elements, including iron, manganese, copper, and zinc, essential microelements in the homeostasis of the human body. Acting as cofactors of enzymes involved in fundamental biological processes, such as cellular respiration, antioxidant protection, protein biosynthesis, and the regulation of immune functions (Pereira et al., 2018). Since the total essential/toxic content of a food does not reflect the beneficial/toxic effects after consumption, it is very important to assess its bioaccessible and bioavailable fractions (Pereira et al., 2018; Ramírez-Ojeda et al., 2017).

In this case, the term bioaccessibility refers to the fraction of a given microelement released from the fruit matrix after ingestion and solubilized in the intestinal lumen (Mingroni et al., 2019; Pereira et al., 2018). An accepted definition of bioavailability is the amount of a nutrient available for normal metabolic and physiological processes, i.e., the amount that becomes available for absorption in the small intestine (Affonfere et al., 2023). The bioaccessibility of a given element can be measured through several experimental models, such as *in vitro* digestion of homogenized foods in a closed system and determination of the soluble nutrient fraction, *in vitro* digestion and dialyzability of soluble nutrients through a semipermeable membrane, or the use of human colorectal adenocarcinoma (Caco-2) cells to mimic many of the characteristics of thin

intestinal cells (Mingroni et al., 2019). This study aims to evaluate the bioaccessibility of the microelements Fe, Mn, Zn, and Cu from wild blueberries and rosehips harvested from the spontaneous flora of hilly and submontane areas in the west of the country.

MATERIALS AND METHODS

Materials

The materials used in this experiment are wild fruits, namely rosehips (*Rosa canina*) and bilberries (*Vaccinium myrtillus*), from the spontaneous flora of a hilly and submontane area in western Romania. Approximately 1 kg of fruit was collected from three different areas, each sample originating from a distinct collector (Provider 1, Provider 2, and Provider 3). Homogeneous samples were then prepared from these batches for analysis. Fruits were cleaned of vegetable residues, washed under running water, rinsed with distilled water, and drained of moisture. They were then dried and ground into a powder. The drying operation was performed in an air-drying oven at 50°C until a constant mass was reached, and the dry samples were ground in a kitchen grinder. Until the moment of analysis, the samples were kept in brown glass bottles at cold temperatures.

Total mineral concentration determination

Total concentrations of four essential trace elements - iron (Fe), manganese (Mn), zinc (Zn), and copper (Cu) - were determined by atomic absorption spectroscopy (AAS), using the calcination method followed by ash solubilization and measurement of element absorbance in the acidic solution, according to the procedure described by Velciov et al. (2024). Samples were calcined at 500°C in two successive stages of 4 hours each. Measurements were performed using a Varian AA-240 FS (Fast Sequential) atomic absorption spectrometer. All determinations were carried out in triplicate, and results were expressed as the mean $\pm$ standard deviation.

Bioaccessible mineral concentration determination

To evaluate the fraction of minerals potentially available for intestinal absorption, the bioaccessible concentrations of Fe, Mn, Zn, and Cu in rosehip and bilberries samples were

determined using an *in vitro* gastrointestinal digestion model described by Souza et al. (2023). Briefly, 300 mg of each sample was subjected to a standardized two-phase *in vitro* digestion procedure simulating gastric and intestinal conditions.

In the gastric phase, 4.5 mL of salivary solution (pH 6.5 ± 0.5) and 6.75 mL of gastric solution (pH 1.1 ± 0.1) were added. The pH was adjusted to 1.20 ± 0.05 with concentrated HCl, and the mixture was incubated for 1 h at 37°C under agitation (100 rpm). For the intestinal phase, 13.5 mL of duodenal solution and 4.5 mL of bile solution were added, and the pH was adjusted to 6.3 ± 0.5 using 1 mol/L NaOH. Incubation continued for 4 h at 37°C under constant shaking (100 rpm). After centrifugation (3000 rpm, 5 min), the supernatant was collected as the bioaccessible fraction.

Concentrations of Fe, Mn, Zn, and Cu were determined by the same AAS method. Bioaccessibility, specifically the bioaccessible fraction (BF, %), was calculated as: $BF (\%) = (MBF/TMC) \times 100$, where MBF represents the mineral concentration in the bioaccessible fraction (mg/100 g, dry weight) and TMC is the total mineral content (mg/100 g, dry weight). All analyses were performed in triplicate.

Statistical Analysis

The mathematical rigor and statistical evaluation of the experimental data were conducted to

ensure the highest reliability of the results. Before performing the analysis of variance (ANOVA), the normality of the data was confirmed using the Shapiro-Wilk test, and the homogeneity of variances was assessed using Levene's test. Following validation of these premises, a one-way ANOVA was performed to assess differences between harvesting sites (providers). When significant differences were detected ($p < 0.05$), the Duncan multiple-range test was used for post hoc pairwise comparisons. All statistical procedures were performed using the R software environment (R Core Team), specifically utilizing the agricolae package for agricultural and biological research data analysis.

RESULTS AND DISCUSSIONS

Before performing the ANOVA, the normality of the data was confirmed using the Shapiro-Wilk test, and the homogeneity of variances was assessed using Levene's test, thereby ensuring a robust mathematical foundation for the data. The extremely low standard deviations highlight the precision of the measurements.

The results obtained for determining the total and bioaccessible concentrations of microelements, as well as the accessible fractions of these microelements in the analyzed bilberries and rosehips, are presented in Tables 1, 2, and 3.

Table 1. Total and bioaccessible concentration (mg /100g DM) of Fe, Mn, Zn, and Cu in bilberry fruits

Fruit origin	Total concentrations				Bioaccessible concentrations			
	Fe	Mn	Zn	Cu	Fe	Mn	Zn	Cu
Provider 1	2.1814 ± 0.07d	1.6219 ± 0.08f	0.8609 ± 0.05d	0.8932 ± 0.03a	0.2142 ± 0.02g	0.4155 ± 0.02i	0.2167 ± 0.02f	0.4262 ± 0.02d
Provider 2	1.8062 ± 0.07e	2.0567 ± 0.05d	0.8913 ± 0.04cd	0.5717 ± 0.33c	0.1694 ± 0.02g	0.5457 ± 0.02i	0.2240 ± 0.02f	0.2936 ± 0.02f
Provider 3	2.2289 ± 0.09d	1.8704 ± 0.05e	1.1104 ± 0.03c	0.6329 ± 3.02b	0.2175 ± 0.02g	0.4878 ± 0.02i	0.2783 ± 0.03f	0.3039 ± 0.02f

The distinct letters in each column indicate statistically significant differences between the samples ($p < 0.05$) according to the Duncan test.

Table 2. Total and bioaccessible concentrations (mg/100g DM) of Fe, Mn, Zn, and Cu in wild rosehip fruits

Fruit origin	Total concentrations				Bioaccessible concentrations			
	Fe	Mn	Zn	Cu	Fe	Mn	Zn	Cu
Provider 1	2.8728 ± 0.09a	3.1155 ± 0.08c	2.7934 ± 0.07b	0.3707 ± 0.01e	0.3453 ± 0.03f	0.8759 ± 0.09h	0.5967 ± 0.30e	0.1802 ± 0.02g
Provider 2	3.1158 ± 0.08b	3.8645 ± 0.06a	2.6081 ± 0.19 ^b	0.4504 ± 0.02d	0.3649 ± 0.04f	1.1157 ± 0.07g	0.5670 ± 0.05e	0.2213 ± 0.03g
Provider 3	3.2426 ± 0.06c	3.7092 ± 0.08b	3.3181 ± 0.19a	0.3629 ± 0.01e	0.3839 ± 0.03f	1.0649 ± 0.09g	0.7313 ± 0.05de	0.1759 ± 0.02g

The distinct letters in each column indicate statistically significant differences between the samples ($p < 0.05$) according to the Duncan test.

Table 3. The bioaccessible fraction of Fe, Mn, Zn, and Cu in bilberry and rosehip fruits

Fruit origin	Bioaccessible fractions (%)							
	Bilberry fruits				Rose hip fruits			
	Fe	Mn	Zn	Cu	Fe	Mn	Zn	Cu
Provider 1	9.81± 0.11b	25.73± 0.13a	25.31± 0.10a	48.71± 0.09a	12.00± 0.12a	28.20± 0.10a	22.11± 0.11a	47.85± 0.12a
Provider 2	9.39± 0.09b	26.56± 0.11a	25.19± 0.10a	49.10± 0.10a	11.68± 0.10a	28.83± 0.08a	21.85± 0.10a	48.30± 0.11a
Provider 3	9.74± 0.08b	26.09± 0.10a	25.13± 0.11a	48.50± 0.11a	11.83± 0.10a	28.75± 0.12a	21.33± 0.10a	48.04± 0.11a

The distinct letters in each column indicate statistically significant differences between the samples ($p < 0.05$) according to the Duncan test.

The results presented in Table 1 show variations in the total and bioaccessible concentrations of Fe, Mn, Zn, and Cu in analyzed bilberries.

Regarding the total concentrations, significant differences are observed for all the microelements investigated. The total Fe content was higher in the samples from Provider 3 (2.2289 mg/100 g DM) and Provider 1 (2.1814 mg/100 g DM) than in Provider 2 (1.8062 mg/100 g DM), the differences being statistically significant ($p < 0.05$). For Mn, the highest total concentration was recorded in Provider 2 (2.0567 mg/100 g DM), followed by Provider 3 (1.8704 mg/100 g DM) and Provider 1 (1.6219 mg/100 g DM), each value differing significantly from the others. In the case of Zn, the blueberries from Provider 3 showed a significantly higher content (1.1104 mg/100 g DM) compared to the other samples, while for Cu, the highest total concentration was observed in Provider 1 (0.8932 mg/100 g DM); the differences compared to the other suppliers were also significant ($p < 0.05$).

The analysis of bioaccessible concentrations shows considerably lower values than the total concentrations; however, even in this case, statistically significant differences are found between the samples.

For Fe, the bioaccessible fraction was relatively low for all providers (9.39-9.81% of total content), with minor but significant differences ($p < 0.05$). In the case of Mn, the highest bioaccessible value was observed in Provider 2 (0.5457 mg/100 g DM), followed by Providers 3 and 1, with significant differences between samples. Zn showed the highest bioaccessible concentration in blueberries from Provider 3 (0.2783 mg/100 g DM), while for Cu, the bioaccessible values were highest at Provider 1 (0.4262 mg/100 g DM), suggesting a relatively

higher proportion of this element available for absorption.

These results show that the origin of bilberries significantly influences both the total content and the bioaccessible fraction of the microelements analyzed ($p < 0.05$). It is also noted that the assessment of the nutritional value of fruits should not be based solely on total mineral concentration, but also on the bioaccessible fraction, which better reflects absorption potential at the gastrointestinal level. By correlating the results for total and bioaccessible concentrations (Table 1) with the bioaccessible fraction values (Table 3), an integrated interpretation of the nutritional potential of the analysed bilberries can be achieved.

The data confirm that although there are statistically significant differences ($p < 0.05$) between suppliers in total and bioaccessible concentrations of micronutrients, the proportion of the potential element available for absorption (the bioaccessible fraction) varies by element.

For Fe, the bioaccessible fraction is relatively small and comparable across samples, at 9.81% (Provider 1), 9.39% (Provider 2), and 9.74% (Provider 3). The statistical calculation indicates the absence of statistically significant differences between providers ($p < 0.05$). These results are consistent with low bioaccessible concentrations (Table 1) and suggest limited Fe availability, probably due to interactions with phenolic compounds or dietary fibres in the fruit matrix.

For Mn, the bioaccessible fraction is considerably higher, ranging from 25.73% to 26.56%, with no significant differences between providers. Although the total concentrations differ significantly (Table 1), the proportion available for absorption remains relatively

constant, indicating a stability of the digestive behavior of this element in the blueberry matrix. A similar profile is observed for Zn, where the bioaccessible fraction varies very little (25.1265–25.31%), with no statistically significant differences ($p < 0.05$). Thus, although Provider 3 showed the highest total and bioaccessible Zn concentrations (Table 1), the proportion of the total that becomes bioaccessible is similar across all samples.

In contrast, Cu has the highest bioaccessible fraction among the microelements analyzed, ranging from 48.50% to 49.10%, with no significant differences between suppliers. These results confirm the observations in Table 1, where Cu recorded relatively high bioaccessible values compared to the total content, suggesting a chemical form more favourable to solubilisation and potential intestinal absorption.

Overall, the data show that although the origin of bilberries significantly influences the total and bioaccessible concentrations of microelements ($p < 0.05$), the bioaccessible fraction is relatively constant for Mn, Zn, and Cu, with no significant differences between suppliers. Iron remains the element with the lowest percentage bioaccessibility (approximate values: 9–10%), while copper has the highest relative availability (approximate values: 48–49%).

These results underline the importance of the simultaneous assessment of the total content, the bioaccessible concentration, and the bioaccessible fraction for a correct assessment of the nutritional value of bilberries, demonstrating that the potential mineral intake depends not only on the total level of the element, but also on the efficiency of its release under simulated gastrointestinal conditions.

The results presented in Table 2 show significant variations in the total and bioaccessible concentrations of Fe, Mn, Zn, and Cu in wild rosehips from the three suppliers analyzed. The statistical scale of the mean values in each column indicates statistically significant differences between the samples ($p < 0.05$), confirming the influence of origin on the mineral composition of the fruits.

Regarding the total concentrations, significant differences are observed for all the microelements investigated. The total Fe

content progressively increases from Provider 1 (2.8728 mg/100 g DM) to Provider 2 (3.1158 mg/100 g DM) and Provider 3 (3.2426 mg/100 g DM), with each value being significantly different (letters in distinct colors, $p < 0.05$). For Mn, the highest total concentration was recorded in Provider 2 (3.8645 mg/100 g DM), followed by Provider 3 (3.7092 mg/100 g DM) and Provider 1 (3.1155 mg/100 g DM), with the differences also being statistically significant.

In the case of Zn, the rosehips from Provider 3 presented the highest total content (3.3181 mg/100 g DM), significantly higher than the other samples ($p < 0.05$), while for Cu, the maximum value was recorded in Provider 2 (0.4504 mg/100 g DM), differing significantly from the other two samples, which do not differ from each other (same letter).

The analysis of bioaccessible concentrations also reveals statistically significant differences between providers ($p < 0.05$). For Fe, bioaccessible values increase from Provider 1 (0.3453 mg/100 g DM) to Provider 3 (0.3839 mg/100 g DM), with the difference being significant. In the case of Mn, the highest bioaccessible concentration is found in Provider 2 (1.1157 mg/100 g DM), followed by Provider 3 (1.0649 mg/100 g DM) and Provider 1 (0.8759 mg/100 g DM), all differences being statistically significant.

For Zn, Provider 3 has the highest bioaccessible value (0.7313 mg/100 g DM), significantly higher than the other providers, whereas for Cu, there are no significant differences between the samples, suggesting a relatively constant availability of this element regardless of origin. Overall, the results demonstrate that the origin of wild rosehips significantly influences the total and bioaccessible concentrations of most microelements analyzed ($p < 0.05$). It is noted that Mn and Zn have relatively high total and bioaccessible levels compared to Fe and Cu, indicating a potentially important contribution of rosehips to the dietary intake of essential microelements. At the same time, the statistical differences highlighted underline the importance of pedoclimatic factors and growing conditions in determining the mineral profile of wild fruits.

The correlation between the results for total and bioaccessible concentrations of trace elements (Table 2) and the bioaccessible fraction values

presented in Table 3 allows a more complete assessment of the nutritional potential of the wild rosehips analyzed.

As previously highlighted, the total and bioaccessible concentrations of Fe, Mn, Zn, and Cu differ significantly between suppliers ($p < 0.05$), as indicated by the distinct letters in each column of Table 2.

The analysis of the bioaccessible fraction (Table 3) indicates that, for all the microelements investigated, there is an absence of statistically significant differences between suppliers ($p < 0.05$).

For iron (Fe), the bioaccessible fraction ranges from 11.68% (Provider 2) to 12.00% (Provider 1), with an intermediate value at Provider 3 (11.83%). Although total and bioaccessible concentrations differ significantly between samples, the proportion of total iron that becomes bioaccessible remains relatively constant, ranging from 11.68% to 12.00%.

For Mn, the bioaccessible fraction is higher than for Fe, ranging from 28.20% to 28.83%. The highest value is recorded in Provider 2 (28.83%), but the differences are not statistically significant ($p < 0.05$). This constancy suggests a similar digestive behavior of Mn in the rosehip matrix, regardless of variations in total content. For zinc (Zn), the bioaccessible fraction ranges from 21.33% (Provider 3) to 22.11% (Provider 1), with no significant differences between providers. Although Provider 3 showed the highest total and bioaccessible Zn concentrations (Table 2), the proportion available for absorption was comparable across all samples.

Copper has the highest bioaccessible fraction among the analysed microelements, with values ranging from 47.85% to 48.30%. Here, too, the lack of statistically significant differences indicates that almost half of the total Cu content becomes potentially available for uptake, independent of changes in the total concentration.

Overall, the results show that although the origin of rosehips significantly influences the total and bioaccessible levels of microelements ($p < 0.05$), the bioaccessible fraction is relatively constant for all elements analyzed. Iron has the lowest bioaccessible proportion, zinc and manganese have intermediate values, and copper has the highest.

These findings underline that the potential bioavailability of microelements in rosehips is determined not only by the total concentration, but also by the efficiency of their release under simulated gastrointestinal conditions, and the relatively constant proportion of the bioaccessible fraction suggests a stable behavior of these elements in the fruit matrix, regardless of provenance.

The comparative analysis of the total concentrations, bioaccessible concentrations, and bioaccessible fractions of the microelements Fe, Mn, Zn, and Cu highlights relevant differences between bilberries and wild rosehips, both quantitatively and in terms of bioavailability potential. In terms of total concentrations, rosehips stand out for superior values for most of the elements analyzed. Thus, the total Fe content is higher in rosehips (2.8728-3.2426 mg/100 g DM) compared to bilberries (1.8062-2.2289 mg/100 g DM). Similar differences are observed for Mn (3.1155-3.8645 mg/100 g DM in rosehips versus 1.6219-2.0567 mg/100 g DM in bilberries) and Zn (2.6081-3.3181 mg/100 g DM in rosehips compared to 0.8609-1.1104 mg/100 g DM in bilberries). On the other hand, for Cu, bilberries show comparable or even higher total values (0.5717-0.8932 mg/100 g DM) compared to rosehips (0.3629-0.4504 mg/100 g DM). In both types of fruit, the distinct letters within each column indicate statistically significant differences between suppliers ($p < 0.05$), highlighting the influence of origin on mineral accumulation.

The trends observed in total concentrations are also reflected in bioaccessible concentrations. Rosehips show higher bioaccessible values for Fe (0.3453-0.3839 mg/100 g DM vs. 0.1694-0.2175 mg/100 g DM in blueberries), Mn (0.8759-1.1157 mg/100 g DM vs. 0.4155-0.5457 mg/100 g DM) and Zn (0.5670-0.7313 mg/100 g DM vs. 0.2167-0.2783 mg/100 g DM). For Cu, the situation is different, with bilberries registering comparable or higher bioaccessible concentration values (0.2936-0.4262 mg/100 g DM) than rosehips (0.1759-0.2213 mg/100 g DM). Again, the differences between suppliers are statistically significant for most elements ($p < 0.05$), unless the same letter indicates no significant difference.

The comparative analysis of the bioaccessibility of the microelements Fe, Mn, Zn, and Cu in bilberry and rosehip fruit powders shows that the specific plant matrix of each fruit influences mineral release under gastrointestinal conditions to a greater extent than variations in sample origin. Both bilberries and rosehips are characterized by a high content of phenolic compounds (anthocyanins, flavonols, condensed tannins, and phenolic acids), as well as a significant intake of dietary fiber and pectins, both of which are known to interact with minerals during *in vitro* digestion. The low iron bioaccessibility values (9.39-12.00%) can be attributed to the formation of stable complexes between Fe and phenolic compounds, which reduce its solubility in the intestinal phase. Similarly, zinc showed moderate bioaccessibility (21.33-25.31%), consistent with its susceptibility to polyphenolic chelation. Manganese showed intermediate bioaccessibility (25.73-28.83%), possibly influenced mainly by interactions with fibers and the plant matrix rather than by specific inhibitors such as phytate, which is virtually absent in these fruits. In contrast, copper showed the highest bioaccessibility (47.85-49.10%), which can be explained by its ability to form more soluble complexes with organic ligands in the fruit matrix.

In the case of rosehips, the high ascorbic acid content could help maintain iron in a more soluble form, explaining the slightly higher values compared to blueberries; however, the favourable effect of vitamin C is probably partially counterbalanced by the high polyphenol concentrations. Overall, the bioaccessibility profile obtained is consistent with the data reported for other berries and reflects the decisive influence of phytochemical composition on the potential availability of microelements.

As regards the bioaccessible fraction, expressed as a percentage, there is a relatively constant behavior across suppliers for each type of fruit ($p < 0.05$), suggesting that the fruit-specific matrix influences the proportion released to a greater extent than variations in origin. Compared between species, rosehips have a slightly higher fraction for Fe (values between 11.68-12.00%) compared to blueberries (values between 9.39-9.81%) and for Mn (values

between 28.20–28.83% compared to 25.73-26.56%). For Zn, blueberries register a slightly higher fraction (values between 25.13-25.31%) compared to rosehips (values between 21.33-22.11%). In the case of Cu, both fruits have the highest bioaccessible fraction (approximate values, between 47.85-49.10%), with no notable differences between them.

Overall, wild rosehips stand out for their superior total and bioaccessible Fe, Mn, and Zn content, suggesting a higher nutritional potential for these microelements. Bilberries, on the other hand, can be an important source of Cu, both in total and bioaccessible concentrations. At the same time, the relative consistency of the bioaccessible fraction for each element indicates that the peculiarities of the plant matrix mainly determine the efficiency of release under gastrointestinal conditions.

Therefore, in order to reflect as accurately as possible the real contribution of bilberries and rosehips to dietary mineral intake, it is necessary to comparatively assess the value of both the total level of trace elements and the concentration and bioaccessible fraction.

The results obtained are consistent with the literature, which reports variability in the mineral content of wild fruits across species and pedoclimatic conditions (Cabrera et al., 2021; Gentscheva et al., 2014). The higher Fe, Mn, and Zn levels in rosehips compared to bilberries confirm the observations of other researchers, who describe rosehips as an important source of essential microelements (Peña et al., 2023). Also, the Cu concentrations in bilberries fall within the previously reported ranges for this type of fruit.

The low bioaccessible fractions for Fe and higher values for Cu are also in agreement with studies showing limited bioavailability of iron in the plant matrix and higher solubility of copper under simulated gastrointestinal conditions (de Lima et al., 2014; Cai et al., 2017). Moderate values for Mn and Zn also support the trends described in the literature (de Lima et al., 2014; Cai et al., 2017).

Overall, wild rosehips stand out for their superior total and bioaccessible Fe, Mn, and Zn content, suggesting a higher nutritional potential for these microelements. Bilberries, on the other hand, can be an important source of Cu, both in total and bioaccessible concentrations.

Therefore, in order to reflect as accurately as possible the real contribution of bilberries and rosehips to the dietary mineral intake, it is necessary to comparatively assess the value of both the total level of trace elements, the concentration, and the bioaccessible fraction.

CONCLUSIONS

The results obtained indicate statistically significant differences ($p < 0.05$) between suppliers in the total and bioaccessible concentrations of the microelements analyzed, both in wild blueberries and in wild rosehips. Overall, rosehips show higher total and bioaccessible concentrations of Fe, Mn, and Zn than blueberries, suggesting a superior nutritional potential for these elements. However, blueberries show comparable or even higher Cu values, both at the total level and in bioaccessible levels.

Bioaccessible concentrations generally follow the same trend as the total ones but represent only a fraction of the original mineral content, underlining the importance of assessing potential bioavailability. The bioaccessible fraction was relatively constant between suppliers for each type of fruit, indicating a determining role of the plant matrix on the release of elements under simulated gastrointestinal conditions. Iron had the lowest bioaccessible fraction, while copper showed the highest release percentages in both types of fruit.

From an applied perspective, these findings suggest that the formulation of dietary supplements or functional foods could strategically combine wild fruits to achieve an optimized, highly bioaccessible mineral profile, harnessing Fe, Mn, and Zn from rosehips and Cu from blueberries. The integration of data on total concentration, bioaccessible concentration, and bioaccessible fraction provides a complete picture of the true nutritional value of these wild fruits.

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TRENDS IN LATE FROST DAMAGE PROBABILITY FOR PLUM AND SWEET CHERRY IN ROMANIA: A COMPARATIVE ANALYSIS OF 2013-2022 VS. 2000-2009

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Abstract

Late frost damage probability (LFDP) was analyzed in Romania for plum and sweet cherry by comparing the decades 2013-2022 and 2000-2009, using a patented warning method for late-frost damage in orchards based on phenoclimatic simulation. An uneven spatial gradient of LFDP tendency was observed, distributed between the southern half of the country (from -1 to 1.5% for plum and from -1 to 3% for sweet cherry) and the submontane depressions located in the eastern part of Transylvania (7.5-9%). Although in plum the areas with almost unchanged LFDP occupied the south, east, and partially the west of Romania's territory, for sweet cherry, they were restricted to the south-western part of the country and the coastal area. The increase in the critical minimum temperature exerted an overriding influence on the increase in late frost damage probability for sweet cherry and plum, compared with the phenological advance. The study analyzes the circumstances that led to the increasing LFDP for plum and sweet cherry in the 2013-2022 decade and identifies the areas with increased late frost damage frequency and intensity.

Key words: climatic accidents, 'Tuleu gras' and 'Germersdorf' cvs., bud swelling, budburst, beginning of flowering, end of flowering.

INTRODUCTION

In recent decades, scientific interest in late-spring frost has increased in a warming climate context, being associated with phenological advance in species such as apple, pear (Chitu & Paltineanu, 2020), sour cherry (Paltineanu & Chitu, 2020; Mazilu et al., 2025a), sweet cherry, peach, apricot (Jakab-Ilyefalvi et al., 2021; Mazilu et al., 2025b), and plum (Chitu et al., 2008; Butac et al., 2025). In this context, the variability of minimum temperatures during spring has increased and consequently, repeated frost episodes have been reported, while the probability of their overlap with sensitive vegetation stages may even rise (Vitasse et al., 2018; Vitasse & Rebetez, 2018). These situations frequently occur during so-called "false springs", when early warm periods are followed by cold air advections, a situation frequently observed in Europe (Zohner et al., 2020).

Two of the main drivers of European climate variability are the North Atlantic Oscillation

(NAO) and the Arctic Oscillation (AO) (Hurrell et al., 2003; Wypych et al., 2017). NAO regulates the intensity of the western circulation over Europe (Trigo et al., 2002; Hurrell et al., 2003), influencing either the transport of milder oceanic air during its positive phase or the intrusion of cold air towards Central and Eastern Europe during its negative phase (Trigo et al., 2002; Chamberlain et al., 2021). AO reflects the stability of the polar vortex (Thompson & Wallace, 1998; Cohen et al., 2014) and the degree to which cold Arctic air remains at high latitudes or may extend over the mid-latitudes in Europe (Cohen et al., 2014; Wypych et al., 2017).

In addition to these mechanisms, spring late frosts are often associated with atmospheric blockages. These blockages, such as the Scandinavian type, favor the cold-air intrusion from northern or northeastern regions into Central and Eastern Europe (Brunner et al., 2017; Kautz et al., 2022). In Romania, the effects of these mechanisms are influenced by the country's position within the intersection of

several regional climate influences (Caian et al., 2019). The eastern and northeastern parts have a more continental character, with larger thermal amplitudes and more frequent cold-air advection (Caian et al., 2019), conditions that favor the occurrence of late spring frost.

Oceanic influences are mainly felt in the west and central region, generally associated with higher temperatures and increased precipitation (Caian et al., 2019), reducing the radiative frost events frequency and intensity. Warm and moist airflows can cross the Southern Carpathians. In the south, they are often associated with higher minimum temperatures and fewer frost events (Dima, 2014). In the southwest, Sub-Mediterranean influences are associated with warmer, cloudier, or rainy conditions, which tend to limit nocturnal radiative cooling and, in turn, reduce the intensity of late frosts. In the southeast, the Pontic influence moderates thermal amplitudes. Frost events are therefore generally less frequent and less severe, as nighttime temperatures remain higher (Bosneagu et al., 2022; Manea & Grigorescu, 2024). Last but not least, the complex relief of the Carpathians strongly influences the distribution of late frosts, acting both as a barrier to the circulation of air masses and as a factor in their redistribution. Within and at the contact with the Carpathian arch, favorable conditions for the accumulation of cold air frequently occur in depressions and at the valley bottoms, where persistent thermal inversions and cold-air pooling episodes can occur (Rusz, 2015; Micu et al., 2016). The advected cold air may become trapped/stagnant, strengthening local frosts, especially in depressions (as in the Transylvanian Plateau) and the Subcarpathian sector. Recent frost episodes, as in the springs of 2017, 2020 (Mazilu et al., 2025b), and 2025, illustrate how Romania's climatic and aims to establish the main contributing factors to the increased late frost damage, geographical particularities can favor severe late frost episodes in fruit-growing regions. Given that these events are difficult to predict, especially over the long term, the present study (LFDP) for plum and sweet cherry during the 2013-2022 decade, compared with the previous 2000-2009 interval, identifies the regions where late frost damage has become more frequent and intense.

MATERIALS AND METHODS

Phenological data and phenophase probabilities. This study used phenological observations made for sweet cherry ('Germersdorf' cv.) and plum ('Tuleu gras' cv.), which differ in phenological timing, with sweet cherry reaching sensitive stages earlier than plum. The choice of the cultivars was motivated by the availability of long-term phenological data (49 years), which is essential for the development and validation of phenological models. Both cultivars are traditional and have been widely cultivated in Romania and, therefore, are representative of pedoclimatic conditions in the main fruit-growing regions. Although they do not fully represent the actual varietal structure, they provide a robust dataset to analyze the climate-phenology relationships and the impact. Phenological stages, bud swelling (51 BBCH), bud burst (53 BBCH), beginning of flowering (61 BBCH), and end of flowering (69 BBCH), were recorded according to the BBCH scale described by Meier (2018). A multiannual minimum, maximum, and average daily temperatures (together with their standard deviations) database for 28 agrometeorological stations in areas representative of fruit growing in Romania was also used to calculate the occurrence date/probability of each of the four phenological phases using the mathematical model and the methodology proposed by Chitu & Paltineanu (2006) and Chitu et al. (2008; 2011). The study focused on 2000-2009 and 2013-2022 periods, with data resulting from standardized interdisciplinary projects developed.

Late frost damage probability. Critical temperature thresholds used to define frost damage for sweet cherry and plum were adopted from Murray (2020) and integrated as input parameters in the LFDP calculation. These thresholds were -3.9°C (sweet cherry) and -6.7°C (plum) for 51-53 BBCH, -2.8°C for 53-61 BBCH, -2.2°C for 61-69 BBCH, and -1.5°C for the post-flowering period. The late frost damage probability (LFDP) was calculated with a patented warning method, following the methodology previously established and improved by Chitu & Paltineanu (2006), and Chitu et al. (2011) for 2000-2009 and 2013-2022

decades. Therefore, a dynamic analysis of the frost-damage probability tendency was presented.

Minimum temperature, phenology, and late frost damage probability trend and correlations. The 5-day minimum temperatures trend was also obtained as the difference between the above-mentioned decades, and the overlap with sensitive phenological stages was assessed.

The dynamics of the maximum 5-day probability of minimum critical temperatures recorded after the pentad in which, in 50% of the years, the onset of the four phenophases was reached was calculated in order to use a biologically relevant reference for frost risk assessment. This threshold better represents the presence of sensitive organs at orchard scale and avoid inclusion of early intervals, with incipient phenophase and limited exposure to damaging temperatures.

The study performed a comprehensive evaluation of the intensity of the correlations between phenophase occurrence date and their critical minimum temperature with late frost damage probability. The research further explored the contribution of the minimum critical temperature dynamics to the increasing late frost damage probability.

Statistical analyses were conducted using IBM SPSS Statistics 20. Pearson correlation coefficients were also calculated in SPSS to assess the relationships between variables, while linear regression models were developed in Microsoft Office Excel to determine the trends in critical minimum temperature and phenology and their influence on the dynamics of late frost damage probability. All data were presented in the manuscript. Statistically significant differences were explicitly indicated where applicable.

RESULTS AND DISCUSSIONS

I. Comparative analysis of the late frost damage probability in sweet cherry between the decades 2000-2009 and 2013-2022

As shown in Figure 1, during the 2000-2009 period, the country-averaged late frost damage probability (LFDP) for sweet cherry was 10.6%. It ranged from a minimum of 2.3% (corresponding to one damaging frost event in

over 50 years), recorded in isolated areas of southeastern Romania, to a maximum of 36.3% (damage occurring more frequent than once every 3 years) in the eastern part of the Transylvanian Plateau.

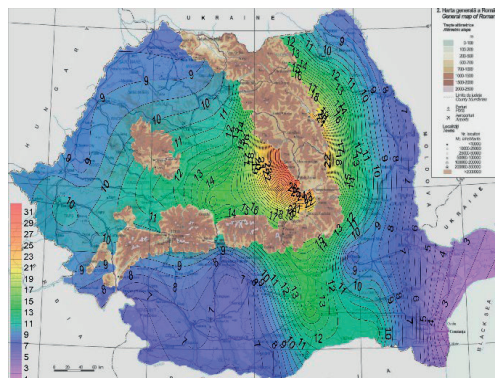


Figure 1. Spatial distribution of LFDP for sweet cherry in Romania between 2000 and 2009

Most of the territory showed LFDP values between 7-10%, although regional differences led to spatial gradients of damage probability. In the western part of the country, damaging frosts could occur once every 10-11 years, while in the southern areas their frequency increased (one event every 7-10 years). Exceptions were observed in the southeastern extremity, where the minimum LFDP values were recorded, and east, where damaging late frosts occurred once every 7-10 years, while more frequent damaging events occurred in the submontane regions, every 4 years. These differences reflect regional climatic influences and local topo-climatic effects.

During the 2013-2022 decade (Figure 2), the spatial distribution of LFDP generally followed a similar pattern, although important differences appeared. The average LFDP increased to 13.4%, and most values ranged from 10% to 15%. At the same time, the variation amplitude increased, with the minimum LFDP decreasing to 2.0% (in the extreme south-east) and the maximum values reaching almost 47% (a damaging frost almost every 2 years) in the east of the Transylvanian Plateau. Additionally, a new area of elevated frost risk was identified in the east, extending from the submontane areas, where frost occurrence rose to 28% towards Iasi (LFDP 15-16%).

The most critical changes were observed in the central part of Romania, where areas with one late frost every 7 years were drastically reduced, forming only a narrow peripheral band towards its western margin.

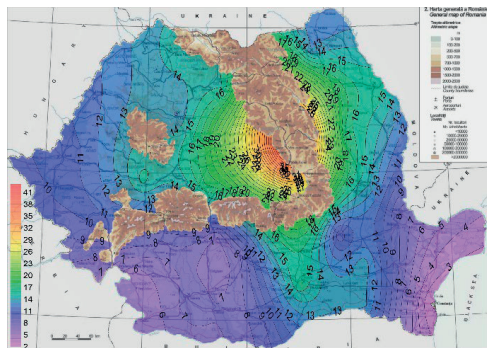


Figure 2. Spatial distribution of LFDP for sweet cherry in Romania between 2013 and 2022

The Transylvanian Plateau showed a sharp LFDP gradient (16-47%, e.g., frosts occurring every 2-6 years) oriented from west to east. Overall, most regions experienced relatively small LFDP increases (by 0-2 percentage points, as presented in Figure 3).

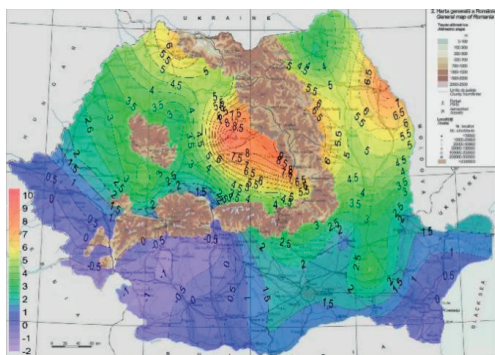


Figure 3. Spatial distribution of LFDP tendency for sweet cherry in Romania (difference between 2013-2022 and 2000-2009)

However, a spatial contrast in damage probability was observed between the southwest (with only a slight decrease in LFDP, especially around Drobeta-Turnu Severin and Targu Jiu) and the northeast (with increases in the LFDP of 8 percentage points around Iasi). The highest LFDP intensity was centered in the east of the Transylvanian Plateau, where both high LFDP values and their strong positive trend were

noted. This increase is consistent with a higher frequency of minimum critical temperatures.

II. Comparative analysis of the late frost damage probability in plum between the decades 2000-2009 and 2013-2022

In plum, during the 2000-2009 period (Figure 4), LFDP was 6%, which corresponded to damaging events occurring less frequently than once every 16 years. LFDP ranged from 1.2% (approximately one event every 80 years) in the extreme southeast to 22% (more frequent damaging frost than once every five years) in the eastern Transylvanian Plateau. Most frequently, LFDP values clustered around 5.5%, indicating a relatively lower frost risk than sweet cherry, likely due to later phenological development.

Spatial variability of LFDP was less pronounced in the west and south-west of Romania (5-7% and 3-6%, respectively), although more pronounced in the other regions (1-8% in the south-east and 3-12% in east), the most pronounced LFDP gradient was observed in the Transylvanian Plateau (5-22%), a region is prone to both cold air accumulation and continental air advection.

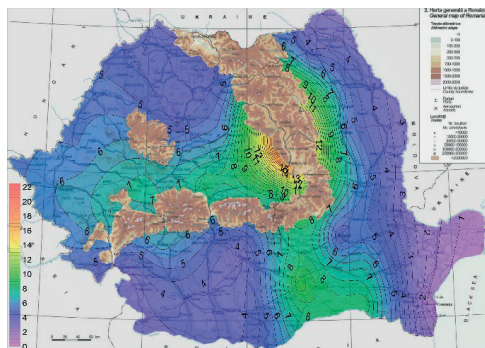


Figure 4. Spatial distribution of LFDP for plum in Romania between 2000 and 2009

Similar to sweet cherry, a secondary core of risk was clearly delineated in the east of the Romanian Plain (7-10%), a region where cold air advection is more frequent. During the decade 2013-2022 (Figure 5), the average LFDP increased slightly to 7%, compared with the previous period, while its maximum values reached the threshold of 36% in the east of the Transylvanian Plateau. Low LFDP values (1-4%) were observed across larger areas of the Romanian Plain, while in the extreme south-

east, as well as in the south of the Western Plain, LFDP remained approximately stable (Figure 6).

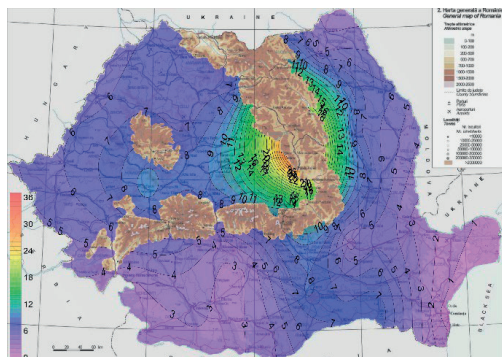


Figure 5. Spatial distribution of LFDP for plum in Romania between 2013 and 2022

LFDP increased in the last analyzed decade by 6-14 percentage units, especially in the risk-prone regions. Low increases in LFDP were observed in the northwest, towards Baia Mare (+2.5 percentage points) and in the east, towards Iasi (+2 percentage points).

However, despite this increase in late frost risk, most of the country showed almost stable or even slightly decreasing LFDP values (by 1.2 percentage points), located particularly across a broad area between the southern half of the Timis Plain and Mangalia.

III. Influence of phenology dynamics and 5-day mean of daily minimum temperatures on the increase in the late frost damage probability

a. Dynamics of late frost damage probability across phenological stages BBCH 51-53, 53-61, 61-69, and post-BBCH 69

Comparing the 2013-2022 and 2000-2009 decades, an increase in the average damage probability, higher for sweet cherry (1.03% vs. 0.55% for plum), was observed. This difference may be explained by the higher sensitivity of sweet cherry to negative minimum temperatures and by its earlier phenological development. These characteristics increase the probability of overlap between sensitive phenological stages and critical damaging temperatures. Nevertheless, significant differences were recorded between the four phenological intervals.

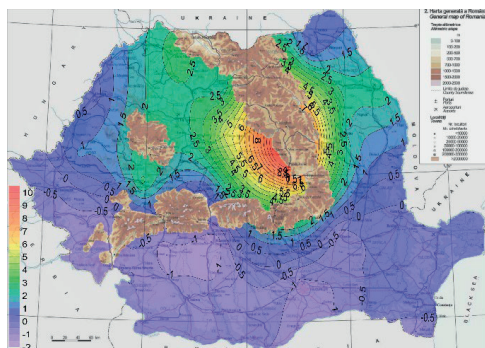


Figure 6. Spatial distribution of LFDP tendency for plum in Romania (difference between 2013-2022 and 2000-2009)

The most pronounced increase in LFDP was observed for BBCH 51-53 (2.78% for sweet cherry and 0.53% for plum) and post-BBCH 69 (0.85% in cherry and 0.96% in plum), while a lower LFDP increase was recorded for BBCH 61-69 (0.56% and 0.55%, respectively). The lowest LFDP variation was found in BBCH 53-61 (-0.07% and 0.17%, respectively), although these values differed significantly from those reported for BBCH 51-53 and post-BBCH 69. These results indicated that late frost risk varied between phenophases, was species-dependent, and was not uniformly distributed across the spring season. To identify the statistically significant drivers of LFDP changes, further analyses regarding phenological dynamics and minimum critical temperature dynamics were performed.

b. Phenological dynamics

The analysis of the average onset date of the four phenophases across 28 representative localities in the decade 2013-2022 highlighted a phenological advance compared to 2000-2009. Sweet cherry showed an average phenological advance of 3.2 days (5 days for BBCH 51 and 3 days for the BBCH stages 53, 61, and 69), while plum exhibited a slightly greater advance of 3.7 days (4 days for BBCH stages 51, 61, and 69 and only 3 days for BBCH 53). However, these phenological shifts alone could not justify the observed increases in LFDP, suggesting that other factors, particularly the temporal variability of minimum temperatures, may play a dominant role in frost risk. Although plum showed a slightly greater advance, its phenological development remai-

ned later than that of sweet cherry. Consequently, sweet cherry stages, especially flowering, are more likely to occur earlier in the season, when the probability of critical minimum temperatures is still relatively high, while the later development of plum may reduce its exposure to early-season cold events.

These interspecific differences in timing did not translate into a consistent effect on LFDP across all phenophases. Phenological advance influenced frost risk mainly during flowering, when sensitive stages overlapped with periods of increased occurrence of critical minimum temperatures.

The increase in LFDP between the two decades was not uniformly distributed across phenophases and followed different patterns. In sweet cherry, the most pronounced increase occurred during bud swelling, whereas in plum it was highest after flowering, followed by flowering, suggesting that the main frost risk window occurs earlier in the season for sweet cherry and later for plum.

Because both species exhibited similar phenological advances, these differences cannot be attributed only to phenological shift, nor solely to differences in temperature sensitivity. Instead, they suggest that LFDP dynamics result from the interaction between phenological timing and the temporal distribution of critical minimum temperatures.

c. Dynamics of minimum 5-day temperatures

In 14 of the 24 analyzed pentads, the minimum temperatures increased, and in 10 pentads they decreased, following a highly irregular temporal distribution (Figure 7).

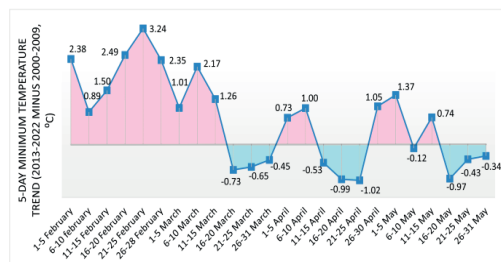


Figure 7. Dynamics of minimum 5-day temperatures from 2000-2009 to 2013-2022

The highest increases, exceeding 2°C, were recorded in four pentads in February and in the

second pentad in March. Smaller increases, of 1-2°C, were also recorded in February, March, April, and May. Such warming of minimum temperatures would generally reduce frost risk even under a phenological advance of 3-5 days. However, two periods showed a 5-day minimum temperature decrease of up to 1.02°C. In the first period (the last three pentads of March), temperatures dropped by 0.73°C, while in the second period, between April 11 and 25, temperatures decreased by up to 1.02°C. The April cooling is critical, as it overlapped with the most sensitive stages in both species: flowering (BBCH 61-69) and young fruit (post BBCH 69). Therefore, the increase in LFDP can be primarily explained by this increasing exposure to late frost events of sensitive phenophase, rather than phenological advance alone.

d. The dynamics of the maximum 5-day probability of minimum critical temperatures recorded after phenophase onset

This indicator also increased in the decade 2013-2022 compared with 2000-2009 by 1.55% for sweet cherry and only by 0.72% for plum (both differences were statistically significant at $P \leq 0.05$), indicating a higher probability of damage after the onset of sensitive stages. The probability of critical minimum temperatures associated with the four phenological intervals followed a similar increasing pattern, present in stages BBCH 61-69 (on average, + 2.05%) and BBCH 51-53 (on average, + 1.99%), reflecting increased exposure to critical temperatures during flowering and bud swelling. The differences observed between species (+3.21% during bud swelling and +1.97% during flowering for sweet cherry; +0.76% during bud swelling and +2.13% during flowering for plum) indicate the role of phenological timing, which influences the degree of exposure to critical minimum temperatures.

Post-BBCH 69, further increases of 1.78% and 1.44%, respectively, were recorded. In contrast, the probability of critical minimum temperatures decreased by 0.74% in sweet cherry and by 1.44% in plum during BBCH 53-61, indicating a temporal window with reduced frost risk just before flowering.

Overall, these patterns suggested that both phenological shift and minimum critical temperature variability influenced frost risk.

e. The contribution of the influence of the phenology dynamics and the critical minimum temperatures to the increase in the late frost damage probability

The increase in the late frost damage probability was strongly associated with the increase in the probability of critical minimum temperatures rather than with the phenological advance.

In sweet cherry (Figure 8), LFDP increased in all four phenological intervals, positively correlated with the increase in the incidence of critical minimum temperatures. The intensity of these relationships increased from early stages (51-53 BBCH, $R^2=0.2813^{**}$) to a maximum during flowering ($R^2=0.7642^{***}$) and remained substantial after fruit set ($R^2=0.5191^{***}$), suggesting that frost risk becomes increasingly controlled by minimum temperature variability as floral organ sensitivity increases.

In contrast, phenological advance displayed a significant effect only during flowering ($R^2=0.223^{**}$), indicating a limited effect of phenological advance.

A similar pattern was observed in plum (Figure 9), where LFDP variation was moderately explained by critical minimum temperatures in early phenological stages ($R^2=0.2391^{**}$ and $R^2=0.2326^{**}$), but showed a stronger relationship during and after flowering ($R^2=0.63693^{***}$ and $R^2=0.47977^{***}$, respectively). Although earlier flowering caused a significant increase in the probability of frost damage ($R^2=0.354^{***}$), its effect was weaker compared to the critical minimum temperatures. Overall, these results demonstrated that LFDP appeared to be largely driven by the timing and frequency of critical minimum temperatures. Phenological advance became relevant only when the temporal coincidence between flowering and cold-air periods is extended.

The spatial distribution of the late frost damage probability reflected the interaction between cold-air advections on a regional scale, the frequency of critical minimum temperatures, external climatic influences, and topo-climatic features of the relief (Bogdan et al., 2010; Caian et al., 2019).

During the 2000-2009 period, the maximum LFDP values for both species were concentrated in the east of the Transylvanian Plateau, while moderate values characterized the remaining parts of Transylvania, the eastern edge of the

Sub-Carpathians, and the southern sector descending toward the Danube.

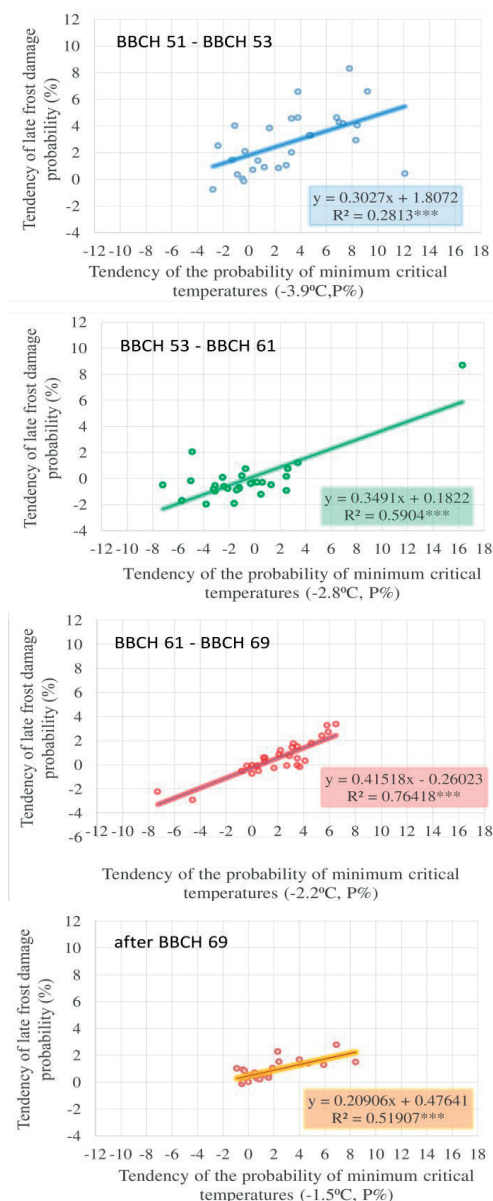


Figure 8. Correlation between LFDP trend and the minimum critical temperatures tendency for sweet cherry (mean of 2013-2022 decade minus that of 2000-2009)

In the Transylvanian Plateau (with high to highest LFDP), the depressional character allows cold-air accumulation through nocturnal radiative cooling, temperature inversions, and cold-air pooling (Geiger et al., 2009).

Meanwhile, intermittent cold-air advection from the north and northeast, with cold-air drainage from the slopes, enhances the accumulation of cold air in valley bottoms, intensifying the occurrence of critical minimum temperatures (Bogdan et al., 2010; Caian et al., 2019). In the east and southeast, increasing LFDP gradients reflect the exposure of the Romanian Plain to advective frost events due to cold-air intrusions from the north and northeast, facilitated by the open relief (Bogdan et al., 2010), which allows the rapid intrusion and spatial expansion of cold-air masses compared with wind-protected regions.

In northeastern regions, higher LFDP values and their increase in the last decade are associated with the predominance of advective frosts driven by northern cold-air masses and facilitated by regional open relief, while wind conditions limit the formation of stable temperature inversions.

In contrast, in the southwestern part of Romania, the low LFDP values are likely related to the sub-Mediterranean influences and the foehn effects produced when moist air masses from the north-western sector cross the Carpathians and reach the south in the form of warmer and drier air masses (Dima, 2014).

In these circumstances, reduced occurrence of the minimum critical temperatures in spring is expected.

In the coastal area, low LFDP could be attributed to the Black Sea influence, which reduces the thermal amplitude and diminishes the frequency of critical minimum temperatures in spring, thereby lowering the incidence and severity of late frosts (Bosneagu et al., 2022; Manea & Grigorescu, 2024). During 2013-2022 decade, high-risk regions intensified.

Comparable trends in spring minimum temperatures have been reported by Mazilu et al. (2025a). The evolution of LFDP, therefore, appears to be linked, at least in part, to increasing critical minimum temperatures. Overall, the spatial distribution of LFDP reflects a transition between radiative frost regimes in intra-Carpathian depressions (Transylvanian Plateau) and advective frost regimes in the eastern part of Romania, with mixed processes in submontane areas (Geiger et al., 2009; Caian et al., 2019).

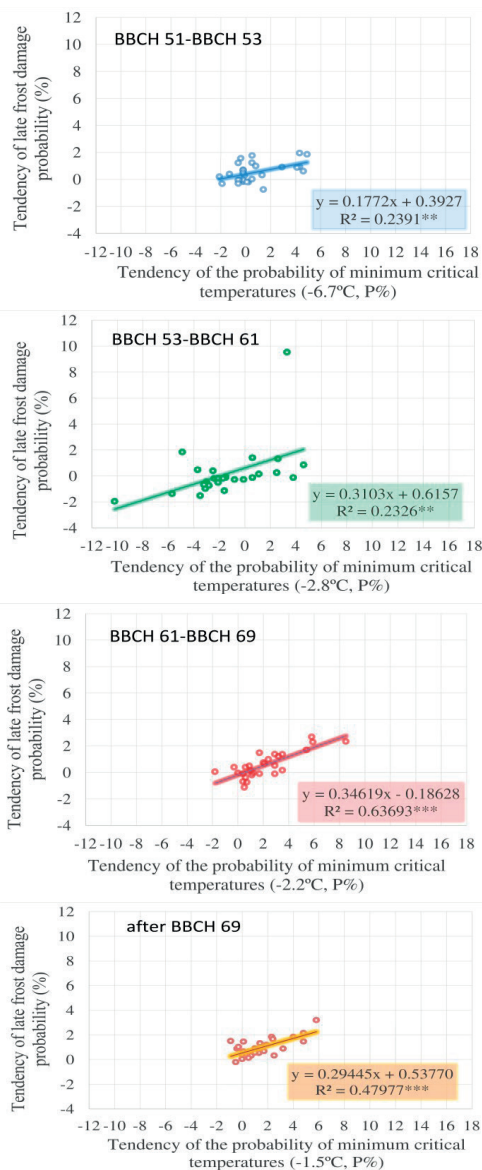


Figure 9. Correlation between LFDP trend and the minimum critical temperatures tendency for plum (mean of 2013-2022 decade minus that of 2000-2009)

CONCLUSIONS

The study performed a comprehensive analysis of the late frost damage probability in two stone fruit species, sweet cherry ('Germersdorf' cv.) and plum ('Tuleu gras' cv.), under recent climate change conditions in Romania. LFDP was higher in sweet cherry.

This was mainly related to a greater exposure to critical minimum temperatures, while advances in flowering seem to play a secondary role. Spatially, high-risk areas remained concentrated, especially in the eastern part of the Transylvanian Plateau, where both the highest values and the strongest increasing trends are observed. At the opposite pole, lower and more stable LFDP values were observed in the southern and western areas, suggesting that regional climatic influences and topoclimatic conditions shape frost risk.

This study shows that the increase in LFDP during 2013-2022, relative to 2000-2009, was mainly driven by the increase in the probability of critical minimum temperatures, rather than by phenological advance. This relationship was statistically significant for both species and across all phenological stages, reaching its maximum intensity during flowering.

Although significant phenological advances were observed (up to 5 days in sweet cherry and 4 days in plum), their influence on frost damage probability was limited and became significant only when flowering coincided with periods of increased occurrence of critical minimum temperatures.

The distribution of frost risk across phenophases differs between species. In sweet cherry, LFDP tends to increase during early stages, while in plum the increase occurs later. These differences reflect a shift in the timing of sensitive periods relative to cold events.

Overall, the study provides new evidence that changes in late frost risk in Romania are more strongly linked to temperature dynamics than to phenological advance alone, highlighting the need to integrate phenological data with detailed analyses of minimum temperature variability to improve frost-risk assessment.

The results are relevant for selecting site-adapted cultivars and improving frost protection strategies. Still, the relative role of critical minimum temperatures and phenological advance may differ between regions, and this needs to be explored further at the regional scale.

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FRUIT QUALITY OF SOME SWEET CHERRY (*PRUNUS AVIUM* L.) CULTIVARS IN THE CONDITIONS OF NORTH-EAST ROMANIA

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Abstract

The sweet cherry (Prunus avium L.) is one of the most appreciated fruit crops worldwide due to the taste, color, and nutritional value of the fruits. The present study was conducted during 2022-2024 and involved five native sweet cherry cultivars ('Alexus', 'Iașirom', 'Bucium', 'Mihailis', and 'Ștefan'), created within the Research Station for Fruit Growing (RSFG) Iași. The aim of the study was to evaluate multiple fruit quality indicators, both physical: fruit weight, length, thickness, width, fruit geometric diameter, sphericity, flesh weight and stone weight, peduncle length, firmness, and biochemical (total soluble solids, titratable acidity, and sugar-acidity ratio). If the cv. 'Andreiș' stood out with the largest fruits in flesh weight (FIW=9.82 g), the cv. 'Ștefan' had the highest values in terms of fruit firmness (F=7.2 N), while the cv. 'Mihailis' had the highest soluble sugar content (TSS=22.87 °Brix). All the five analyzed cultivars represent valuable phenotypic resources, with an average weight of 9.4 g and a soluble dry matter content of 21.23 °Brix.

Key words: *Prunus avium L., fruit quality, traits, physical, biochemical.*

INTRODUCTION

Among the cultivated fruit tree species, the sweet cherry (*Prunus avium* L.) finds optimal conditions in Romania to manifest its agrobiological potential. The precocity of the species, the production yield, and the lack of fruiting alternation give this fruit species a priority place in the cultivated area, holding an average area of 3,600 ha and an average production of 37,500 tons in the period 2020-2023 (FAO, 2025).

The biochemical composition, as well as the physical properties of the fruit (dimensions, color, firmness of the pulp, size of the stone) are the main criteria according to which the destination of valorization is established. The values of the physical properties, but also the biochemical composition, can fluctuate depending on the cultivar, the annual climatic conditions, and the agrotechnical measures applied (Quero-Garcia et al., 2017; Corneanu et al., 2020). The unique properties of sweet cherries influence consumer acceptance of these fruits and lead to an increased demand for them in both global and European markets, which has led to continuously increasing production (Sen et al., 2014; Szpadzik et al., 2019).

In recent years, fluctuations in the fruit production of this fruit species have been

observed; new cultivars have appeared with an increase in the season, and modern cultivation techniques have been implemented (Schuster et al., 2014). However, as highlighted in similar research, currently, special interest should be given to the quality of the fruit to the detriment of the quantity, because this is a main factor, in addition to the regularity of the production yield, that determines the profitability of sweet cherry cultivation (Vignati et al., 2022). Thus, external attributes such as dimensions or caliber and color determine the category in which each fruit is classified and, consequently, its price, while nutritional and gustatory parameters emphasize the consumer's perception of high quality (Ballistreri et al., 2013; Ricardo-Rodrigues et al., 2023).

The chemical composition of sweet cherries also greatly affects the sensory quality of the fruit (Fazzari et al., 2008). The sugar and other acid content, as well as the color, influence consumer acceptance of sweet cherry cultivars, as do the weight, diameter, and firmness (Serrano et al., 2009).

The objective of this study was to analyze and compare some sweet cherry cultivars of Romanian autochthonous origin regarding the fruit quality parameters, both physical and biochemical, and consequently, to identify the

most valuable ones. These sweet cherry cultivars can also serve as a basis for further research on improving sweet cherry quality in the climatic conditions of North-Eastern Romania and beyond.

MATERIALS AND METHODS

The study was conducted over three years, between 2022-2024, using as plant material five sweet cherry cultivars: 'Alexus', 'Iașirom', 'Bucium', 'Mihailis' and 'Ștefan' created and registered at the Research Station for Fruit Growing (RSFG) Iași, Romania (47°20' N; 27°60' E). The orchard that included the studied cultivars was established in 2010 and is organized in variants of three repetitions, with the placement of the variants in randomized blocks. The trees were grafted onto a high-vigor rootstock (*Prunus mahaleb* L.) and managed in the form of an improved vase-type crown. The experimental plantation was carried out in a semi-intensive culture system, with a planting distance of 4 × 5 m without a support and irrigation system.

The general growing conditions in the experimental area include predominantly cambic chernozem soils with good natural drainage, a pronounced temperate-continental climate, with an average annual temperature over the last 20 years of +10.2°C and total precipitation of 568.7 mm.

Meteorological conditions during the study were monitored using the FieldClimate system, a specialized equipment for monitoring the microclimate in the crop area and consisting of a set of meteorological indicators for measuring and transmitting data from the crop areas served to the central receiving station. The average values were compared with a database of the unit, as a multi-annual reference value for the period 1970-2020.

Sweet cherry samples were collected at full maturity from several areas of the crown. For each cultivar, 3 replicates (30 fruits each) were performed annually (n=3) and used for physicochemical analyses.

The following physical parameters were studied: fruit weight (FW), fruit length (FL), fruit width (FWD), fruit thickness (FT), fruit geometric diameter (FGD), stone weight (SW), sphericity (Ø), flesh weight (FIW), peduncle length (LP) and firmness.

Geometric diameter (mm) = $[(FL \times FWD \times FT)^{0.333}]$

Sphericity (mm²) = (FGD/FL)

Flesh weight (g) = (FW – SW)

Physical parameters regarding fruit and stone weight (g), diameter (mm) and peduncle dimensions (mm) were determined by weighing using an analytical balance with an accuracy of 0.01 g Radwag WLC C/2 (RADWAG Development Studio, Radom, Poland) and a 0.02 mm digital caliper Luumytools LT15240 (LUMY TOOLS TRANS SRL, Suceava, Romania).

Fruit firmness (F) was determined using the Fruit Hardness Tester ML-41050 (STEP Systems GmbH, Nuremberg, Germany) as the force required to deform the fruit by 3 mm. The determinations were performed twice on each fruit, in the horizontal and vertical planes, and the resulting fruit firmness value was expressed in newtons.

Total soluble solids (TSS) were determined refractometrically using a Hanna Instruments model HI96800 digital refractometer (Woonsocket, RI, USA). The results were expressed in Brix and reflect the sugar content of the fruits, along with other soluble organic acids, minerals, and amino acids. (Ricardo Rodrigues et al., 2023). Titratable acidity (TA) was determined by homogenizing the samples with distilled water and titrating with 0.1 N NaOH until reaching a pH of 8.1. The results were obtained using the formula and expressed in g malic acid 100 g⁻¹.

TA (%malic acid) = $(V \times N \times 100 \times 0.0067)/m$, where N is the normality of NaOH, V is the volume of NaOH (mL) used in the titration, m is the sample weight (g), and 0.0067 is the conversion factor for malic acid. The values are expressed in g malic acid 100 g⁻¹ (Hayaloglu and Demir, 2016).

The TSS/TA ratio, expressed as Brix/g malic acid per 100 g⁻¹ is a good indicator of the sweetness and, consequently, the degree of ripeness of the fruit for consumption (Chockchaisawasdee et al., 2016).

The data were interpreted and statistically processed using the Duncan multiple range test at $p \leq 0.05$ and to estimate the relationship between the analyzed and monitored indices, the Pearson correlation coefficient (r^2) was also calculated at $p \leq 0.05$. Depending on the mean value of the results, the standard deviation

(STDEV) and the coefficient of variation (COVAR) were also calculated.

RESULTS AND DISCUSSIONS

Weather conditions are extremely important for fruit crops, especially from a production perspective (Blanco et al., 2020).

The climatic data obtained during the study years are presented in Table 1 as average values and frame the climatic regime in the NE area of Romania in the context of global warming through the increase in the average annual temperature and an abnormal distribution of precipitation and/or major deficit (Chmielewski and Götz, 2016).

Table 1. Monthly temperature (°C) and precipitation (mm) in the studied years (RSFG, 2022-2024)

Period	Air temperature (°C)			Precipitation (mm)		
	av.	M.a	Dev	sum	M.a	Dev.
I	1.1	-3.3	4.4	13.9	28.9	-15.0
II	4.2	-1.5	5.7	14.8	27.4	-12.6
III	4.8	3.1	1.7	44.8	28.1	16.7
IV	10.9	10.3	0.6	46.8	40.3	6.5
V	16.3	16.1	0.2	46.0	52.5	-6.5
VI	21.6	19.4	2.2	45.7	75.1	-29.4
VII	23.5	21.3	2.2	55.7	69.2	-13.5
VIII	23.2	20.5	2.7	59.6	57.6	2.0
IX	16.3	16.3	0.0	60.1	40.8	19.3
X	12.4	10.1	2.3	25.3	34.4	-9.1
XI	5.1	4.0	1.1	48.5	34.6	13.9
XII	1.8	-0.9	2.7	22.8	28.9	-6.1
Av./Sum	11.8	9.6	+2.2	484.0	517.8	-33.8

The evolution of air temperature was similar in the study years but increasing as an average compared to the multiannual average in all months, by +2.2°C.

Although the total amount of precipitation was sufficient in terms of the annual average, with a deficit of -33.8 mm, its distribution was uneven throughout the growing season, with abundant amounts recorded at phenological stages when they represent a risk for the cherry crop in achieving pollination (March and April).

The physical characteristics of the fruit, stone, and peduncle length are given in Table 2. Fruit weight depends on the crop load (Goncalves et al., 2006; Usenik et al., 2010) and the stage of fruit maturity (Usenik et al., 2008; Serrano et al.,2009).

In the study, the average fruit weight ranged from 8.7 g ('Mihailis') to 10.2 g ('Alexus'), and a length from 20.06 mm ('Mihailis') to 27.99 mm ('Ștefan').

Research carried out on cherry cultivars from NE Romania (Corneanu et al., 2020; Perju et al., 2024) highlights smaller fruit sizes belonging to

the local assortment created within RSFG Iași, ranging between 6.1 g and 8.7 g.

The 'Iașirom' and 'Ștefan' cultivars are found in the study conducted by Corneanu et al., 2020, where, under the climatic conditions of 2015-2018, they recorded an average weight of 6.4 g ('Iașirom') and 8.2 g ('Ștefan'), lower values compared to the present study.

Sphericity ranged between 0.8 ('Ștefan') and 1.03 mm² ('Mihailis') in the studied cultivars, and the weight of the pulp, the edible part of the sweet cherries, was on average 9.07 g.

Firmness is also an important attribute that influences the quality of a fruit (Table 3), and for the cherry cultivars in this case, it had values ranging from 3.67 ('Mihailis') to 7.23 N ('Ștefan'). A large study conducted on 24 sweet cherry cultivars grown in Italy (Ballistreri et al., 2013) notes that firmness in cherries can range from 3.25 ('Bigarreau Moreau') to 27.00 N ('Minnulara'). According to Pereira et al., 2020 the firmer and larger the fruits, the more they are affected by cracking than smaller fruits with soft pulp.

The biochemical properties of total soluble solids content and acidity are presented in Table 3. The TSS content exceeded the value of 20°Brix in all cultivars, with a maximum of 22.87°Brix

in the 'Mihailis' cultivar, while TA values ranged from 0.65 ('Iașirom') to 1.06 mg/100 g⁻¹ malic acid ('Mihailis').

Table 2. The physical parameters of the fruits in the studied sweet cherry cultivars (RSFG, 2022-2024)

Cultivar	FW	FL	FT	FWD	FGD	ø	FIW	SW	LP
	(g)	(mm)	(mm)	(mm)	(mm)	(mm ²)	(g)	(g)	(mm)
Alexus	10.19 ^a	26.55 ^b	25.28 ^a	21.93 ^a	24.42 ^a	0.92 ^{bc}	9.82 ^a	0.37 ^a	36.00 ^a
Iașirom	9.47 ^{ab}	25.34 ^b	23.95 ^a	22.36 ^a	23.77 ^b	0.94 ^{ab}	9.13 ^{ab}	0.33 ^a	34.67 ^a
Bucium	9.07 ^b	26.66 ^b	25.34 ^a	20.31 ^b	23.86 ^b	0.90 ^{bc}	8.73 ^b	0.34 ^a	37.33 ^a
Mihailis	8.73 ^b	20.06 ^c	20.41 ^b	21.72 ^a	20.65 ^c	1.03 ^a	8.40 ^b	0.33 ^a	36.33 ^a
Ștefan	9.62 ^{ab}	27.99 ^a	24.45 ^a	22.07 ^a	24.64 ^a	0.88 ^c	9.29 ^{ab}	0.33 ^a	38.33 ^a
Average	9.41	25.32	23.88	21.68	23.47	0.93	9.07	0.34	36.53
Min	8.73	20.06	20.41	20.31	20.65	0.88	8.40	0.33	34.67
Max	10.19	27.99	25.34	22.36	24.64	1.03	9.82	0.37	38.33
STDEV	0.55	3.09	2.03	0.80	1.62	0.06	0.54	0.02	1.39
COVAR	5.88	12.19	8.50	3.69	6.88	6.29	5.97	4.86	3.80

¹fruit weight (FW); fruit length (FL); fruit thickness (FT); fruit width (FWD); fruit geometric diameter (FGD); sphericity (ø); flesh weight (FIW); stone weight (SW); peduncle length (LP).

²different letters after the number correspond with statistically significant differences for *p* 5% - Duncan test.

The biochemical properties of the total soluble substances content and acidity are presented in Table 3. The TSS content exceeded the value of 20.0°Brix in all cultivars, with a maximum of 22.87°Brix in the Mihailis cultivar, while TA values ranged from 0.65 ('Iașirom') to 1.06 mg /100 g⁻¹ malic acid ('Mihailis'). The TSS/TA ratio of the Romanian studied cultivars had values ranging between 21.56 ('Mihailis') and 31.02°Brix/mg/100 g⁻¹

('Iașirom'), a standard deviation of 3.98 and a coefficient of variation COVAR of 14.89. All fruit quality parameters analyzed in the study were correlated to establish the degree of interdependence between them (Table 4). The Pearson correlation values of the quality profile of the analyzed cherries reflect a predominantly strong correlation (*p* < 0.05) with more than 18 statistically significant values.

Table 3. Fruit quality of the studied sweet cherry depending on the cultivar (RSFG, 2022-2024)

Cultivar	Firmness	TSS	TA	TSS/TA
	(N)	(°Brix)	(mg /100 g ⁻¹)	(°Brix/ mg /100 g ⁻¹)
Alexus	5.70 ^b	21.27 ^{bc}	0.72 ^{cd}	29.89 ^{ab}
Iașirom	5.73 ^b	20.13 ^c	0.65 ^d	31.02 ^a
Bucium	5.93 ^b	20.17 ^c	0.74 ^c	27.18 ^b
Mihailis	3.67 ^c	22.87 ^a	1.06 ^a	21.56 ^d
Ștefan	7.23 ^a	21.73 ^{ab}	0.91 ^b	23.91 ^{cd}
Average	5.65	21.23	0.82	26.71
Min	3.67	20.13	0.65	21.56
Max	7.23	22.87	1.06	31.02
STDEV	1.28	1.15	0.17	3.98
COVAR	22.60	5.40	20.62	14.89

¹total soluble solids (TSS); titrable acidity (TA); sugar-acidity ratio (TSS/TA).

²different letters after the number correspond with statistically significant differences for *p* 5% - Duncan test.

Total insignificant correlations were obtained predominantly between the physical dimensions

of length, width, geometric diameter, and sphericity.

Table 4. Correlation matrix between the sweet cherry fruits characteristics

	FL	FT	FWD	FGD	Ø	FIW	SW	F	TSS	TA
FW	0.674*	0.680*	0.426 ^{ns}	0.774**	-0.564*	1.000***	0.723**	0.571*	-0.265 ^{ns}	-0.571*
FL		0.936***	-0.071 ^{ns}	0.984***	-0.989***	0.683*	0.198 ^{ns}	0.958***	-0.612*	-0.614*
FT			-0.238 ^{ns}	0.942***	-0.921***	0.682*	0.402 ^{ns}	0.798**	-0.749**	-0.782**
FWD				0.046 ^{ns}	0.190 ^{ns}	0.434 ^{ns}	0.064 ^{ns}	0.051 ^{ns}	0.269 ^{ns}	0.021 ^{ns}
FGD					-0.948***	0.782**	0.302 ^{ns}	0.922***	-0.638*	-0.700**
Ø						-0.573*	-0.106 ^{ns}	-0.955***	0.614*	0.559*
FIW							0.708**	0.585*	-0.269 ^{ns}	-0.572*
SW								-0.031 ^{ns}	-0.053 ^{ns}	-0.397 ^{ns}
F									-0.484 ^{ns}	-0.439 ^{ns}
TSS										0.914***

¹fruit weight (FW); fruit length (FL); fruit width (FWD); fruit thickness (FT); fruit geometric diameter (FGD); flesh weight (FIW); stone weight (SW) sphericity (Ø); firmness (F); total soluble solids (TSS); titrable acidity (TA);

²***: high significant correlation; **: distinct significant correlation; *: significant correlation at $p < 0.05$; ns: not significant correlation;

CONCLUSIONS

In the research conducted between 2022–2024, the five native sweet cherry genotypes created at RSFG Iași presented significant variability from a biometric and biochemical point of view.

In this study, fruit quality parameters and chemical attributes of the sweet cherry cultivars (*P. avium* L.) created and cultivated at RSFG Iași (Romania) were evaluated. The research results showed variability between cultivars in terms of their physico-chemical characteristics. Quantitatively, the 'Mihailis' cv. was found to have the highest amounts of the main compounds of total soluble solids (22.87°Brix) and titratable acidity (1.06 g malic acid·100 g⁻¹) being suitable for industrial processing.

The 'Alexus' cv. stood out for its large fruit mass (10.19 g) and high pulp content, being valuable for fresh consumption, and 'Ștefan' presented the highest pulp firmness (7.23 N), which gives it superior resistance to handling and transport.

The results confirm the high potential of native sweet cherry creations for the production of high-quality fruits, adapted to current climatic conditions characterized by increasing average annual temperatures.

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THE EFFECT OF PILARHANCE, SL GROWTH REGULATOR ON YIELD AND QUALITY OF SOUR CHERRY FRUITS

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Abstract

The research was carried out in the orchard of the “Ceteronis ST SRL” enterprise, founded in the spring of 2022, with trees of Erdi Botermo variety, grafted on Mahaleb cherry rootstock. The planting distance was 4 x 2 m, and the crown shape was a free-growing spindle. The soil between the rows and between the trees in a row was maintained as clean cultivation and irrigated. The following variants were investigated in the research plot: 1. Control (spraying with water); 2. Pilarhance, SL, 0.8 l/ha; 3. Pilarhance, SL, 1.3 l/ha. Two treatments were carried out during the growing season. During the reference period, it was established that the greatest intensity of growth processes in the tree crown and sour cherry fruits was recorded in the variant treated with Pilarhance, SL at a dose of 1.3 l/ha, applied twice by spraying. The first treatment should be carried out at the end of the flowering period, and the second during the period when the fruit color changes from green to yellow.

Key words: growth regulator, leaf area, production, fruit setting, *Prunus cerasus*.

INTRODUCTION

Along with the use of mineral and organic fertilizers and plant protection products, the application of plant growth regulators has recently become of great importance in agriculture (Neamțu & Irimie, 1991; Peșteanu et al., 2000). Growth regulators are capable of causing various changes in plant growth and development in small quantities (Peșteanu et al., 2023; Peșteanu & Calestru, 2025).

Modern plant growth regulators can boost plant immunity, resistance to unfavorable growing conditions and stressful situations, accelerate fruiting, increase yields, and ensure the ecological safety of crops. All this makes plant growth regulators simply indispensable in the cultivation of agricultural crops (Buban, 1996). When using growth regulators, it is necessary to take into account that each of them is designed to stimulate the growth, development, and increase the productivity of certain crops at the corresponding doses, times, and methods of application (Peșteanu et al., 2018).

To the present time, hundreds of regulators of various chemical nature have been synthesized, possessing a wide spectrum of action on fruit plants (Kurlus et al., 2017; Neamțu & Irimie, 1991).

Many of them are already used in agriculture. There are six known groups of phytohormones: auxins, gibberellins, cytokinins, abscisic acid, ethylene, and brassinosteroids, which differ in their chemical nature and effect on plant physiological processes (Neamțu & Irimie, 1991).

Gibberellins are the most extensive class of natural growth stimulants. More than a hundred varieties are known. The main advantages of preparations based on them are high efficiency and safety. They are completely safe for humans, do not accumulate in plants, and have no post-effects (Kurlus et al., 2017; Peșteanu et al., 2023).

Gibberellic acid has many physiological effects on plants. It significantly increases the number of flower buds and fruits, influences plant growth by promoting cell division in meristematic zones and their elongation, stimulates the production of enzymes, increases productivity and improves product quality, and reduces damage from diseases and fruit cracking (Пештяну, 2022).

Brassinosteroids are steroid phytohormones that support the normal function of the plant's defense system, especially in unfavorable conditions, such as soil salinization, low temperatures, frost, drought, and pathogen

infection (Chai et al., 2013; Mandava & Wang, 2016).

Brassinosteroids change enzyme activity and membrane potential, stimulate protein and nucleic acid synthesis, regulate plastid gene expression and fatty acid metabolism, and influence the plant's hormonal status, which is reflected in the whole plant in the form of enhanced growth and increased productivity (Mandava & Wang, 2016; Muradoglu et al., 2025)

The most common and studied is 24-epibrassinolide (EPB), a polyhydroxylated steroid compound that plays a significant role in regulating physiological processes. It is the main active ingredient in various preparations used in agriculture as an adaptive agent (Ahammed et al., 2020).

24-epibrassinolide is a plant growth regulator that acts through the hormonal system, influencing the activity and biosynthesis of oxidative cycle enzymes and hydrolytic enzymes (proteases), having a multifaceted effect on plants: it increases resistance to biotic and abiotic factors, enhances growth and shape-forming processes, increases yield, and improves its quality (Sun et al., 2020).

Combined growth regulators based on gibberellic acid and 24-epibrassinolide can promote crop growth and cell elongation, annual growth, and increase leaf surface area, reduce flower and fruit drop, promote fruit growth and increase productivity in orchards, and improve product quality (Buban, 1996; Kurlus et al., 2017).

Therefore, the aim of this research was to determine the effect of Pilarhance SL growth regulator on tree and fruit development of the Erdi Botermo sour cherry variety, grafted on Mahaleb cherry rootstock in an intensive sour cherry orchard, and to maintain a physiological balance between growth, fruiting, and obtaining consistent high-quality yields.

MATERIALS AND METHODS

The current research was carried out in the orchard of "Ceteronis ST SRL" during the growing season of 2025.

The experimental plot was located in the central part of the Republic of Moldova and had a

favorable influence on the growth and fruiting of the sour cherry crop.

The region is characterized by warm weather and average humidity, with an active temperature sum of 3000-3100°C, where the thermohydrological coefficient is 0.6-0.8.

The average positive monthly temperature is maintained for 9 months (March-November). According to long-term data, late-spring frosts are observed until the first decade of May. Average daily air temperatures above 10°C are observed for 175-180 days. The average annual air temperature is 9.6°C. July is the warmest month of the year (21.4°C), and January is the coldest (-2.4°C).

Annual precipitation is 502.1 mm, with 342 mm falling during the growing season, of which approximately 75 mm falls in June. There is a difference in precipitation between dry and rainy months.

The weather conditions during the experiment were favorable for the growth and fruiting of sour cherry trees, except for the months when there was very little rainfall; however, growth processes were maintained with the aid of drip irrigation (Figure 1).



Figure 1. Climatic indicators recorded in the SRL "Ceteronis ST" orchard during the period during 01.03 - 30.06.2025 (iMetos meteorological station)

The orchard was established in 2022 with trees of the Erdi Botermo variety, grafted on Mahaleb cherry rootstock. The planting density was 4 x 2 m, and the crown shape was a free-growing spindle. The soil between rows and between the trees was maintained as clean cultivation by mechanical loosening and was irrigated.

The experiment was started in spring (April 25) according to the experimental design presented

in (Table 1). Sour cherry tree crowns of the control plants were sprayed with water.

Two treatments were carried out during the growing season with Pilarhance SL products at a dose of 0.8 and 1.3 l/ha. The first treatment was applied at the end of the flowering period (April 25, 2025), and the second during the fruit coloration period, when the fruit color changed from green to yellow (May 12, 2025).

Table 1. Experimental design for determining the effectiveness of Pilarhance SL growth regulator on sour cherry trees

Variants	Active substance	Method of application
Control	Spraying with water	The first treatment was performed at the end of the flowering period, and the second during the period when the fruit color changed from green to yellow
Pilarhance, SL, 0.8 l/ha	Gibberellic acid, 3.98 g/l+24-	
Pilarhance, SL, 1.3 l/ha	epibrassinolide, 0.02 g/l 750 g/kg	

Every treatment included four replicates, and each replicate consisted of 8 trees.

The treatments were carried out with the help of a knapsack sprayer in the morning hours, in the absence of wind. The amount of solution per tree when treating with the studied preparations was 0.8 litres, based on the number of trees per unit area and the recommended amount of water of 1000 l/ha.

The studies were conducted under field and laboratory conditions according to the accepted methodology for conducting experiments with fruit crops and growth regulators (Моисейченко et al., 1994).

To determine the effect of the growth regulator at various doses on the leaf area of sour cherry trees, the method described by Balan V. et al. (2024) was used. This indicator has a direct effect on the photosynthesis process and, finally, on increasing crop yield and its quality.

The average and total length of annual growth was determined by measuring four model trees in each variant at the end of the growing season. The fruit-setting rate was calculated as the ratio between the number of fruits set on the tree after petal fall and the total number of flowers on the tree, multiplied by 100%.

The sour cherry yield was determined at the stage of fully ripened fruits by determining fruit weight. The total yield from each tree was collected separately and weighed. Then, for each variant, the average yield per tree was

determined, followed by conversion to yield per unit area.

The average fruit weight was determined in four repetitions for each variant by weighing 100 fruits picked consecutively from the tree, followed by division of the result by this number.

The firmness of the sour cherry fruits was assessed using a GY 1 penetrometer, which measures the resistance of the pulp to the penetration of a 3 mm² plunger. Ten fruits were selected for each variant to determine fruit firmness. In the equatorial region, on two opposite sides of the fruit (on the side exposed to the sun and the other from the shade), 10 firmness assessments were performed, and the average value was determined.

The total soluble solids content in sour cherry juice was determined using a ATA60N-20E digital refractometer.

Total acidity was determined by titration with a 0.1% NaOH solution using the TitroLine+ 5000 equipment.

The vitamin C content of the fruits was determined by the titrimetric method using 2,6-dichlorophenolindophenolate sodium.

The statistical processing of the main indicators was carried out using the one-factor analysis of dispersion using the ANOVA test and the STATGRAPHICS 18.0 software package.

RESULTS AND DISCUSSIONS

Among the main photometric characteristics of fruit plantations, leaf surface area and annual growth length are particularly important, as the productive potential of the orchard depends on these indicators.

The results (Table 2) demonstrate that the smallest leaf area per tree was recorded in the control, at 6.0 m²/tree.

The use of the growth regulator Pilarhance, SL, at doses of 0.8 l/ha and 1.3 l/ha increased the leaf area compared with the control. In the variants treated with Pilarhance, SL, at a dose of 0.8 l/ha, the leaf area increased by 3.3% compared with the control.

When the trees were treated with the Pilarhance, SL growth regulator at a dose of 1.3 l/ha, the leaf surface area was 6.7 m²/tree, which was 10.0% higher than in the control variant and 8.1%

higher than in the variant with Pilarhance, SL at a dose of 0.8 l/ha.

The difference between the variants treated with the Pilarhance, SL growth regulator at a doses of 0.8 l/ha and 1.3 l/ha was 0.5 m²/tree, which is confirmed by mathematical data processing.

To obtain stable and high yields, fruit trees in the orchard crown should form 15-20 thousand m²/ha of leaf surface.

The research conducted proves that the control variant had the smallest leaf surface area (7.5 thousand m²/ha). While the leaf surface area in the Pilarhance SL variant at a dose of 0.8 l/ha increased by 0.3 thousand m²/ha compared to the control, in the Pilarhance SL variant at a dose of 1.3 l/ha, this increase was 0.9 thousand m²/ha, or 8.4 thousand m²/ha.

Table 2. Effect of Pilarhance, SL growth regulator on leaf area and annual growth of Erdi Botermo sour cherry trees

Variants	Foliar surface area		Length of annual growth	
	m ² /tree	thousand m ² /ha	cm	m/tree
Control	6.0	7.5	49.3	17.3
Pilarhance, SL, 0.8 l/ha	6.2	7.8	51.8	18.1
Pilarhance, SL, 1.3 l/ha	6.7	8.4	55.2	19.3
LSD 5%	0.26	0.35	2.4	0.86

Another indicator influenced by the growth regulator under study was the annual growth. Abiotic stresses during the growing season, such as dry weather, high temperatures, insufficient soil moisture, and blocked mineral activity, increase the intensity of physiological processes in plants and reduce annual growth. Treating trees only with growth regulators can improve biochemical processes in plants and promote more uniform annual growth (Ahammed et al., 2020).

Studies on the average length of annual growth confirm that the growth regulator had different effects on the studied indicator.

The minimum average length of annual growth was observed in the control variant (49.3 cm), and the most developed shoots were observed in trees treated with the growth regulator Pilarhance, SL at a dose of 0.8 and 1.3 l/ha (51.8-55.2 cm).

When treating trees with Pilarhance, SL growth regulator at a dose of 0.8 l/ha, the average length of annual growth was 51.8 cm, which was 5.1%

higher than in the control variant. This difference is also confirmed by statistical processing.

In the Pilarhance, SL variant at a dose of 1.3 l/ha, the average length of annual growth was the longest (55.2 cm). This indicator was 6.6% higher than in the Pilarhance, SL variant at a dose of 0.8 l/ha and 12.0% than in the control. The difference between these variants is also confirmed by statistical analysis.

The total length of annual growth is directly related to its average length on the tree, which changes under the influence of the studied growth regulators.

The shortest total length of annual growth during the study was observed in the control, and the longest in the Pilarhance, SL variant at a dose of 1.3 l/ha. If the total length of annual growth in the control variant was 17.3 m/tree, then in the Pilarhance, SL variant at a dose of 1.3 l/ha it was 19.3 m/tree, or 2.0 m/tree more. This is explained by the fact that treatment with a growth regulator based on gibberellic acid + 24-epibrassinolide improved the physiological processes occurring in the tree crown during shoot growth.

When treating with Pilarhance, SL at a dose of 0.8 l/ha, the total length of annual growth decreased by 1.2 m/tree compared to Pilarhance, SL at a dose of 1.3 l/ha, but increased by 0.8 m/tree compared to the control variant. The increase in the total length of annual growth is also confirmed by statistical analysis of the data. Analysis of the experimental data allows us to conclude that the studied indicators are directly dependent on treatment with the growth regulator under study, but the highest values were recorded in the variant where the sour cherry trees were sprayed with the growth regulator Pilarhance, SL at a dose of 1.3 l/ha.

The results obtained prove that the number of flowers on the trees in the studied variants varied from 3319 units/tree in the Pilarhance, SL variant at a dose of 1.3 l/ha to 3364 units/tree in the control variant (Table 3). The difference between the number of flowers obtained in the experimental variants is insignificant and could not fundamentally have affected the results of the conducted research.

This increase in the number of fruits in the variant treated with the growth regulator Pilarhance, SL at a dose of 1.3 l/ha occurred due

to a slight increase in the percentage of fruit set compared to the other studied variants.

The lowest percentage of fruit set was recorded in the control variant (21.4%), followed by the Pilarhance, SL variant at a dose of 0.8 l/ha (22.2%), where the increase in the studied indicator was 0.8%.

Table 3. The effect of the growth regulator Pilarhance, SL on the number of fruits and their percentage of fruit set in Erdi Botermo sour cherry trees

Variants	Number of flowers, pcs./tree	Number of fruits, pcs./tree	Percentage of fruit set, %
Control	3364	720	21.4
Pilarhance, SL, 0.8 l/ha	3324	738	22.2
Pilarhance, SL, 1.3 l/ha	3319	780	23.5
LSD 5%	159	31.4	-

The highest percentage of fruit set (23.5%) was recorded in the variant treated with Pilarhance, SL at a dose of 1.3 l/ha, i.e., the increase was 1.3-2.1% compared to the previous variants.

The results obtained prove that trees treated with Pilarhance, SL at a dose of 1.3 l/ha had a higher percentage of fruit set compared to the other experimental variants.

Productivity is an indicator that demonstrates how all agrotechnical measures were carried out in the sour cherry orchard of the Erdi Botermo variety.

The conducted studies proved that the studied growth regulator Pilarhance, SL affected the average weight of fruits and yield as a whole, but in different ways.

During the research, the lowest fruit weight was observed in the control variant (5.45 g) (Table 4).

Table 4. The effect of the growth regulator Pilarhance, SL on the average weight and productivity of sour cherry trees of the Erdi Botermo variety

Variants	Average weight, g	Productivity		As a % of the control group
		kg/tree	t/ha	
Control	5.45	3.92	4.90	0.0
Pilarhance, SL, 0.8 l/ha	5.55	4.10	5.13	104.7
Pilarhance, SL, 1.3 l/ha	5.77	4.50	5.62	114.7
LSD 5%	0.24	0.19	0.25	-

When using the growth regulator Pilarhance, SL at a dose of 0.8 l/ha, the average fruit weight was insignificantly higher than the control variant,

i.e., by 1.8%, and amounted to 5.55 g. The results show that the growth regulator Pilarhance, SL at a dose of 0.8 l/ha does not have such a significant effect on the average fruit weight as in the Pilarhance, SL – 1.3 l/ha variant, where this indicator was higher and amounted to 5.77 g, or 4.0% compared to the previous variant and 5.9% compared to the control variant. The change in the average fruit weight in the Pilarhance, SL – 1.3 l/ha variant occurred as a result of improved growth processes in the tree crown and increased plant resistance to unfavorable environmental conditions (frost, drought, diseases).

The control variant exhibited the lowest number of fruits and the lowest average weight per fruit. Accordingly, this variant also showed the lowest yield both per tree (3.92 kg/tree) and per unit area (4.9 t/ha).

When treating the variant with the growth regulator Pilarhance, SL at a dose of 0.8 l/ha, the fruit yield was higher than the control variant, but much lower than the Pilarhance, SL variant at a dose of 1.3 l/ha. The resulting yield in this variant was 5.22 kg per tree and 6.52 tons per hectare, representing an increase of 4.7% compared to the control variant.

In the case of the tested variant with Pilarhance, SL at a dose of 1.3 l/ha, a significant increase in the number of fruits (780 pieces/tree) and their average weight (5.77 g) was recorded, which led to a significant increase in yield both per tree (4.5 kg) and per unit area (5.62 t/ha).

The treatment doses had different effects on the yield obtained in the experimental variants. That is, the difference between the variants treated with the growth regulator Pilarhance, SL at doses of 0.8 and 1.3 l/ha was more significant, which is confirmed by statistical analysis.

The statistical analysis data on productivity indicate an insignificant difference between the control variant and the Pilarhance, SL variant at a dose of 0.8 l/ha, and a significant effect compared with the Pilarhance, SL variant at a dose of 1.3 l/ha. That is, if the difference in fruit yield obtained between the control and the variant treated with Pilarhance, SL at a dose of 1.3 l/ha reached 14.7%, then between the variant with Pilarhance, SL at a dose of 0.8 l/ha and the previous variant, this value was 10.0%.

The results obtained prove that among the experimental variants, the highest fruit yield was

recorded when Pilarhance, SL at a dose of 1.3 l/ha was applied compared to the other variants included in this study.

The observations (Table 5) show that the firmness of the sour cherry pulp of the Erdi Botermo variety at the time of harvest in the studied variants ranged from 0.38 to 0.46 kg/cm².

The firmness of Erdi Botermo sour cherry fruits changes under the influence of the growth regulator Pilarhance, SL, meaning that the corresponding preparations, whose active ingredient is gibberellic acid + 24-epibrassinolide, increase the firmness of sour cherries to some extent. While, in the control variant, fruit firmness was 0.38 kg/cm², in the variants treated with the growth regulator Pilarhance, SL, the corresponding indicator increased, reaching parameters of 0.41-0.46 kg/cm².

Comparing the variants treated with gibberellic acid + 24-epibrassinolide, it can be noted that the lowest fruit firmness was observed in the Pilarhance, SL variant at a dose of 0.8 l/ha - 0.41 kg/cm², i.e., an increase of 0.03 kg/cm² compared to the control variant. When the dose of the tested growth regulator Pilarhance, SL was increased to 1.3 l/ha, this indicator was 21.1% higher than in the control variant, reaching 0.46 kg/cm².

Table 5. The effect of the growth regulator Pilarhance, SL on the firmness of sour cherry fruits of the Erdi Botermo variety and their biochemical parameters

Variants	Firmness, kg/cm ²	TSS, %	TA, %	Vitamin C, mg%,
Control	0.38	16.5	0.64	15.9
Pilarhance, SL, 0.8 l/ha	0.41	16.8	0.60	16.1
Pilarhance, SL, 1.3 l/ha	0.46	17.2	0.57	16.4

Studying the effect of the treatment dose on the studied parameter, it can be noted that increasing the dose of the Pilarhance, SL preparation from 0.8 to 1.3 l/ha increased fruit firmness by 0.05 kg/cm², meaning the sour cherry fruit peel becomes more elastic and the pulp firmer.

The content of soluble solids is a characteristic of the variety, and the value of this indicator can be used to determine the optimal harvest time. As the harvest period approaches, the intensity of soluble solids accumulation increases.

The conducted studies prove that the amount of soluble solids in the fruits of Erdi Botermo sour cherry variety was ranging between 16.5-17.2%. The lowest proportion of soluble solids content was recorded in the control – 16.5%. When using the growth regulator Pilarhance, SL, an increase in the studied indicator to 16.5-17.2% was noted. The growth regulator Pilarhance, SL increased the content of soluble solids in fruits by 0.3-0.7% compared to the control.

Studying the effect of the treatment dose on the amount of soluble solids in fruits, it can be noted that with an increase in the dose of the Pilarhance, SL preparation from 0.8 to 1.3 l/ha, the studied indicator increased. If the difference between the variants treated with the growth regulator Pilarhance, SL at doses of 0.8 and 1.3 l/ha was 0.4%, then between the control variant and the variant treated with Pilarhance, SL at a dose of 1.3 l/ha, the increase was 0.7%. Thus, treatment with the growth regulator Pilarhance, SL at a dose of 1.3 l/ha significantly influenced the increase in the amount of soluble solids in the fruits. The proportion of titratable acids in fruits is directly dependent on the amount of soluble solids. Simultaneously, as the amount of soluble solids in fruits decreases, the proportion of titratable acids increases, reaching its highest value in the control - 0.64%.

Applying the growth regulator Pilarhance, SL at a dose of 1.3 l/ha to the trees reduced the proportion of titratable acids in this variant to 0.57%, which is 0.07% less than in the control variant. In the variant treated with the growth regulator Pilarhance, SL at a dose of 0.8 l/ha, the studied indicator was 0.60%, which was 0.04% lower than the control variant.

Vitamin C is one of the main indicators of sour cherry fruit quality. The conducted studies prove that the lowest amount of vitamin C was recorded in the control variant, where its content was 15.9 mg%.

Treatment of the experimental variants with the growth regulator Pilarhance, SL increased the vitamin C content to 16.1-16.4 mg%, which is 0.2-0.5 mg% more compared to the control variant. The highest amount of vitamin C was observed in the Pilarhance, SL variant at a dose of 1.3 l/ha (16.4 mg%), which is 0.5 mg% more than the control variant and 0.3 mg% more than the Pilarhance, SL variant at a dose of 0.8 l/ha.

The results show that the application of the growth regulator Pilarhance, SL at a dose of 1.3 l/ha increased the firmness of fruits, while soluble solids, titratable acidity, and vitamin C content in this variant were more balanced in the sour cherry fruits compared to the other experimental variants.

CONCLUSIONS

The effectiveness of using the growth regulator Pilarhance, SL at a dose of 0.8 l/ha to stimulate growth processes and fruit development was insignificantly higher than the control variant, while with an increase in the dose to 1.3 l/ha, the studied indicator was much higher compared to the other tested variants.

The greatest intensity of growth processes in the tree crown and sour cherry fruit development was recorded in the variant with Pilarhance, SL at a dose of 1.3 l/ha, treated twice by spraying. The first treatment should be carried out at the end of the flowering period, and the second during the period when the fruit color changes from green to yellow.

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EVALUATION OF THE PRODUCTIVITY AND MORPHOMETRIC CHARACTERISTICS OF THE SIBERIAN BLUEBERRY FRUITS (*LONICERA CAERULEA* L.)

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Abstract

Siberian blueberry (*Lonicera caerulea* L.) is an emerging berry crop, which is attracting increasing interest due to its high nutritional value and adaptability to various climatic conditions. This study evaluated the productive performance and morphometric characteristics in Siberian blueberry fruits in the fifth year of cultivation under pedoclimatic conditions of the Bucharest-Băneasa area. Productivity was analysed using descriptive statistics and Pearson correlations, highlighting a very strong and positive correlation between number of fruits and total production ($r = 0.956$), while the average fruit mass had an insignificant influence on the yield. The analysis of the fruit morphometric characteristics of the fruits included determinations of the height, diameter, and individual mass, complemented by Pearson correlations and linear regressions, highlighting that the mass of the fruit was strongly correlated with its height ($r = 0.763$), compared to a moderate correlation with the diameter ($r = 0.465$). Therefore, production was determined by fruit load, and fruit mass accumulation was associated with fruit elongation. The results confirm the productive stability and adaptability of Siberian blueberry to the real conditions in south-eastern Romania.

Key words: Siberian blueberry, *Lonicera caerulea* L., productivity, fruit morphometry, Pearson correlation, linear regression.

INTRODUCTION

The global transition to healthy, predominantly plant-based diets has driven a sharp increase in demand for berries, recognized as important sources of bioactive compounds and antioxidants (Bujor et al., 2025; Lobos & Hancock, 2015). In this context, the tall blueberry (*Vaccinium corymbosum* L.) and the Siberian blueberry (*Lonicera caerulea* L.) have become species of major interest for horticulture and the functional food industry (Xu et al., 2025). If the blueberry is a pillar of the global berry market (Bujor et al., 2025), *Lonicera caerulea* L. stands out for its very rich nutraceutical profile, characterized by high contents of polyphenols, anthocyanins, and ascorbic acid, with documented beneficial effects on human health (Boyarskikh, 2017; Grobelna et al., 2020; Svarcova et al., 2007). The expansion of the cultivation of these species outside their traditional areas is, however, limited by pedoclimatic and biological

constraints. For acidophilic species, the high soil pH constitutes a major agronomic barrier, which has led to the adoption of soilless cultivation systems, in containers or raised beds, with controlled substrates, as a viable technological solution in pedologically unfavorable areas (De Salvador et al., 2017; Çelik & Acar, 2025). Although these systems favor vigorous early vegetative growth, their sustainability and productive performance in the medium and long term depend on the adaptation of the cultivated genotypes (Pinto et al., 2017; Starast et al., 2017). Fruit development is a complex process that follows distinct growth patterns (e.g., a double “S” curve) and involves coordinated cell division and expansion, ultimately determining the final morphology (Wan et al., 2024).

The productivity of both species is also closely linked to reproductive biology, with fruit set being dependent on efficient cross-pollination, influenced by pollen viability and floral morphology (Lyrene, 1994; Boyarskikh, 2017). The use of inappropriate combinations of

pollinator varieties can lead to significant reductions in yield and fruit mass (Boyarskikh, 2017).

Overcoming these limitations requires rigorous selection and improvement of genetic material, based on the phenotypic and genetic diversity existing within both genera (Qi et al., 2025; Truta et al., 2013). The main objectives of breeding are aimed at phenological adaptation to local conditions and optimization of yield, fruit quality, and stress tolerance (Lobos & Hancock, 2015; Pavliuk et al., 2025; Gerbrandt, 2018), by evaluating germplasm in target environments (Malodobry et al., 2013; Ochmian et al., 2012). In this context, fruit size and weight are essential economic traits, directly correlated with commercial acceptability and yield, and are determined both genetically and by environmental factors and the cultivation system (Jorquera-Fontena et al., 2017; Starast et al., 2017).

Although there has been recent progress, research on the species *Lonicera caerulea* L. remains limited in the specific conditions of southern Romania. Although data are available on the biochemical composition, reproductive biology, and phenotypic diversity of the species (Boyarskikh, 2017; Grobelna et al., 2020; Qi et al., 2025), integrated studies that simultaneously evaluate plant productivity and morphometric determinants of fruit weight in cropping systems are lacking.

Therefore, the objective of the present study was to analyse, at two complementary levels, the productive performance of *Lonicera caerulea* L. plants cultivated in south-eastern Romania, as well as the quantitative relationships between fruit dimensions (height and diameter) and their individual weight. The results obtained contribute to clarifying the morphological bases of fruit yield and quality and provide relevant information both for growers and for breeding programs oriented towards the optimization of this emerging crop.

MATERIALS AND METHODS

The study was conducted on Siberian blueberry plants (*Lonicera caerulea* L.) in their fifth year of cultivation under field conditions in the Bucharest-Băneasa area, Romania. The study area is characterized by pedoclimatic conditions

specific to south-eastern Romania, favourable for fruit shrub crops.

The soil on which the blueberry experimental plot was located, in the Băneasa, area of Bucharest, is of the cambic chernozem type, with a weakly acidic reaction (pH = 6.6). The chemical analysis of the soil indicated heavy metal concentrations below the alert thresholds, namely Pb < 17.0 mg/kg, Ni 24.7 mg/kg, Cu 27.84 mg/kg, Cd 0.20 mg/kg and Zn 62.92 mg/kg, as well as a high content of Mn (591.5 mg/kg). The soluble salt content was moderate, with values of 108.49 mg/kg for chlorides and 39.0 mg S-SO₄²⁻/kg, the soil being classified as non-saline. From a physical point of view, the soil presented an apparent density of 1.31 g/cm³, a total porosity of 53% and a loamy texture, characteristics that ensure adequate conditions for the development of the blueberry root system.

The experimental blueberry plot, with an area of approximately 500 m², was organized according to the agrotechnical norms specific to the species. The plants were arranged in rows, at a distance of 1.0 m between plants in a row and 3.0 m between rows, ensuring good air circulation, optimal exposure to light, and easy access for maintenance work. The crop was maintained by grassing with alfalfa (*Medicago sativa* L.) between the rows, keeping the strip in the row free of vegetation. Agricultural work was carried out uniformly over the entire surface, so that all plants benefited from similar conditions of nutrition and natural humidity, minimizing the influence of technological differences on the experimental results.

The biological material was represented by an experimental lot consisting of a single plot, without experimental variants or treatments applied. The analysis was observational in nature, following the natural expression of the production and morphological characters of the fruits under real cultivation conditions. The evaluations were carried out during the period of full maturity of the fruits, during the harvest season, mid-May 2025.

Determination of production at plant level

Seven individual plants (n = 7) were selected for production analysis. The following parameters were determined for each plant:

- total number of fruits per plant;

- Total production (g/plant), determined by weighing the harvested fruits;
- Average fruit weight (g), calculated as the ratio of total production to the number of fruits per plant.

The data obtained at the plant level were used exclusively for the evaluation of productive performance and were not combined with the data obtained at the fruit level to avoid pseudoreplication.

Morphometric analysis of fruits

The morphometric characteristics of the fruits were evaluated on a sample of 35 fruits ($n = 35$), randomly harvested from several plants in the experimental plot. For each fruit, the following were determined:

- fruit height (mm);
- fruit diameter (mm);
- individual fruit mass (g).

Fruit dimensions were measured using a digital calliper, and individual mass was determined with a precision electronic balance. Measurements were performed on fresh fruits, immediately after harvest.

Aspects of the experimental research, including plants in the fifth year of cultivation, fruit harvesting, and the measurement of morphometric characteristics, are illustrated in Figure 1.



Figure 1. Aspects during experimental research on *Lonicera caerulea* L.

Statistical analysis

The experimental data were subjected to descriptive statistical analysis, calculating the mean, standard deviation, minimum and maximum values, as well as the coefficient of variation (CV, %). The relationships between the analysed parameters were evaluated by the Pearson correlation coefficient.

To quantify the influence of morphometric traits on fruit mass, simple linear regression models were applied between:

- fruit mass and fruit height;
- fruit mass and fruit diameter;
- fruit diameter and fruit height.

Statistical analyses were performed separately for plant-level and fruit-level data to ensure correct biological interpretation. Statistical significance was assessed at $p < 0.05$. Results were plotted to highlight relationships between variables.

RESULTS AND DISCUSSIONS

The fifth year of cultivation represents a critical stage in the productive maturation of *Lonicera caerulea* L., when plants are expected to express a stable yield potential and characteristic fruit morphology.

1. Yield performance at plant level

The main yield components and their descriptive statistics at plant level are presented in Table 1.

Table 1 - Descriptive statistics of yield-related traits at plant level ($n = 7$)

Plant	No. of fruits	Yield (g/plant)	Average fruit weight (g)
1	74	44.7	0.60
2	174	106.6	0.61
3	102	58.8	0.58
4	89	49.0	0.55
5	34	21.3	0.63
6	180	86.2	0.48
7	125	83.1	0.67
Mean $\pm$ SD	111.14 $\pm$ 52.90	64.24 $\pm$ 29.21	0.59 $\pm$ 0.06
Min.-Max.	34-180	21.3-106.6	0.48-0.67
CV (%)	47.59	45.47	10.23

It can be seen from Table 1 that Siberian bilberry (*Lonicera caerulea* L.) recorded an average production of approximately 64 g per plant, with significant individual variations ranging from 21.3 g to 106.6 g per plant. The number of fruits per plant fluctuated between 34 and 180, indicating a moderate variability in productive load between plants. The average mass of a fruit was relatively stable, ranging from 0.48-0.67 g, which reflects a good development of the morphological characters specific to the species in the pedoclimatic conditions of the Bucharest-Băneasa area.

Regression equation:

$$\text{Total yield (g)} = 5.58 + 0.528 \times \text{Number of fruits}$$

In Figure 2 it shows relationship between the number of fruits per plant and total yield in *Lonicera caerulea* L.

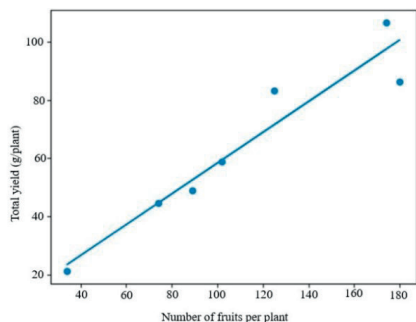


Figure 2. Relationship between number of fruits per plant and total yield in *Lonicera caerulea* L.

The coefficient of variation (CV) was high for both fruit number and total yield, indicating substantial biological variability among individual plants, whereas the low CV for average fruit weight reflected good morphological stability. A strong positive correlation was observed between fruit number and total yield ($r = 0.956$), while the correlation between fruit weight and total yield was weak and biologically negligible ($r = -0.117$). A moderate negative relationship was detected between fruit number and average fruit weight ($r = -0.395$), indicating a tendency toward reduced fruit mass at higher fruit loads. Each additional fruit contributed, on average, approximately 0.53 g to total yield per plant, and fruit number explained 91.4% ($R^2 = 0.914$) of the total yield variation. Overall, yield variation was primarily driven by fruit number rather than individual fruit weight, indicating that fruit load was the main determinant of productivity in the fifth year of cultivation.

The coefficient of variation calculated for total production indicates acceptable uniformity of the crop, characteristic of plantations at biological maturity, according to observations in the literature on blueberry crops (Qi et al., 2025). Correlation analysis revealed a significant positive relationship between the number of fruits and the total mass of production, suggesting that variation in production is mainly determined by fruit load, and variation in the individual mass of the fruit has a much smaller

impact on total production (Jorquera-Fontena et al., 2017).

An increase in fruit number was associated with an almost proportional increase in total yield, whereas average fruit weight showed limited variation and therefore had a negligible influence on yield formation. From a biological perspective, higher productivity resulted primarily from increased fruit load rather than from an increase in individual fruit size.

The results obtained confirm previous observations regarding the importance of fruit number as a determining factor of total production in Siberian blueberry (*Lonicera caerulea* L.). The moderate variability of fruit load and the stability of fruit mass are consistent with studies by Qi et al. (2025), who highlighted that in mature plants, variations in total production are mainly explained by fluctuations in fruit number, rather than by changes in average fruit mass.

On the other hand, Jorquera-Fontena et al. (2017) highlighted that the relationship between morphological dimensions of fruits and their weight can vary depending on the species and growing conditions, and in the case of Siberian blueberry, the stability of individual mass suggests a potential for selection to maintain fruit quality, even under intensive production conditions.

2. Fruit morphometric characteristics

In addition to yield-related traits, fruit morphometric characteristics were evaluated on a sample of 35 fruits. Individual characteristics of blueberry fruits (1-35): height, diameter, and mass, with the means of each parameter indicated by dotted lines, were graphically represented in Figure 3.

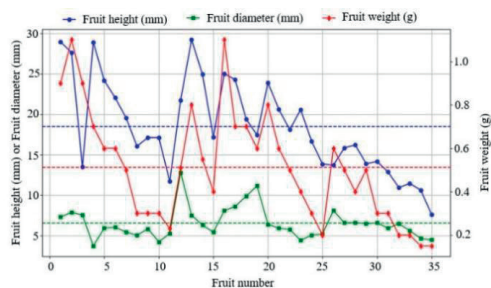


Figure 3. Individual characteristics of blueberry fruits: height, diameter, and mass

Following the analysis of the 35 blueberry fruits, significant variability in the measured

morphometric characters is observed, namely the height, diameter, and mass of the fruit. The height of the fruits presents values ranging between 7.61 mm and 29.2 mm, with the majority of the fruits falling within the range of 15-25 mm, which indicates a relatively uniform development of the analyzed population, with the exception of some less developed fruits. The diameter of the fruits varies between 3.73 mm and 12.8 mm, with most values being concentrated around the range of 5–7 mm. The presence of fruits with a larger diameter is observed, which can be considered better developed from a morphological point of view, suggesting potential superior quality. The mass of the fruits ranges between 0.15 g and 1.1 g, with the most common values being between 0.3 g and 0.8 g. Fruits with reduced mass generally correspond to smaller sizes, while fruits with greater mass are associated with high values of diameter and height. Overall, a direct relationship is observed between fruit size and mass, with diameter having a more pronounced influence on mass than height. The variability recorded can be explained by differences in maturity, fruit position on the plant, or environmental conditions, confirming the non-uniform character of the development of the analyzed fruits (Jorquera-Fontena et al., 2017; Lobos & Hancock, 2015; Malodobry et al., 2013). Statistical analysis of fruit morphometric traits revealed moderate to high variability among the analyzed samples, are presented in Table 2.

Table 2. Descriptive statistics of fruit morphometric characteristics (n = 35)

Parameter	Medie	SD	Min.	Max.	CV (%)
Fruit height (mm)	18.48	5.77	7.61	29.20	31.20
Fruit diameter (mm)	6.52	1.91	3.73	12.80	29.28
Fruit weight (g)	0.511	0.257	0.15	1.10	50.28

To quantify the relationships between fruit morphometric parameters, simple linear regression was applied between individual mass and fruit height, individual mass and fruit diameter, as well as between diameter and height, allowing the evaluation of the influence of each character on fruit mass and shape.

In Figure 4 it is shown between fruit height and individual fruit weight in *Lonicera caerulea* L. (n = 35).

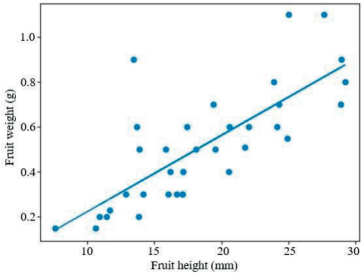


Figure 4. Linear regression between fruit height and individual fruit weight

The regression equation is:

$$\text{Fruit weight (g)} = -0.117 + 0.034 \times \text{Fruit height (mm)}$$

It is therefore observed that each additional millimeter in fruit height corresponds, on average, to an increase of ~0.034 g in mass. The coefficient of determination $R^2 = 0.582$ suggests that 58.2% of the variation in fruit mass is explained by height, which highlights its major role in determining individual mass. The negative intercept has no direct biological relevance, being only a statistical consequence. Therefore, fruit height is an important predictor of individual mass in *Lonicera caerulea* L., highlighting that longitudinal growth contributes significantly to mass accumulation. Data on longitudinal and transverse dimensions of blueberry fruits demonstrate that some morphological dimensions may be differently correlated with the actual weight of the fruits (Wan et al., 2024).

In Figure 5 it is shown a linear regression between fruit diameter and individual fruit weight in *Lonicera caerulea* L. (n = 35).

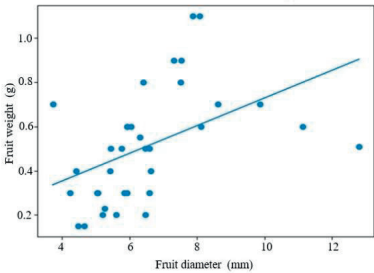


Figure 5. Linear regression between fruit diameter and individual fruit weight

The regression equation is:

$$\text{Fruit weight (g)} = 0.103 + 0.063 \times \text{Fruit diameter (mm)}$$

A moderate positive relationship was observed ($R^2 = 0.216$), indicating that fruit diameter contributes to fruit mass but explains a limited proportion of weight variability.

The regression analysis revealed a positive linear relationship between fruit diameter and individual fruit weight ($R^2 = 0.216$), indicating that radial growth contributes to fruit mass. However, the relatively low coefficient of determination suggests that diameter alone explains only a limited proportion of weight variability among fruits. In contrast, fruit height exhibited a substantially stronger relationship with fruit weight ($R^2 = 0.582$), demonstrating a higher predictive capacity for mass accumulation. This marked difference highlights fruit elongation as the primary morphological driver of fruit weight in the studied material.

The stronger association between fruit height and mass may reflect species-specific growth patterns in *Lonicera caerulea* L., where longitudinal cell expansion and berry elongation play a dominant role during fruit development. Similar findings have been reported in other elongated berry species, where fruit length has been shown to be a better predictor of fresh weight than diameter, due to asymmetric growth dynamics and differential cell division along the longitudinal axis (Jorquera-Fontena et al., 2017; Wan et al., 2024). The relatively weaker influence of diameter suggests that radial expansion occurs to a lesser extent or contributes less consistently to total fruit biomass.

In Figure 6 it is shown a linear regression between fruit height and individual fruit diameter in *Lonicera caerulea* L. ($n = 35$).

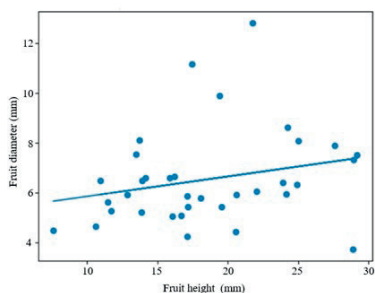


Figure 6. Linear regression between fruit height and individual fruit diameter

The regression equation is:

$$\text{Fruit diameter (mm)} = 5.051 + 0.080 \times \text{Fruit height (mm)}$$

The very low R^2 value ($R^2 = 0.058$) indicates that only ~6% of the variation in fruit diameter is explained by height variation. The relationship is very weak, although the slope is positive. Biologically, this result shows that fruit height and diameter are relatively independent characters: longitudinal growth does not automatically imply radial growth. This is typical for *Lonicera caerulea* L., a species with elongated fruits, where shape is determined more genetically than by proportional growth. Pearson correlations between the analyzed parameters show that fruit mass is more closely related to height ($r = 0.763$, strongly positive correlation) than to diameter ($r = 0.465$, moderate correlation), suggesting that more elongated fruits tend to be heavier, and diameter influences mass to a lesser extent. Height and diameter have a weak positive correlation ($r = 0.241$), indicating that fruit shape varies independently to some extent.

From a physiological perspective, the dominance of fruit height over diameter in explaining fruit weight may be related to vascular architecture and the distribution of assimilates. Longitudinal growth potentially allows for a more efficient allocation of photoassimilates along the fruit axis, resulting in greater mass accumulation without proportional increases in diameter (Wan et al., 2024; Boyarskikh, 2017). In addition, environmental and genetic variability among fruits may further modulate radial growth, thus reducing the explanatory power of diameter-based models (Starast et al., 2017; Lobos & Hancock, 2015; Lyrene, 1994).

The moderate relationship observed between fruit diameter and mass should not be ignored, as it still indicates a positive contribution to fruit weight. However, when considered in isolation, diameter appears insufficient as a reliable predictor of yield-related traits. These results highlight the importance of multivariate approaches when evaluating fruit quality parameters, especially in breeding and selection programs aimed at improving fruit size and uniformity (Truță et al., 2013; Xu et al., 2025).

The relatively high variability observed between individual fruits may also reflect differences in

plant vigor, fruit position, and environmental conditions. As the fruits analyzed came from multiple plants, interplant variability likely contributed to the dispersion of morphological traits. However, the consistent superiority of fruit height as a predictor of mass in the data set underscores the robustness of this relationship. From an applied perspective, the results have practical implications for cultivar evaluation and agronomic management. Selection strategies focused on increasing fruit elongation, rather than diameter alone, may be more effective in improving individual fruit weight and overall yield. Furthermore, fruit height could serve as a rapid, non-destructive indicator of fruit mass in field assessments and phenotypic screening.

CONCLUSIONS

Fruit height was identified as the main morphological determinant of fruit weight in *Lonicera caerulea* L., and can be used as a rapid and non-destructive indicator of fruit quality.

Fruit weight is explained to a greater extent by fruit height than by diameter, which highlights the dominant role of fruit elongation in mass accumulation.

Fruit diameter showed a positive relationship with fruit mass, but its predictive capacity was limited compared to that of height.

Fruit shape proved stable, with a low correlation between morphological axes, supporting the importance of height as a distinct morphometric parameter.

The results obtained provide relevant information for cultivar evaluation, fruit shape-oriented breeding programs, and agronomic assessment of marketable yield under open-field conditions.

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ANALYSIS OF BIOMETRIC AND ECONOMIC INDICATORS IN TWO PEACH CULTIVARS (*PRUNUS PERSICA* L.) – GLOUGHAVEN AND CRESTHAVEN

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Abstract

The study was conducted during the period 2023-2024 at the Institute of Fruit Growing in Plovdiv. A comparative analysis of the peach varieties 'Gloheaven' and 'Kresthaven' was performed based on biometric and economic indicators. In 2023, the average fruit weight ranged from 0.239 g for the 'Glowhaven' cultivar to 0.256 g for the 'Cresthaven' cultivar. The yield per decare for 'Glowhaven' was 1265 kg, followed by 'Cresthaven' with 1201 kg. The average fruit weight in the second year ranged from 0.159 g for 'Glowhaven' to 0.163 g for 'Cresthaven'. The Randemana as follows: 2023 - 96% for 'Cresthaven' and 95% for 'Glowhaven'; 2024 - 95% for both cultivars. The economic analysis shows that the 'Glowhaven' cultivar has a higher average annual profitability (233%) compared to 'Cresthaven' (62%), which indicates a better ratio between the resources invested and the yield. The results obtained from the yield per decare for both cultivars in 2024 (3624.70 kg for 'Glowhaven' and 1768.13 kg for 'Cresthaven'), all other conditions being equal, emphasize the need to continue observations and conduct a multifactorial analysis over a long period.

Key words: *Prunus persica* (L.) Batsch, peach, biometric indicators, yield, market potential.

INTRODUCTION

The right choice of peach cultivars is crucial for successful and profitable fruit growing. In the context of changing climatic factors and growing competition in the sector, research into cultivar differences and their impact on productivity is becoming increasingly relevant. The peach (*Prunus persica* (L.) Batsch) has a wide distribution range and finds favorable conditions for development in Southern Europe, North Africa, Asia, America, and Oceania. According to the latest FAOSTAT data for 2023, peaches and nectarines rank fourth after plums - 57,740 tons (18%), cherries - 46,230 tons (14%), and apples - 34,930 tons (11%), with a yield of 10,800 tons (3%) from fruit plantations in the country. Despite their lower percentage share, peaches occupy an important place in the structure of fruit growing due to the potential of a crop in which Bulgaria has a comparative economic advantage.

Peach production is in line with the OAP's objectives of supporting local cultivars and traditions, with an emphasis on preserving agrobiodiversity and sustainable rural development. The crop is preferred by small and medium-sized producers, while providing

income diversification and increasing farm sustainability (European Commission, 2018; Bélanger & Pilling, 2019).

To achieve optimal production results in peach cultivation, it is necessary to have in-depth knowledge of the characteristics of the cultivars and their behavior under specific conditions. In recent years, there has been growing interest in the scientific literature in comparative studies that allow the evaluation of different peach cultivars (Surányi, 2020; Pashova, 2024; Barayschuk et al., 2025) in terms of the sustainability and profitability of fruit farms.

Cipriani et al. (2013) implemented a twenty-year breeding program, within which they comparatively analyzed 535 selections and 125 cultivars of peaches and nectarines. The evaluation of pomological and phenological characteristics using a multi-criteria method allows for the selection of the most promising cultivars in view of market requirements.

Mihaylova et al. (2021) investigated the biological activity and mineral composition of early and mid-season peach cultivars, finding significant differences in the nutrient profile related to the phenological ripening period.

Ondrášek et al. (2022) conducted a comparative analysis of phenological and pomological

indicators of 35 traditional and modern peach cultivars from the national genetic collection in the Czech Republic. The results identified cultivars with the potential to renew the cultivar composition and increase the efficiency of commercial production.

Research in the field demonstrates the influence of a number of factors on the production potential of fruit crops, including genetic predisposition (Tareen et al., 2022; Lesmes-Vesga et al., 2022; Vuković et al., 2025; Altan et al., 2025), climatic conditions (Malyshev, 2020; Alexandrov & Hoogenboom, 2000; Vanalli et al., 2021;), agrotechnical measures (Staneva et al., 2018, 2020; Rankova et al., 2021; Drew and Wang, 2025; Harhash et al., 2025) and others.

Established peach cultivars such as Glowhaven, Cresthaven, Redhaven, Evmolpia, Lascava, etc. are grown for mass production in the country. Although Glowhaven and Cresthaven were created in the last century, these cultivars have proven agronomic and consumer advantages, making them some of the most preferred for cultivation and consumption in Bulgaria.

This study aims to compare the cultivars 'Glowhaven' and 'Cresthaven' in terms of biometric and economic indicators.

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MATERIALS AND METHODS

The experimental field is located at the Fruit Growing Institute in Plovdiv. The subject of the study are two peach cultivars: 'Glowhaven' and 'Kresthaven'. The orchard was established in 2018. The study period is 24 calendar months (2023-2024). The trees are in full fruiting and

are grafted onto seed peach rootstock. The orchard is planted according to a 5x3 m scheme (67 trees per decare). They are grown according to an identical set of agronomic practices.

Annual phenological observations are carried out, and the individual phases are determined using the BBCH scale from bud swelling to leaf fall. The biometric indicators of height, width, thickness, and weight of the fruits of both cultivars were recorded. Each sample consisted of 25 fruits selected from all parts of the tree. The dimensions of the fruits were measured using a Mitutoyo CD-15DAX digital caliper and electronic scales.

The results obtained were statistically processed using the method of David B. Duncan (Duncan, 1955; Harter, 1960). The software used was "R3.1.3", in combination with "RStudio-0.98" and the installed package "agricolae 1.2-2" (Mendiburu, 2021). The percentage of fruit flesh yield was determined. Yields were calculated using the average method. The average yields from three trees were taken as reference values, on the basis of which the total harvest volume per decare was calculated.

Within the framework of this study, a financial assessment of the profitability of the studied cultivars was carried out. For each of them, the costs associated with cultivation, including planting material, fertilizers, preparations, labor, and others, were calculated. The potential revenues from the sale of the production at current market prices were assessed, taking into account the quantitative indicators of the fruits. The results should help producers make informed decisions when choosing a suitable variety and an investment activity.

RESULTS AND DISCUSSIONS

One of the most important economic characteristics of fruit crops is fruit size and yield. According to research in the field, these depend on the genetic potential of the cultivar (Petrov & Grigorov, 1981), growing conditions (Iglesias & Echeverria, 2022), nutrient supply (Cai, et al., 2023), disease resistance (Manganaris et al., 2022), accumulation of low positive temperatures during dormancy (chill hours) (Penso et al., 2020), etc.

The results of the two-year study of the two peach cultivars show significant differences in

both the biometric indicators of the fruit and the yields per decare. In 2023, the growing season began on February 27 (BBCH 51) and continued until early November. The duration of the entire growing season was 253 days for Glowhaven and 248 days for Cresthaven (Table 1).

Table 1. Phenological phases and duration of vegetation in days in 2023

Cultivars		BBCH 51	BBCH 62	BBCH 63-65	BBCH 67	BBCH 97	Continued of vegetation in days
Glowhaven	2023	27 Feb	20 Mar	22 Mar	25 Mar	7 Nov	253
Cresthaven		27 Feb	21 Mar	23 Mar	26 Mar	2 Nov	248
Glowhaven	2024	23 Feb	19 Mar	21 Mar	26 Mar	4 Nov	256
Cresthaven		22 Feb	19 Mar	22 Mar	27 Mar	10 Nov	263

In 2024, the beginning of phenological development occurred on February 23 (BBCH 51) for both cultivars observed. The beginning of dormancy (BBCH 97) was observed on November 4 for Glowhaven and on November 10 for Cresthaven. The duration of the entire growing season was longer than in the previous year, ranging from 256 to 263 days (Table 1). According to Ivanova (2015), critical temperatures for flower buds are between -2.7° and -3.5°C, and during flowering they are between -1.6° and -2.2°C. In 2023, damage from late spring frosts was observed, and at the beginning of flowering, negative temperatures reaching -2.5°C were recorded. The negative temperatures measured on March 20, 2023, were sufficient to partially compromise yields (Figure 1).

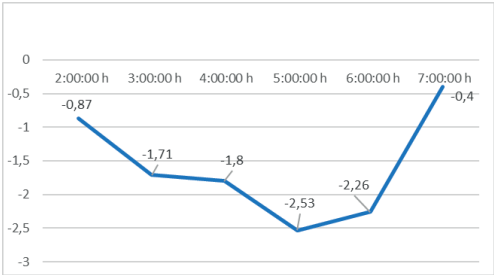


Figure 1 Minimum temperatures recorded in 2023 Source: Author's calculations based on measurements in 2023-2024

An analysis of average monthly temperatures for the period 2023-2024 shows that the winter period provides optimal conditions for accumulating the necessary number of cooling hours (Figure 2).

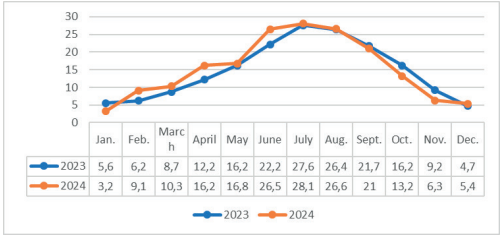


Figure 2. Average monthly temperatures for 2023 and 2024
Source: Author's calculations based on measurements in 2023-2024

In 2023, the average fruit weight ranged from 239.07 g for the Glowhaven cultivar to 256.51 g for the Cresthaven cultivar (Table 2). The study by İkinci and Bolat (2018) on the pomological characteristics of different peach cultivars found that the average fruit weight of the Glowhaven cultivar was 0.115 g. In our study, the values obtained are significantly higher, which is due to the lower load on the trees. The average yields per decare obtained this year range from 1201 kg for Cresthaven to 1265 kg for Glowhaven. In the second year of the study, the average fruit weight ranged from 159.13 g for the Glowhaven cultivar to 163.38 g for the Cresthaven cultivar. The average fruit weight was lower than in the previous year, as a large number of fruit buds differentiated without competition from the limited number of fruits in the previous season. The highest yield was recorded for the Cresthaven cultivar (96.08%), with both cultivars within the acceptable percentage range.

Table 2. Average annual pomological characteristics of fruits

Cultivars	Fruit weight (g), 2023		Fruit weight (g), 2024		Randoman % 2023	Randoman % 2024	Weight of stone (g), 2023		Weight of stone (g), 2024	
Glowhaven	239.0	a	159.1	a	95.7	95.0	10.0	a	7.65	a
Cresthaven	256.5	a	163.3	a	96.0	95.4	9.8	a	7.08	b

Source: Author's calculations based on measurements in 2023-2024.

The values for fruit thickness in the two cultivars studied are similar (Table 3). In terms of stone size, the Cresthaven cultivar stands out with the highest values for all three measured indicators (Table 4). Similar values were found in the study by Pashova (2024), where the stone weight was 9.79 g, while in our study it ranged from 9.83 g for the Cresthaven cultivar to 10.01 g for the Glowhaven cultivar.

The weight of the stone ranges from 7.06 g for Kresthaven to 7.65 g for Glowhaven. The lower fruit weight of the fruit corresponds to smaller dimensions in height, width, and thickness. This year, the measured average weight is lower, which means that the biometric dimensions of the mesocarp and endocarp are smaller compared to the previous year.

Table 3. Average biometric data for fruits for 2023-2024

Cultivars	Fruit size											
	hight (mm)		hight (mm)		width (mm)		width (mm)		thickness (mm)			
	2023		2024		2023		2024		2023		2024	
Glowhaven	71.96	a	64.38	b	78.14	b	67.50	a	77.39	a	67.45	a
Cresthaven	73.15	a	67.07	a	81.01	a	67.41	a	76.54	a	65.67	a

Source: Author's calculations based on measurements in 2023-2024.

Table 4. Average biometric data for stones for 2023-2024

Cultivars	Stone size											
	hight (mm) 2023		hight (mm) 2024		width (mm) 2023		width (mm) 2024		thickness (mm) 2023		thickness (mm) 2024	
Glowhaven	38.24	a	37.73	a	19.84	b	19.13	a	27.76	b	26.09	a
Cresthaven	38.92	a	37.21	a	22.30	a	19.24	a	29.40	a	25.90	a

Source: Author's calculations based on measurements in 2023-2024.

In 2024, despite the smaller size of the fruit, the quantity per tree is greater, with a significant increase in yields for both cultivars. The average yield per tree increases to 54.10 kg for Glowhaven and 26.39 kg for Cresthaven.

During dormancy, consistently low, positive temperatures play an important role in the accumulation of phospholipids in cell membranes, which ensure the proper functioning of vegetative and reproductive buds. During this period, when warm and cold days alternate, the process of phospholipid accumulation is disrupted, leading to disturbances in the normal physiology of the buds (Erez et al., 1997).

The results show that, despite the low positive temperatures required by both cultivars being met, during the period November 2023 to February 2024 during winter dormancy, higher daily temperatures (above 7°C) had an adverse effect on the physiological activity of the fruit buds of the Cresthaven cultivar, which is a prerequisite for a reduction in yield in 2024 (Figure 3).

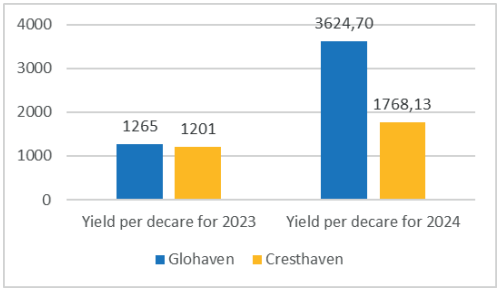


Figure 3. Average yield per hectare, by cultivar, for 2023 and 2024
Source: Author's calculations based on measurements in 2023-2024

With identical costs per decare, the Glowhaven cultivar provides significantly higher yields and profits, with average annual profitability exceeding that of Cresthaven, demonstrating higher economic potential and better adaptation to regional climatic conditions (Table 5).

Table 5. Economic calculations for growing peaches of the Glowhaven and Cresthaven cultivars, at an average selling price of BGN 3.50/kg

Cultivars	Yield per hectare in kg in 2024.	Expense per decare in BGN	Revenue per decare in BGN	Profit per decare in BGN	Average annual profitability per decare in
Glowhaven	3624,70	3813,62	12686,45	8871,83	233%
Cresthaven	1768,13	3813,62	6188,46	2372,84	62%

Source: Author's calculations based on measurements in 2023-2024.

CONCLUSIONS

The comparative analysis between Glowhaven and Cresthaven reveals statistically significant differences between the cultivars. Glowhaven is distinguished by a larger stone weight and a smaller fruit height and width. Other statistically significant differences are the smaller width and thickness of the stone. Despite the larger fruit

size of the fruit in Cresthaven, the Glowhaven cultivar is distinguished by a more than twofold higher yield at the end of the analyzed period. The Glowhaven cultivar demonstrates higher adaptability to climatic conditions. The results obtained define it as more economically profitable for cultivation in the conditions of the Plovdiv region. The reported differences in yields, under equal agrotechnical conditions, are a prerequisite for a subsequent multifactorial analysis in which yields are considered as the dependent variable over the long term.

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STATISTICAL EVALUATION OF BIOMETRIC AND PHYSICOCHEMICAL VARIABILITY IN THE FRUITS OF FIVE SWEET CHERRY CULTIVARS (*PRUNUS AVIUM* L.)

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Abstract

The cherry (Prunus avium L.) is a fruit tree belonging to the genus Prunus of the Rosaceae family, originating around the Caspian and Black Seas, on the border between Europe and Asia, and thriving best in temperate or Mediterranean climates. Sweet cherries are grown commercially in over 40 countries worldwide (Webster and Looney, 1996). In 2025, cherry production in Romania was severely affected by April frosts, which compromised orchards by up to 100% in some areas. For this study, 5 cherry varieties were analyzed (Inima de Porumbel, Van, Boambe de Cotnari, Bigarreau Burlat, Rosii de Bistrita), originating from a family plantation established 7 years ago in the town of Bistrita, Bistrita-Nasaud County, Romania. The following fruit quality indicators were studied for these varieties: large fruit diameter (mm), small diameter (mm), fruit height (mm), weight (g), soluble dry matter ($^{\circ}$ Bx), sugar percentage (%), fruit pH, and firmness (25 fruits of each variety, from the experimental year 2025). All statistical analyses were performed using R statistical software [v4.4.1; RCoreTeam, 2024]. Comparisons of means were performed using Duncan's multiple-range test (DMRT).

Key words: cherry varieties, biometric characteristics, physicochemical, fruit quality.

INTRODUCTION

Sweet cherries are part of the Rosaceae family, subfamily Prunoideae, and are among the most important and delicious fruits in temperate regions of the world (Wunsch and Hormaza, 2004). They are available on the market from mid-May to July, and there is little competition among different fruit crops in temperate areas (Zheng et al., 2016). Although characterization and evaluation of selected sweet cherry varieties have been previously carried out by several researchers (Scedei et al., 2023; Asanica et al., 2013).

The objective of this study was to verify the quality and physico-chemical performance of the fruits in the context of current climate change (Perju et al., 2024), in the experimental year 2025, the fruits of five varieties (Inima de Porumbel, Van, Boambe de Cotnari, Bigarreau Burlat, Rosii de Bistrita) from a family plantation with an age of 7 years since planting, located in the Bistrita locality, Bistrita – Năsaud County, Romania (47°08'20"N 24°30'01"E).

In Romania, for a long time, cherry cultivation was limited in extent to the few trees existing in family gardens or in small orchards around large cities, intended to satisfy the requirements of strictly local markets where small quantities of fruit were valued (Budan and Grădinariu, 2000; Corneanu et al., 2021; Petre et al., 2005). Currently, in our country, about 7 thousand ha are still cultivated, most of them in the private sector, yielding a production of about 60 thousand tons (Budan and Grădinariu, 2000; Petre et al., 2005).

Sweet cherry (*Prunus avium* L.) is a crop of great importance in the northwest region of Romania due to the favorable cultural conditions and tradition found in this area. Romanian sweet cherry production shows a growth trend (Hrotko, 2016; Bujdosó and Hrotko, 2017). Sweet cherry orchards, according to FAO data, occupy approximately 7,058 ha and produce 90,837 tons annually (FAOSTAT, 2018; FAOSTAT 2021), placing our country among the world's top sweet cherry-producing countries, along with Turkey, Chile, Italy,

Spain, and others. Of the area occupied by sweet cherry orchards in Romania, the main producing counties are Iași, Galați, Vrancea, Vaslui, Botoșani, Neamț, and Arad (Coman & Chițu, 2014; Petre et al., 2005).

According to Grădinaru (2002), sweet cherry cultivation has specific requirements, and their fulfillment is necessary for the success of orchards. In terms of soil characteristics, cherries require well-drained, aerated soil but can tolerate soils ranging from sandy to loamy, as long as drainage is good (Roper and Frank, 2004). Cherry has also been successfully cultivated and has yielded well in soils with organic matter content ranging from less than 1% in sandy soils of warm, semi-arid regions to 10% in cooler temperate regions (Nielsen et al., 2017).

The addition of new local varieties, developed at research stations in the area, together with foreign varieties, is a method of improving the sweet cherry assortment across different orchard management systems (Zlati et al., 2019). For cherry growers, the concept of “fruit quality” depends on the product itself and, of course, on consumer preferences (Romano et al., 2006). It is widely accepted that the main characteristics of sweet cherry quality are fruit weight, color, firmness, sweetness, acidity, taste, and aroma, with important differences between varieties (DíazMula et al., 2009). Pérez-Sánchez et al. (2008 and 2010) show that for consumers, they must have distinct agronomic characteristics (Chivu et al., 2024), such as resistance to fruit cracking, high levels of soluble matter (⁰Brix), ripening time at the extremes of the sweet cherry ripening season, and high resistance (Corneanu et al., 2012).

Sweet cherry is an important fruit species, cultivated and distributed globally (Palasciano et al., 2022). In addition to the traditional and modern varieties known in Europe, a multitude of varieties have been developed through genetic improvement in growing countries, providing a means to expand and enhance the current assortment (Iurea et al., 2019). Sweet cherries are among the first fresh fruits of the year and among the most appreciated for their organoleptic characteristics (color, aroma) and recognized benefits for human health (biochemical composition) (Blando & Oomah, 2019; Meland et al., 2014). Although sweet

cherry is an economically profitable crop, it also has several characteristics that subject it to multiple risks in the context of current climate change (Lang, 2019; Păltineanu & Chitu, 2020; Quero-Garcia et al., 2017).

To limit climatic stress factors (high temperatures, drought) and improve production and its quality, genetic (rootstocks) and horticultural (training systems) research is needed (Whiting et al., 2005; Asănică et al., 2013).

Regarding the rootstock, the adaptability of *Prunus mahaleb* to the continental climate, drought tolerance, and hot summers due to the root system increases its importance for sweet cherry cultivation in the future (Martins et al., 2021; Hrotkó et al., 2023), becoming increasingly used in European countries, Central Asia, and Northwest China (Ercisli et al., 2006; Hrotkó, 2016; Sansavini and Lugli, 2008). These traits are gaining greater importance due to accelerated climate change and replanting conditions. Although researchers agree that rootstocks affect fruit quality, the growth control of mahaleb rootstocks can be managed through frequent pruning and maintaining an optimal crown for optimal production (Tabakov et al., 2020; Martins et al., 2021).

The recent increase in cherry production worldwide has given new importance to fruit quality (Malchev et al., 2022). Thus, recent studies emphasize the value of balancing crop load but also increasing fruit size and quality. For sweet cherries, fruit size and color remain the most important attributes, and the development of strategies to improve fruit quality by researching rootstocks, cultivars, crop load, environmental factors, and final production is of great interest (Whiting & Ophardt, 2005; Zhang & Whiting, 2011; Díaz-Mula et al., 2009).

The objective of this study was to compare five cherry varieties originating from a family plantation in Bistrita, Bistrita-Nasaud County, Romania, in terms of the biometric and physicochemical variability of the fruits, in the context of current climate change (Perju et al., 2024).

The physicochemical characteristics of the fruit are the first elements that attract the consumer and are the basis for varietal selection. The success of the variety is ensured by fruits with

attractive color, large size, firm, tasty pulp, and resistance to transportation (Pérez-Sánchez et al., 2010). These characteristics generally depend on the genetic profile and are also strongly influenced by the environment. Finally, the quality of cherries and consumer preferences are influenced by chemical profiles such as titratable acidity (TA) and total soluble solids (TSS), which are very important parameters for determining the harvest date (Fazzari et al., 2008; Pérez-Sánchez et al., 2010; El Kettabi et al., 2025)

Modern sweet cherry orchards are equipped with rain cover systems, which can affect fruit quality, especially by reducing rain-induced fruit cracking.

The positive effect of covered plantations is that the fruits are larger and heavier, which increases cherry prices and the marketability of the production.

Nowadays, retail chains and consumers demand large-sized cherries, with a fruit width of more than 30 mm (Measham et al., 2014). This size (size) can be achieved by using rain cover systems (Vávra Radek, 2020). These trends in improving the assortment structure should be followed by positive developments in cultivation technology and the application of appropriate pomotechnical and agro-management practices (Radičević et al., 2022). In the marketing process, fruit size is one of the most important quality indicators, with larger cherries being more valuable on the market. Thus, factors that limit production and satisfy market demands for superior cherry quality, such as rootstock choice and tree pruning system, become of major importance (Balan et al., 2022; Cimpoieș, 2018).

MATERIALS AND METHODS

The biological material

For this study, fruits from five cherry varieties with different origins were analyzed. They were harvested from the Bistrita locality, Bistrita-Nasaud County (in 2025), Romania. This year, cherry production was very low due to spring frost. The varieties studied were Inima de Porumbel (origin: Germany), Van (origin: Canada), Boambe de Cotnari (origin: Romania),

Bigarreau Burlat (origin: France), and Rosii de Bistrita (origin: Romania). The biometric and physico-chemical characteristics of the fruits were studied for these varieties, such as average fruit weight, height, and diameter (large diameter, small diameter) of the fruits, fruit firmness, soluble dry matter content, sugar percentage, and fruit acidity (pH).

All statistical procedures to evaluate the biometric and physicochemical parameters of the five sweet cherry cultivars were conducted in R Statistical Software (v4.4.1; R Core Team, 2024). For post-hoc pairwise comparisons among cultivars, Duncan's Multiple Range Test (DMRT) was performed to establish significance levels for differences between any pair of means. This procedure operates independently of a prior significant F-value from the initial ANOVA. Furthermore, the relationships between the fruit quality indicators were analyzed through Pearson linear correlation coefficients and linear regression models.

The analyzed biometric and physicochemical properties

The fruits (25 fruits/variety) were harvested from different parts of the trees (10 trees/variety). The diameter and length of the fruits were measured using a caliper. The weight was determined using an analytical balance. The sugar content of the fruits, expressed as soluble carbohydrates (Brix %), was measured with a digital refractometer (Kruss DR 201-95). The firmness of the fruit pulp was determined with a penetrometer (Force Gauge PCE-FM 200), and the results were expressed in pounds (lbs). The acidity of the fruit juice was determined using a multiparameter analyzer (pH, Consort C-933). The variability in biometric and physicochemical characteristics among cherry varieties is essential for understanding their quality, nutritional value, and suitability for different uses.

This analysis synthesizes results from several studies to provide a comprehensive overview of differences in biometric traits (diameter, weight, height) and physicochemical traits (firmness, Brix index, sugar, and pH) across different cherry cultivars.

RESULTS AND DISCUSSIONS

From the data in Figure 1, it can be seen that the Bigarreau Burlat cherry variety had the highest average large fruit diameter in 2025.

A one-way ANOVA was performed to compare the effect of different types of cherry on the large diameter. It was revealed that there was a statistically significant difference in large-diameter measurements across at least two groups. The main effect of cherry varieties is statistically significant and large ($F(4, 45) = 13.91$, $p < .001$; $\text{Eta}^2 = 0.55$, 95% CI [0.36, 1.00]). Duncan's test was performed to compare group means. Significant differences ($p < 0.05$) were found between the following groups: Bigarreau Burlat and Boambe de Cotnari, with a mean difference of 2.72 ($p = 0.0107$, 95% CI [0.65, 4.79]). A significant difference was observed between Bigarreau Burlat and Inima de Porumbel, with a mean difference of 5.26 ($p < .001$, 95% CI [3.12, 7.4]). A significant difference was observed between Bigarreau Burlat and Rosii de Bistrita, with a mean difference of 6.36 ($p < .001$, 95% CI [4.17, 8.55]). A significant difference was observed between Boambe de Cotnari and Inima de Porumbel, with a mean difference of 2.54 ($p = 0.0127$, 95% CI [0.57, 4.51]). A significant difference was observed between Boambe de Cotnari and Rosii de Bistrita, with a mean difference of 3.64 ($p < .001$, 95% CI [1.57, 5.71]). A significant difference was observed between Inima de Porumbel and Van, with a mean difference of -3.46 ($p = 0.0014$, 95% CI [-5.53, -1.39]).

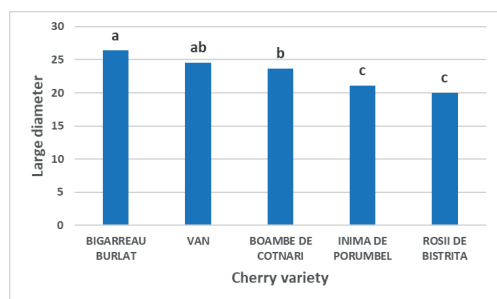


Figure 1. Evaluation of the large diameter (mm) of the fruits of the studied cherry varieties

A significant difference was observed between Rosii de Bistrita and Van, with a mean

difference of -4.56 ($p < .001$, 95% CI [-6.7, -2.42]) (Figure 1).

In the experimental year 2025, the cherry varieties Bigarreau Burlat and Boambe de Cotnari recorded the highest average small-diameter values, according to measurements (Figure 2).

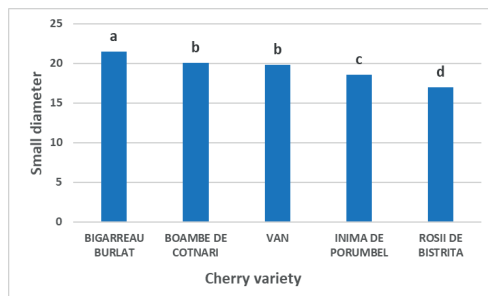


Figure 2. Evaluation of the small diameter (mm) in the fruits of the studied cherry varieties

A one-way ANOVA was performed to compare the effect of different types of cherry on the small diameter. It was revealed that there was a statistically significant difference in small-diameter measurements across at least two groups. The main effect of cherry varieties is statistically significant and large ($F(4, 45) = 22.07$, $p < .001$; $\text{Eta}^2 = 0.66$, 95% CI [0.51, 1.00]) (Figure 2). Duncan's test was performed to compare group means. Significant differences ($p < 0.05$) were found between the following groups: Bigarreau Burlat and Boambe de Cotnari, with a mean difference of 1.47 ($p = 0.0067$, 95% CI [0.43, 2.51]). A significant difference was observed between Bigarreau Burlat and Inima de Porumbel, with a mean difference of 2.95 ($p < .001$, 95% CI [1.82, 4.08]). A significant difference was observed between Bigarreau Burlat and Rosii de Bistrita, with a mean difference of 4.57 ($p < .001$, 95% CI [3.41, 5.73]). A significant difference was observed between Bigarreau Burlat and Van, with a mean difference of 1.69 ($p = 0.0029$, 95% CI [0.6, 2.78]). A significant difference was observed between Boambe de Cotnari and Inima de Porumbel, with a mean difference of 1.48 ($p = 0.0086$, 95% CI [0.39, 2.57]). A significant difference was observed between Boambe de Cotnari and Rosii de Bistrita, with a mean difference of 3.1 ($p < .001$, 95% CI [1.97, 4.23]). A significant difference was observed between

Inima de Porumbel and Rosii de Bistrita, with a mean difference of 1.62 ($p = 0.003$, 95% CI [0.58, 2.66]). A significant difference was observed between Inima de Porumbel and Van, with a mean difference of -1.26 ($p = 0.0188$, 95% CI [-2.3, -0.22]). A significant difference was observed between Rosii de Bistrita and Van, with a mean difference of -2.88 ($p < .001$, 95% CI [-3.97, -1.79]).

Regarding the average height of cherry fruits, we can see from Figure 3 that the Van and Bigarreau Burlat varieties had the highest average values for this parameter.

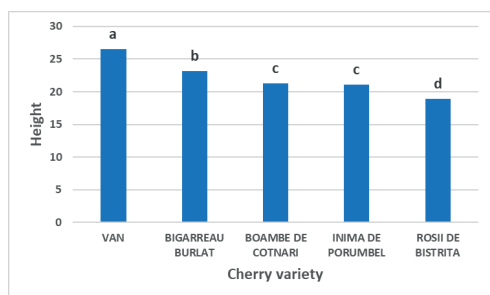


Figure 3. Evaluation of fruit height (mm) in the studied cherry varieties

A one-way ANOVA was performed to compare the effect of different types of cherry on height. It was revealed that there was a statistically significant difference in height between at least two groups. The main effect of cherry varieties is statistically significant and large ($F(4, 45) = 63.18$, $p < .001$; $\text{Eta}^2 = 0.85$, 95% CI [0.78, 1.00]). Duncan's test was performed to compare group means. Significant differences ($p < 0.05$) were found between the following groups: Bigarreau Burlat and Boambe de Cotnari, with a mean difference of 1.89 ($p < .001$, 95% CI [0.86, 2.92]). A significant difference was observed between Bigarreau Burlat and Inima de Porumbel, with a mean difference of 2.03 ($p < .001$, 95% CI [0.95, 3.11]). A significant difference was observed between Bigarreau Burlat and Rosii de Bistrita, with a mean difference of 4.26 ($p < .001$, 95% CI [3.14, 5.38]). A significant difference was observed between Bigarreau Burlat and Van, with a mean difference of -3.43 ($p < .001$, 95% CI [-4.46, -2.4]). A significant difference was observed between Boambe de Cotnari and Rosii de Bistrita, with a mean difference of 2.37 ($p < .001$, 95% CI [1.29, 3.45]).

A significant difference was observed between Boambe de Cotnari and Van, with a mean difference of -5.32 ($p < .001$, 95% CI [-6.4, -4.24]). A significant difference was observed between Inima de Porumbel and Rosii de Bistrita, with a mean difference of 2.23 ($p < .001$, 95% CI [1.2, 3.26]). A significant difference was observed between Inima de Porumbel and Van, with a mean difference of -5.46 ($p < .001$, 95% CI [-6.58, -4.34]). A significant difference was observed between Rosii de Bistrita and Van, with a mean difference of -7.69 ($p < .001$, 95% CI [-8.83, -6.55]).

From the results, we can conclude that the highest average fruit weight was observed in the Bigarreau Burlat and Van varieties (Figure 4).

A one-way ANOVA was performed to compare the effect of different types of cherry on weight. It was revealed that there was a statistically significant difference in the weight between at least two groups. The main effect of cherry varieties is statistically significant and large ($F(4, 45) = 22.49$, $p < .001$; $\text{Eta}^2 = 0.67$, 95% CI [0.52, 1.00]). Duncan's test was performed to compare group means. Significant differences ($p < 0.05$) were found between the following groups: Boambe de Cotnari and Burlat, with a mean difference of -2.71 ($p < .001$, 95% CI [-3.9, -1.52]). A significant difference was observed between Boambe de Cotnari and Pietroase, with a mean difference of 2.08 ($p < .001$, 95% CI [0.89, 3.27]). A significant difference was observed between Boambe de Cotnari and Van, with a mean difference of -1.73 ($p = 0.0034$, 95% CI [-2.86, -0.6]). A significant difference was observed between Burlat and Inima de Porumbel, with a mean difference of 3.15 ($p < .001$, 95% CI [1.93, 4.37]). A significant difference was observed between Burlat and Pietroase, with a mean difference of 4.79 ($p < .001$, 95% CI [3.54, 6.04]). A significant difference was observed between Inima de Porumbel and Pietroase, with a mean difference of 1.64 ($p = 0.0053$, 95% CI [0.51, 2.77]). A significant difference was observed between Inima de Porumbel and Van, with a mean difference of -2.17 ($p < .001$, 95% CI [-3.36, -0.98]). A significant difference was observed between Pietroase and Van, with a mean difference of -3.81 ($p < .001$, 95% CI [-5.03, -2.59]) (Figure 4).

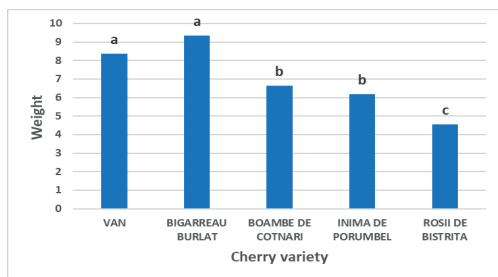


Figure 4. Evaluation of fruit weight (g) of the studied cherry varieties

Among the cherry varieties studied, we can see in Figure 5 that the highest values were obtained by the Boambe de Cotnari and Rosii de Bistrita varieties for the soluble dry matter parameter ($^{\circ}\text{Brix} - \%$).

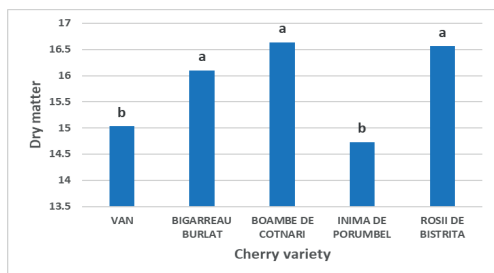


Figure 5. Soluble dry matter ($^{\circ}\text{Brix} - \%$) of the fruits of the studied cherry varieties

A one-way ANOVA was performed to compare the effect of different types of cherry on dry matter. It was revealed that there was a statistically significant difference in the dry matter between at least two groups. The main effect of cherry varieties is statistically significant and large ($F(4, 45) = 7.78$, $p < .001$; $\text{Eta}^2 = 0.41$, 95% CI [0.19, 1.00]). Duncan's test was performed to compare group means. Significant differences ($p < 0.05$) were found between the following groups: Bigarreau Burlat and Inima de Porumbel, with a mean difference of 1.37 ($p = 0.0049$, 95% CI [0.43, 2.31]). A significant difference was observed between Bigarreau Burlat and Van, with a mean difference of 1.07 ($p = 0.0206$, 95% CI [0.17, 1.97]). A significant difference was observed between Boambe de Cotnari and Inima de Porumbel, with a mean difference of 1.9 ($p < .001$, 95% CI [0.9, 2.9]). A significant difference was observed between Boambe de Cotnari and Van, with a mean difference of 1.6 ($p = 0.0015$,

95% CI [0.63, 2.57]). A significant difference was observed between Inima de Porumbel and Rosii de Bistrita, with a mean difference of -1.83 ($p < .001$, 95% CI [-2.8, -0.86]). A significant difference was observed between Rosii de Bistrita and Van, with a mean difference of 1.53 ($p = 0.0018$, 95% CI [0.59, 2.47]) (Figure 5). Regarding the average sugar percentage, the data (Figure 6) show that the varieties Inima de Porumbel and Van recorded the lowest value for this parameter, while the opposite pole are the varieties Boambe de Cotnari and Rosii de Bistrita.

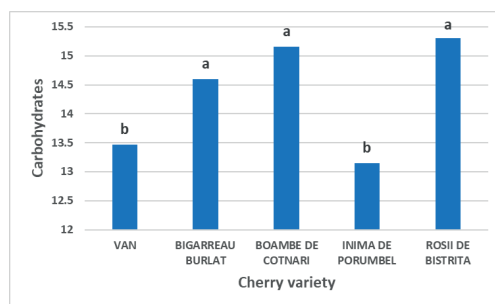


Figure 6. Evaluation of the sugar percentage (%) in the fruits of the studied cherry varieties

A one-way ANOVA was performed to compare the effect of different types of cherry on carbohydrates (Figure 6). It was revealed that there was a statistically significant difference in the carbohydrates between at least two groups. The main effect of cherry varieties is statistically significant and large ($F(4, 45) = 8.51$, $p < .001$; $\text{Eta}^2 = 0.43$, 95% CI [0.22, 1.00]). Duncan's test was performed to compare group means. Significant differences ($p < 0.05$) were found between the following groups: Bigarreau Burlat and Inima de Porumbel, with a mean difference of 1.46 ($p = 0.0053$, 95% CI [0.44, 2.47]). A significant difference was observed between Bigarreau Burlat and Van, with a mean difference of 1.14 ($p = 0.0217$, 95% CI [0.17, 2.1]). A significant difference was observed between Boambe de Cotnari and Inima de Porumbel, with a mean difference of 2.02 ($p < .001$, 95% CI [0.97, 3.06]). A significant difference was observed between Boambe de Cotnari and Van, with a mean difference of 1.7 ($p = 0.0013$, 95% CI [0.69, 2.71]). A significant difference was observed between Inima de Porumbel and Rosii de Bistrita, with a mean

difference of -2.17 ($p < .001$, 95% CI [-3.23, -1.1]). A significant difference was observed between Rosii de Bistrita and Van, with a mean difference of 1.85 ($p < .001$, 95% CI [0.8, 2.89]). From the results obtained regarding the pH of the analyzed cherry fruits, we can state that the varieties Boambe de Cotnari and Rosii de Bistrita recorded the highest values (Figure 7).

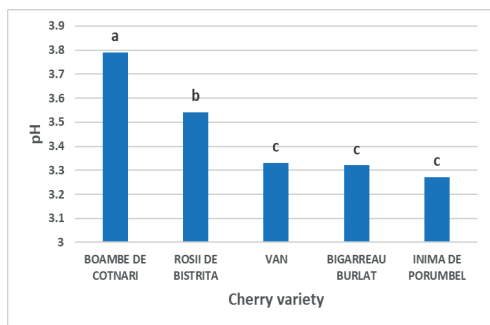


Figure 7. Evaluation of average pH values in the fruits of the studied cherry varieties

A one-way ANOVA was performed to compare the effects of different cherry types on pH (Figure 7). It was revealed that there was a statistically significant difference in the pH between at least two groups. The main effect of cherry varieties is statistically significant and large ($F(4, 10) = 172.72$, $p < .001$; $\eta^2 = 0.99$, 95% CI [0.96, 1.00]). Duncan's test was performed to compare group means. Significant differences ($p < 0.05$) were found between the following groups: Bigarreau Burlat and Boambe de Cotnari, with a mean difference of -0.47 ($p < .001$, 95% CI [-0.53, -0.42]). A significant difference was observed between Bigarreau Burlat and Rosii de Bistrita, with a mean difference of -0.22 ($p < .001$, 95% CI [-0.27, -0.17]). A significant difference was observed between Boambe de Cotnari and Inima de Porumbel, with a mean difference of 0.52 ($p < .001$, 95% CI [0.46, 0.57]). A significant difference was observed between Boambe de Cotnari and Rosii de Bistrita, with a mean difference of 0.25 ($p < .001$, 95% CI [0.2, 0.31]). A significant difference was observed between Boambe de Cotnari and Van, with a mean difference of 0.46 ($p < .001$, 95% CI [0.41, 0.52]). A significant difference was observed between Inima de Porumbel and Rosii de Bistrita, with a mean difference of -0.26 ($p <$

$.001$, 95% CI [-0.32, -0.21]). A significant difference was observed between Rosii de Bistrita and Van, with a mean difference of 0.21 ($p < .001$, 95% CI [0.16, 0.26]).

Regarding the average firmness of the cherry fruits studied, the varieties Inima de Porumbel and Van recorded the highest values (Figure 8).

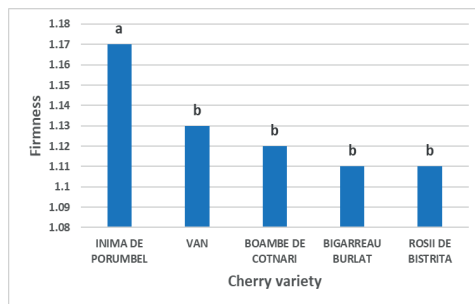


Figure 8. Evaluation of average firmness values in the fruits of the studied cherry varieties

A one-way ANOVA was performed to compare the effect of different types of cherries on firmness (Figure 8). It was revealed that there was a statistically significant difference in firmness between at least two groups. The main effect of cherry varieties is statistically significant and large ($F(4, 20) = 4.08$, $p = 0.014$; $\eta^2 = 0.45$, 95% CI [0.08, 1.00]). Duncan's test was performed to compare group means. Significant differences ($p < 0.05$) were found between the following groups: Bigarreau Burlat and Inima de Porumbel, with a mean difference of -0.06 ($p = 0.0047$, 95% CI [-0.09, -0.02]). A significant difference was observed between Boambe de Cotnari and Inima de Porumbel, with a mean difference of -0.05 ($p = 0.0147$, 95% CI [-0.08, -0.01]). A significant difference was observed between Inima de Porumbel and Rosii de Bistrita, with a mean difference of 0.06 ($p = 0.0041$, 95% CI [0.02, 0.1]). A significant difference was observed between Inima de Porumbel and Van, with a mean difference of 0.04 ($p = 0.0321$, 95% CI [0, 0.07]).

The Pearson's product-moment correlation between CHO and DM (Carbohydrates and Dry Matter) is positive, statistically significant, and very large ($r = 0.99$, 95% CI [0.91, 1.00], $t(3) = 15.47$, $p < .001$) (Figure 9).

Pearson's product-moment correlation between FW and LD (Weight and Large Diameter) is positive, statistically significant, and very large

($r = 0.96$, 95% CI [0.51, 1.00], $t(3) = 5.95$, $p = 0.009$).

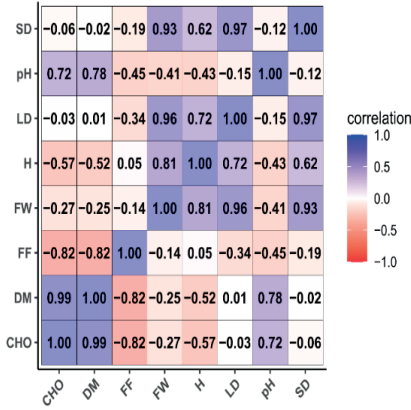


Figure 9. Correlation between biometric and physicochemical characteristics of the studied cherry varieties

Pearson's product-moment correlation between FW and SD (Weight and Small Diameter) is positive, statistically significant, and very large ($r = 0.93$, 95% CI [0.26, 1.00], $t(3) = 4.33$, $p = 0.023$) (Figure 10). Pearson's product-moment correlation between LD and SD (Large Diameter and Small Diameter) is positive, statistically significant, and very large ($r = 0.97$, 95% CI [0.58, 1.00], $t(3) = 6.59$, $p = 0.007$).

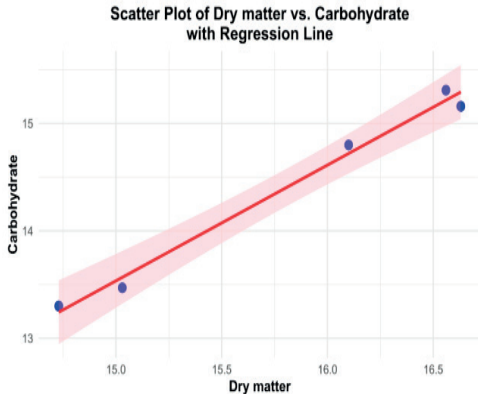


Figure 10. Scatter Plot of Dry Matter vs. Carbohydrate with Regression Line

Simple linear regression analysis was conducted to evaluate the extent to which Dry matter could predict Carbohydrate (Figure 10).

A significant regression was found ($F(1, 3) = 239.36$, $p < 0.001$). The R^2 was 0.99, indicating that Dry matter explained approximately 99% of the variance in Carbohydrate.

The regression equation was: Carbohydrate = $-2.64 + 1.08$ (Dry matter). That is, for each one % increase in Dry matter, the predicted Carbohydrate increased by approximately 1.08%. Confidence intervals indicate that we can be 95% certain that the slope predicting Carbohydrate from Dry matter is between 0.86 and 1.30.

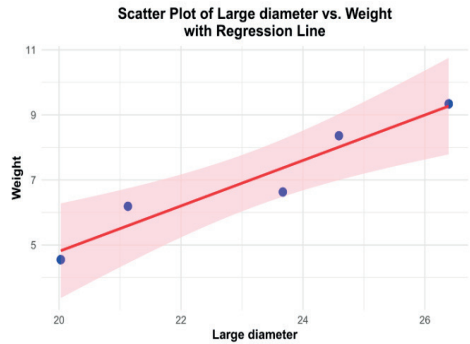


Figure 11. Scatter Plot of Large Diameter vs. Weight with Regression Line

Simple linear regression analysis was conducted to evaluate the extent to which Large diameter could predict Weight (Figure 11). A significant regression was found ($F(1, 3) = 35.40$, $p = 0.009$). The R^2 was 0.92, indicating that Large diameter explained approximately 92.2% of the variance in Weight. The regression equation was: Weight = $-9.17 + 0.70$ (Large diameter). That is, for each one mm increase in Large diameter, the predicted Weight increased by approximately 0.70 g. Confidence intervals indicate that we can be 95% certain that the slope for predicting Weight from Large diameter is between 0.33 and 1.07.

Simple linear regression analysis was conducted to evaluate the extent to which Small diameter could predict Weight (Figure 12).

A significant regression was found ($F(1, 3) = 18.76$, $p = 0.023$). The R^2 was 0.86, indicating that Small diameter explained approximately 86.2% of the variance in Weight. The regression equation was: Weight = $-12.69 + 1.02$ (Small diameter).

That is, for each one mm increase in Small diameter, the predicted Weight increased by approximately 1.02 g.

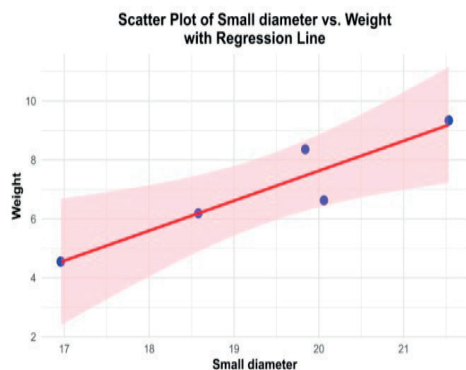


Figure 12. Scatter Plot of Small Diameter vs. Weight with Regression Line

Confidence intervals indicate that we can be 95% certain that the slope for predicting Weight from Small Diameter is between 0.27 and 1.76.

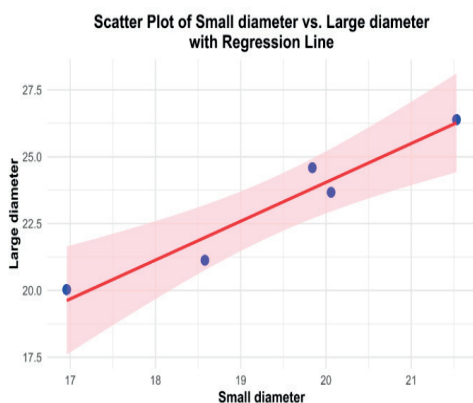


Figure 13. Scatter Plot of Small Diameter vs. Large Diameter with Regression Line

Simple linear regression analysis was conducted to evaluate the extent to which Small Diameter could predict Large Diameter (Figure 13). A significant regression was found ($F(1, 3) = 43.49$, $p = 0.007$). The R^2 was 0.94, indicating that Small Diameter explained approximately 93.5% of the variance in Large Diameter. The regression equation was: Large Diameter = $-5.04 + 1.45(\text{Small Diameter})$. That is, for each one mm increase in Small Diameter, the predicted Large Diameter increased by approximately 1.45 mm. Confidence intervals

indicate that we can be 95% certain that the slope predicting Large Diameter from Small Diameter lies between 0.75 and 2.16.

CONCLUSIONS

In conclusion, the evaluation of the five sweet cherry cultivars during the 2025 experimental year revealed significant biometric and physicochemical variability, highlighting their distinct phenotypic responses in a post-frost context. From a commercial sizing perspective, **'Bigarreau Burlat'** and **'Van'** demonstrated superior biometric performance, recording the highest values for fruit weight, height, and diameter, making them highly suitable for markets demanding large-caliber fruits. Conversely, regarding the internal fruit quality and taste profile, the local Romanian varieties **'Boambe de Cotnari'** and **'Rosii de Bistrita'** exhibited exceptional physicochemical traits, yielding the highest concentrations of soluble dry matter (Brix) and total sugars. **'Inima de Porumbel'**, alongside **'Van'**, distinguished itself through superior pulp firmness, an essential trait for post-harvest handling and transport durability.

Furthermore, the robust statistical modeling conducted in this study provides valuable predictive tools for orchard management. The highly significant linear regressions demonstrate that non-destructive field measurements (such as large and small equatorial diameters) can reliably predict final fruit weight ($R^2 > 0.86$), while dry matter content acts as an almost perfect predictor for total carbohydrate accumulation ($R^2 = 0.99$). These correlations offer growers and researchers efficient, rapid assessment methods for sweet cherry quality monitoring under changing climatic conditions.

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LOCAL CLIMATIC REGIME: TEMPERATURE AND PRECIPITATION TRENDS IN THE CONTEXT OF GLOBAL WARMING

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Abstract

The climatological analysis for the period 2021-2025 highlights a clear warming trend in southeastern Romania compared with the multiannual averages for 1975-2022. The annual mean temperature ranged between 12.2°C and 13.7°C, exceeding in most years the climatological norm of 12.7°C, with more pronounced positive deviations during 2023-2025. The mean maximum temperatures reached 19.2°C in 2025, while extreme summer values exceeded 32.0°C, reflecting increasingly hot summers. The average minimum temperatures, between 6.9°C and 8.2°C, indicate warmer nights and milder winters, emphasizing thermal variability particularly in winter and spring months and an earlier onset of the warm season. The pluviometric regime shows significant variability, alternating between very wet and dry years, with uneven precipitation distribution concentrated in short, intense episodes. The 2021-2025 precipitation average is slightly above the multiannual mean; however, rising temperatures and altered rainfall patterns suggest an intensification of hydric stress. These climatic changes have a significant impact on fruit growing, influencing phenology, productivity, and water management, and requiring the adaptation of agricultural strategies and careful monitoring of the thermo-hydric regime in southeastern Romania.

Key words: climate variability, regional adaptation, analysis.

INTRODUCTION

Climate change represents one of the most significant challenges faced by Romanian agriculture, particularly the fruit-growing sector, which is highly dependent on local climatic conditions. Although several studies have documented climate change at national and regional scales, limited information is available regarding the recent thermo-hydric evolution at local fruit-growing stations in southeastern Romania. This study addresses this gap by providing a detailed assessment of temperature, precipitation, and aridity indicators recorded at Valu lui Traian during 2021-2025. In the southeastern region of the country, characterized by an excessively temperate-continental climate, the thermo-hydric regime has undergone significant changes in recent decades, with direct implications for fruit production (Bandoc & Pravalie, 2015; Croitoru et al., 2016). The increase in average annual temperatures, combined with the reduction in summer precipitation and the growing frequency of extreme weather events, leads to intensified thermal and water stress on fruit tree species

(Pörtner et al., 2022; Li et al., 2023; Roussos, 2024). In Dobrogea, one of the most vulnerable regions in southeastern Romania, the frequency of pedological and atmospheric droughts has increased, causing physiological imbalances in trees, reduced vegetative growth, and a decline in fruit quality (Paltineanu et al., 2015; Barbulescu & Postolache, 2023). Severe water stress limits photosynthesis and transpiration, affecting the accumulation of dry matter and sugar content, which are essential for fruit quality (Ripoll et al., 2014; Mihaljević et al., 2021; Ma et al., 2022; Kang et al., 2024). These physiological disturbances are reflected in measurable agronomic outputs such as reduced fruit size, lower yield, altered sugar accumulation, and increased incidence of fruit defects. Moreover, high temperatures during the ripening period can cause sunburn on fruits, reducing their commercial value. On the other hand, changes in the thermal regime during winter have led to a decrease in the number of days with temperatures below 0°C, which can disrupt the dormancy period of fruit trees (Chitu et al., 2015; Croitoru et al., 2016; Paltineanu & Chitu, 2020). Premature vegetative growth, followed by late spring

frosts, negatively affects the flowering and fruiting phases, leading to significant yield losses (Rodrigo et al., 2006; Gu et al., 2008; Luedeling & Brown, 2011; Drepper et al., 2022; Cebulj et al., 2022; Chamberlain and Wolkovich, 2021; Žnidaršič et al., 2023). In this context, adapting fruit growing to new climatic conditions has become a strategic necessity. The selection of cultivars and rootstocks tolerant to thermal and water stress, the optimization of irrigation regimes, and the application of water-conserving technologies represent priority solutions (Pörtner et al., 2022). Additionally, monitoring the thermo-hydric regime and integrating climatic data into technological planning can help reduce risks and maintain the sustainability of orchards. The present study aims to analyze the impact of climate change on the thermo-hydric regime in the southeastern region of Romania. The objective of this study was to analyze recent changes in mean, maximum, and minimum air temperatures, the precipitation regime, the climatic water balance, and 'De Martonne' Aridity index during 2021-2025, using descriptive and correlation analyses, in order to evaluate the implications of climate change for fruit-growing systems in southeastern Romania.

MATERIALS AND METHODS

The study area selected for analyzing the impact of climate change on the thermo-hydric regime is located in southeastern Romania, in Valu lui Traian commune, Constanța County, with the following coordinates: 44°10' N latitude, 28°40' E longitude, and an altitude ranging from 40 to 80 m above sea level. This region is characterized by a temperate-continental steppe climate, marked by hot and dry summers and relatively mild winters, with average annual precipitation below 450 mm (Paltineanu et al., 2015; Bandoc & Pravalie, 2015). Fruit-growing has a long-standing tradition in the area, mainly represented by apricot, peach, apple, and sour cherry orchards species sensitive to thermal and hydric variations. The study area is representative of southeastern Romania due to its steppe climatic conditions, frequent drought occurrence and extensive fruit-growing activities, making it suitable for evaluating climate change impacts at the

regional level. Apricot and peach are particularly sensitive to spring frosts and water deficits, while apple and sour cherry require sufficient winter chilling accumulation and adequate water availability during fruit development. For the analysis of the thermo-hydric regime during the 2021-2025 period, climate data series from the IMT 300 meteorological station, operated by the local unit, were used. The datasets included monthly and annual values of mean, maximum, and minimum temperatures and total precipitation. These values were compared with multiannual climatological averages calculated for the 1975-2022 period. Climatic data underwent routine quality control procedures, including consistency checks, detection of missing values, and comparison with neighboring stations when available. No major gaps were identified in the analyzed series. For the determination of the Climatic Water Balance (CWB), the formula proposed by Thornthwaite (1948) ($CWB = P - ETP$, where P - precipitation in mm and ETP - potential evapotranspiration in mm) was used, which expresses the difference between effective precipitation and potential evapotranspiration. Potential evapotranspiration was estimated using the Thornthwaite method based on monthly mean air temperature. Although this approach may underestimate evapotranspiration under semi-arid conditions, it remains widely used for comparative climatic assessments and long-term regional studies. Negative values of the CWB indicate periods of water deficit, which are relevant for assessing stress on fruit crops. The analysis was complemented by calculating the 'De Martonne' Aridity Index ($I_a = P / (T + 10)$, where P - precipitation, mm and T - mean air temperature, °C), used to classify the degree of climatic dryness of the region (Paltineanu et al., 2007).

The primary data were centralized and organized into monthly and annual tables using Microsoft Excel. To examine the relationships between climate variables, bivariate statistical analyses were performed using IBM SPSS Statistics software (version 26.0). Pearson correlation analysis was used to assess relationships among climatic variables. Statistical significance was evaluated at $p < 0.05$ and $p < 0.001$ levels. Due to the relatively short study period, no formal time-series trend analysis was applied.

RESULTS AND DISCUSSIONS

1. Air temperatures in the 2021-2025 period compared to the long-term averages for the reference interval 1975-2022

1.1. Analysis of the evolution of annual and monthly mean air temperatures

The mean annual air temperature varied between 12.2°C in 2021 and 13.7°C in 2023, values that exceed, in most cases, exceed the mean multiannual temperature of 12.7°C. This evolution indicates a progressive increase in the mean annual temperature, with a maximum positive deviation of approximately +1.0°C in the year 2023. In particular, the years 2023-2025 stand out for constant mean annual temperatures above the climatological average, confirming the warming trend of the analyzed region (Table 1). The observed warming is consistent with previous studies conducted in southeastern Romania and the Black Sea region, which reported increases in mean annual temperature of approximately 0.3-0.5°C per decade (Croitoru et al., 2016; Barbulescu & Postolache, 2023). The analyzed period covers only five years; therefore, the identified warming tendency should be interpreted as a recent climatic signal rather than a definitive long-term trend. At the monthly level, significant thermal variations are observed, especially in the transitional seasons (spring and autumn). January and February 2025 saw notable deviations from climatological values, with a mean temperature of 4.8°C in January (1.7°C above normal) and -0.8°C in February (below average 4.1°C). These thermal oscillations indicate an increase in climate variability during the cold period of the year. March, April, and May were generally warmer than the multi-year normal. In particular, the month of April 2024 recorded a value of 14.6°C, +4.0°C above the climatological average (10.6°C). This pattern is consistent with regional observations of an advancing warm season onset and an increasing frequency of early warming episodes. The months of June, July and August show relative thermal stability, with the values being close to or slightly higher than the multi-year averages. In July 2024, the mean air temperature reached 25.8°C, exceeding the norm by +1.9°C, and in

June 2024, 23.5°C was recorded, compared with the multiannual air temperature of 21.2°C respectively. These positive deviations confirm the tendency of intensifying heat waves and extending hot periods. In the months of September, October, November, and December, the mean air temperature oscillated around the climatological values, but a slight decrease is noted in the months of October, November, and December 2024-2025, which can be attributed to changes in the regional atmospheric circulation and to an increase in the frequency of cold air episodes (Table 1). The comparative analysis of thermal data for the period 2021-2025 indicates an increase in the average annual temperature compared with the multiannual average 1975-2022, with constant positive deviations in most years. Seasonal analysis showed warmer springs and summers compared with the multiannual averages. These results support the hypothesis of local climate warming, consistent with trends observed on a global scale, and underline the need for continuous monitoring of climate parameters to identify and quantify thermal regime in the context of current climate change.

Table 1. Annual and monthly mean air temperatures for the 2021-2025 period, compared with the long-term mean temperatures for the reference interval 1975-2022

Month	Mean air temperature (°C)					Multiannual mean air temperature 1975-2022 (°C)
	2021	2022	2023	2024	2025	
January	3.4	2.0	5.6	1.9	4.8	1.7
February	3.2	4.5	3.3	7.7	-0.8	4.1
March	4.7	2.7	7.2	7.7	9.2	5.6
April	9.2	11.4	9.9	14.6	9.8	10.6
May	16.2	16.4	15.5	15.0	14.9	16.4
June	20.0	21.1	20.7	23.5	22.1	21.2
July	24.2	23.7	24.5	25.8	24.8	23.9
August	23.5	24.5	24.9	24.0	22.8	24.0
September	17.5	18.6	21.3	19.3	18.9	19.1
October	11.1	13.6	16.6	12.5	12.0	13.3
November	8.7	9.4	9.3	5.6	10.9	8.2
December	4.1	4.5	5.4	4.3	3.7	4.3
Mean	12.2	12.7	13.7	13.5	12.8	12.7

1.2. Analysis of the evolution of annual and monthly mean maximum air temperatures

The annual average of the mean maximum air temperature for the interval 2021-2025 varied between 18.0°C and 19.6°C, values higher than the multiannual average (18.7°C) in most of the analyzed years. The year 2023 recorded the highest annual average (19.6°C), suggesting a warmer thermal character (Table 2). Overall,

the analyzed period confirms the climate warming trend, manifested by the increase in the frequency and intensity of high thermal values. The regime of monthly mean maximum temperatures shows a progressive increase from January to July, with minimum values in January and February and maximum values in July and August. The summer months consistently exceeded 30°C, indicating a pronounced warm character. The mean number of summer days with absolute maximum temperatures between 30°C and 40°C was 59 days in 2021-2025 period. Increasing maximum temperatures may accelerate fruit maturation, increase evapotranspiration demand, and enhance the risk of sunburn damage, requiring improved irrigation scheduling and canopy management. The transition months, April-May and September-October, show significant interannual variations, reflecting seasonal instability. Compared to the multiannual mean (1975-2022), frequent positive deviations are observed, especially in the summer and early autumn months.

Table 2. Annual and monthly mean maximum air temperatures for the 2021-2025 period, compared with the long-term mean maximum temperatures for the reference interval 1975-2022

Month	Mean maximum air temperature (°C)					Multiannual mean maximum air temperature 1975-2022 (°C)
	2021	2022	2023	2024	2025	
January	7.2	6.3	9.6	6.7	10.5	6.1
February	8.1	10.3	8.1	12.7	3.4	9.4
March	10.1	7.8	12.6	12.6	15.5	11.3
April	15.1	18.2	15.2	21.2	16.2	17.3
May	22.9	24.2	21.9	20.9	21.3	23.2
June	26.4	28.0	27.9	30.6	29.6	27.9
July	31.5	31.3	31.8	32.8	31.9	31.2
August	31.4	32.1	31.4	31.1	30.5	31.3
September	24.9	26.0	27.6	25.6	25.5	26.1
October	16.1	21.3	24.3	19.7	16.4	19.3
November	14.0	14.6	14.2	11.4	15.2	13.0
December	7.9	9.3	10.2	7.5	14.9	8.2
Mean	18.0	19.1	19.6	19.4	19.2	18.7

1.3. Analysis of the evolution of annual and monthly mean minimum air temperatures

The annual average of the mean minimum temperature for the analyzed interval varied between 6.7°C (2025) and 8.2°C (2023), values close to or slightly higher than the multiannual mean minimum temperature of 7.2°C. The increase in minimum temperatures agrees with regional climate projections for southeastern

Europe. Warmer winters may reduce chilling accumulation, affecting dormancy release and flowering synchronization in fruit trees. The years 2023 and 2024 are notable for higher mean minimum temperatures, compared to the rest of the analyzed period. The monthly mean minimum temperature shows negative values in the winter months, especially in January (-2.4°C, -1.8°C) and February (-4.9°C, -1.1°C), followed by a progressive increase in the warm season. In the months of July and August, the highest mean minimum temperature values are recorded, 16.5°C and 16.9°C respectively, indicating persistent warm nights (Table 3). Future analyzes will include frost frequency and number of frost days, which are critical indicators for risk assessment in fruit production.

Table 3. Annual and monthly mean minimum air temperatures for the 2021-2025 period, compared with the long-term mean minimum temperatures for the reference interval 1975-2022

Month	Mean minimum air temperature (°C)					Multiannual mean minimum air temperature 1975-2022 (°C)
	2021	2022	2023	2024	2025	
January	0.0	-1.8	2.1	-2.4	0.4	-2.2
February	-1.1	-0.3	-0.8	3.6	-4.9	-0.7
March	-0.1	-2.2	2.3	3.3	3.7	0.4
April	4.2	5.9	5.0	8.3	3.9	4.5
May	10.2	8.9	9.3	9.4	9.0	10.0
June	15.1	14.6	13.4	16.0	13.9	15.3
July	17.2	15.8	17.5	18.8	17.2	16.5
August	15.9	17.9	18.5	17.5	14.6	16.9
September	10.7	12.1	15.6	13.9	13.0	12.4
October	6.9	6.8	9.6	7.0	8.7	8.0
November	4.5	4.7	4.4	0.8	7.6	4.0
December	0.8	0.7	1.5	1.4	-7.0	0.9
Mean	7.0	6.9	8.2	8.1	6.7	7.2

Considering the general trend of increasing mean air temperatures and the intensification of seasonal variability observed in the 2021-2025 period, the simultaneous analysis of the pluviometric regime is necessary to understand the current climate trends and their impact on fruit growing.

2. Analysis of the pluviometric regime in the 2021-2025 period compared with the long-term values for the reference interval 1975-2022

The pluviometric regime is one of the main indicators of local climate characteristics and an essential element in the assessment of the water balance. The present analysis follows the

annual and monthly evolution of precipitation for the 2021-2025 period, compared to the multiannual pluviometric regime (1975-2022), with the aim of highlighting deviation trends from the climatic norm and possible signs of aridity. The pronounced variability of precipitation observed during the study period reflects the increasing climatic instability characteristic of southeastern Romania. Although annual precipitation totals occasionally exceeded the multiannual average, the rainfall distribution became increasingly irregular, with prolonged dry intervals interrupted by short episodes of intense precipitation. The annual values of the pluviometric regime varied significantly in the analyzed interval, from 816.0 mm (2021), with 345.2 mm above the multiannual value indicating favorable water conditions, to 355.0 mm (2022) representing only 75% of the multiannual value, which suggests a pronounced drought (Table 4). The annual average for the 2021-2025 is 554.6 mm, slightly above the multiannual value (470.8 mm), but with large interannual fluctuations, a sign of increased climatic instability. The monthly analysis reveals clear seasonal variations between the cold and warm periods of the year. The period January - March experienced pronounced oscillations from one year to another. If, in 2021 there were abundant precipitation episodes, 86 mm in January, two and a half times more than the multiannual value (33.2 mm) and 65.8 mm in March, twice more than the multiannual value (32.2 mm), starting from the year 2022, for the same interval, precipitation decreased considerably, reaching up to 1.2 mm in February 2024 indicating a deficient regime. This highlights a sudden reduction in water supply during the winter months and the beginning of spring. The spring months show large variations, from 11.0 mm in May 2023 to 105.8 mm in April 2023. In general, 2021 (April and May), 2023 (April), 2024 (April) and 2025 (May) were characterized by high amounts of precipitation above the multiannual values (31.9 mm in April and 42.1 mm in May), which suggests a reactivation of the wet regime in spring. June and August are the most variable months, with precipitation above the multiannual values in June 2021, 124.2 mm respectively, in July 2022 with 68.4 mm and in August 2024 with 155.2 mm. The lowest

amount of precipitation was recorded in June 2025, 1.2 mm respectively, followed by August and July 2023 with 7.2 mm and 11.0 mm, respectively, indicating an accentuated dry period in the height of summer, associated with high temperatures and increased evapotranspiration. The months of September and October mark an improvement in the rainfall regime (101.8 mm in September 2024, 138.6 mm in October 2021) with values above the multiannual average (47.5 mm in September and 48.6 mm in October), with the exception of October of the years 2022 (6.8 mm) and 2023 (21.0 mm) when the pluviometric regime was below the multiannual value. This monthly analysis confirms a seasonal reorientation of precipitation, with episodes of heavy rain in autumn. The end-of-year months (November and December) show large variations, with a pronounced maximum in November 2023 (159.2 mm), almost six times above the multiannual value (27.9 mm) indicating an extreme rainfall event, and a minimum in December 2022 (16.0 mm), almost three times below the multiannual average (44.0 mm) (Table 4). Extreme precipitation events were likely associated with Mediterranean cyclones and atmospheric blocking patterns, while severe drought periods were linked to persistent anticyclonic conditions and enhanced summer evapotranspiration.

Table 4. Annual and monthly precipitations for the 2021-2025 period, compared with the long-term values of precipitation for the reference interval 1975-2022

Month	Pluviometric regime (mm)					Multianual pluviometric regime 1975-2022 (mm)
	2021	2022	2023	2024	2025	
January	86.0	13.6	32.2	11.2	17.6	33.2
February	18.8	26.4	7.6	1.2	3.6	46.2
March	65.8	10.4	20.8	30.8	5.2	32.2
April	66.8	30.8	105.8	84.8	20.8	31.9
May	87.8	22.8	11.0	37.6	57.2	42.1
June	124.2	47.6	22.8	18.8	1.2	63.6
July	30.4	68.4	11.0	19.6	39.8	42.0
August	17.6	10.4	7.2	155.2	18.2	11.6
September	62.6	76.0	68.4	101.8	53.2	47.5
October	138.6	6.8	21.0	81.0	100.8	48.6
November	40.8	25.8	159.2	56.4	56.4	27.9
December	76.6	16.0	44.6	97.2	20.6	44.0
Sum	816.0	355.0	511.6	695.6	394.6	470.8

3. Analysis of the climatic water balance for the 2021-2025 Period

The climatic water balance (CWB) is an indicator that provides a comprehensive view of the soil moisture regime and implicitly of the

availability of water resources for vegetation. During the 2021-2025 period, the CWB exhibits negative annual values in all analyzed years, ranging from -48.6 mm in 2021 to -566.8 mm in 2022. The multiannual mean for this period is -370.6 mm, indicating a deficient water regime. The monthly evolution of the CWB reflects a typical seasonal distribution for areas with a continental climate. The November-January interval is characterized by moderate water surpluses due to reduced evapotranspiration. Positive values in January (+15.4 mm), November (+45.5 mm), and December (+37.4 mm) indicate a consistent replenishment of soil water reserves. Spring (March-May) marks the transition to a water deficit, with mean values ranging from -21 mm to -77 mm, driven by rising temperatures and intensified evapotranspiration. The June-August interval represents the most critical period, characterized by extreme water deficits reaching -131.1 mm in July. These values indicate severe drought conditions, which strongly affect fruit crops. Severe summer deficits highlight the increasing importance of irrigation systems and water-saving technologies for maintaining orchard productivity. September and October bring a partial improvement in water availability, with values close to equilibrium (-20 mm in September and +23.8 mm in October), driven by reduced evapotranspiration and the resumption of precipitation (Table 5). The five-year CWB analysis highlights a progressive aridification. Nevertheless, because the analyzed period is relatively short, the apparent tendency toward increasing aridity should be interpreted cautiously and verified using longer climatic records.

Table 5. Annual and monthly values of the climatic water balance for the 2021-2025 period

Month	CWB (mm)					Mean (2021-2025)
	2021	2022	2023	2024	2025	
January	73.6	-5.0	13.6	-7.4	2.1	15.4
February	-3.6	-4.4	-23.2	-39.4	-18.8	-17.9
March	16.2	-39.2	-31.9	-18.8	-56.8	-26.1
April	-5.2	-62.2	36.8	-14.2	-60.2	-21.0
May	-36.2	-107.4	-106.8	-77.1	-57.5	-77.0
June	-1.8	-81.4	-118.2	-146.2	-157.8	-101.1
July	-127.7	-95.9	-150.2	-150.9	-130.7	-131.1
August	-121.9	-126.0	-135.4	18.2	-127.5	-98.5
September	-27.4	-14.0	-36.6	14.8	-36.8	-20.0
October	101.4	-39.7	-44.1	37.6	63.6	23.8
November	19.8	4.8	129.2	38.4	35.4	45.5
December	64.2	3.6	26.0	84.8	8.2	37.4
Sum	-48.6	-566.8	-440.8	-260.2	-536.8	-370.6

4. Analysis of the 'De Martonne' Aridity Index for the 2021-2025 Period

The analysis of the 'De Martonne' aridity index for the period 2021-2025 indicates significant monthly and interannual variations. Monthly mean values range between 0.9 (February) and 3.6 (November) (Table 6). The months of July ($I_a=1.0$) and August ($I_a=1.2$) show extreme drought, while October 2021 ($I_a=6.6$), November 2023 ($I_a=8.2$), and December 2021 ($I_a=6.8$) indicate relatively higher humidity in late autumn and winter. According to the classical De Martonne classification, values below 10 characterize arid climatic conditions, values between 10 and 20 indicate semi-arid environments, while higher values correspond to increasingly humid climates. The predominance of low index values throughout the analyzed period confirms the persistence of arid conditions in the study area.

Table 6. 'De Martonne' Aridity Index for the 2021-2025 period

Month	De Martonne Aridity Index					Mean (2021-2025)
	2021	2022	2023	2024	2025	
January	6.4	1.1	2.1	0.9	1.2	2.3
February	1.4	1.8	0.6	0.1	0.4	0.9
March	4.5	0.8	1.2	1.7	0.3	1.7
April	3.5	1.4	5.3	3.4	1.1	2.9
May	3.4	0.9	0.4	1.5	2.3	1.7
June	4.1	1.5	0.7	0.6	0.0	1.4
July	0.9	2.0	0.3	0.5	1.1	1.0
August	0.5	0.3	0.2	4.6	0.6	1.2
September	2.3	2.7	2.2	3.5	1.8	2.5
October	6.6	0.3	0.8	3.6	4.6	3.2
November	2.2	1.3	8.2	3.6	2.7	3.6
December	5.4	1.1	2.9	6.8	1.5	3.5
Mean	3.4	1.3	2.1	2.6	1.5	2.2

Spring presents moderate values, with a peak in April 2022 ($I_a=5.3$). The mean annual index $I_a=2.2$ confirms the predominance of arid conditions, with short periods of more pronounced moisture (Table 6). The consistency between the De Martonne Aridity Index and the Climatic Water Balance demonstrates the reliability of both indicators in characterizing moisture availability and drought severity within fruit-growing ecosystems.

5. Statistical analysis of relationships between climatic parameters

- Correlation P vs. CWB (Precipitation vs. Climatic Water Balance). The relationship between precipitation and the Climatic Water Balance (CWB) is positive and statistically

significant ($p < 0.001$). However, the moderate value of the R^2 coefficient (0.3935) suggests that precipitation explains only approximately 40% of the water balance (Table 7, Figure 1). The remaining 60% is massively influenced by potential evapotranspiration. This indicates a climate where, even in rainy months, water loss through evaporation (caused by high temperatures) can maintain a water balance deficit. The relatively short observation period may limit the robustness of the identified statistical relationships.

- Correlation T_{med} vs. CWB (Mean Temperature vs. Climatic Water Balance). There is a distinct negative correlation between mean temperature and the climatic water balance ($r = -0.677$). As the temperature rises, the CWB decreases, frequently entering the zone of negative values (deficit). This inverse relationship confirms the arid character of the summers during the 2021-2025 interval, where high temperatures accelerate evapotranspiration at a rate faster than the pluviometric input can compensate (Table 7, Figure 2).

- Correlation I_a vs. T_{mean} ('De Martonne' Aridity Index vs. Mean Temperature). This is the only correlation that does not reach the standard threshold of statistical significance ($p = 0.068 > 0.05$). The weak negative relationship shows that the aridity index is not dictated exclusively by temperature. Although high temperatures tend to lower the index (towards aridity), the influence of precipitation is much more determinant in the calculation of this index than that of temperature (Table 7).

- Correlation I_a vs. P ('De Martonne' Aridity Index vs. Precipitation). The very strong correlation ($r = 0.906$) confirms that the pluviometric regime is the main factor controlling aridity in the studied area. Variations in the 'De Martonne' Aridity Index faithfully follow the precipitation curve ($R^2 = 0.820$), demonstrating that any decrease in precipitation quantity leads directly and immediately to an accentuation of the degree of aridity, without other factors being able to significantly temper this effect (Table 7, Figure 3).

- Correlation I_a vs. CWB ('De Martonne' Aridity Index vs. Climatic Water Balance). The close relationship between these two indicators validates the consistency of the climatic data. Since both indices measure, in different forms,

the availability of water in the ecosystem, the strong positive correlation ($r = 0.792$) shows that periods classified as "arid" or "semi-arid" according to 'De Martonne' correspond faithfully to periods with a major soil water deficit (negative CWB), confirming the agricultural vulnerability of the area during drought intervals (Table 7, Figure 4).

Table 7. Pearson correlation (r), determination coefficients (R-squared), and significance levels (p-value) for the analyzed climatic parameters

Variable Pair	Pearson Correlation (r)	R-squared (R ²)	Significance (p-value)
P vs. CWB (Precipitation vs. Climatic Water Balance)	0.627	0.3935	< 0.001
T_{med} vs. CWB (Mean Temperature vs. Climatic Water Balance)	-0.677	0.4583	< 0.001
I_a vs. T_{med} ('De Martonne' Aridity Index vs. Mean Air Temperature)	-0.237	0.056	> 0.05
I_a vs. P ('De Martonne' Aridity Index vs. Precipitation)	0.906	0.8201	< 0.001
I_a vs. CWB ('De Martonne' Aridity Index vs. Climatic Water Balance)	0.792	0.6271	< 0.001

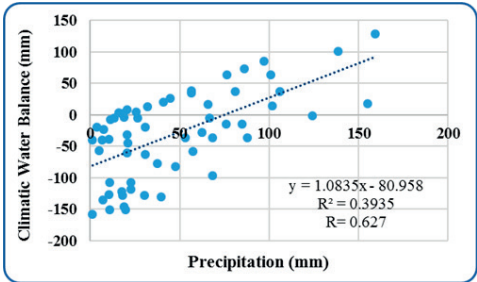


Figure 1. Correlation between the Precipitation (P) and the Climatic Water Balance (CWB) during the 2021-2025

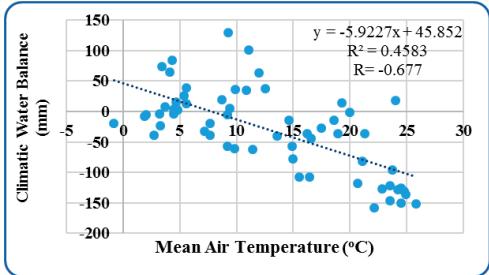


Figure 2. Correlation between the Mean Air Temperature (T_{med}) and the Climatic Water Balance (CWB) during the 2021-2025

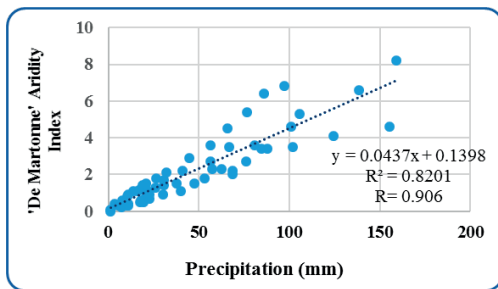


Figure 3. Correlation between the Precipitation (P) and the 'De Martonne' Aridity Index (I_a) during the 2021-2025

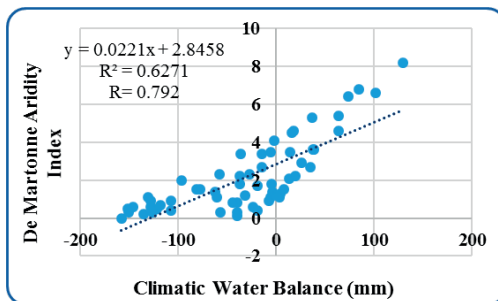


Figure 4. Correlation between the Climatic Water Balance (CWB) and the 'De Martonne' Aridity Index (I_a)

The identified relationships can support drought risk assessment and the optimization of irrigation management in fruit-growing systems.

CONCLUSIONS

Mean air temperatures during 2021-2025 generally exceeded the long-term average, confirming a warming trend and an increased frequency of high-temperature events. The adoption of heat-tolerant cultivars and improved orchard management practices is recommended to reduce thermal stress.

Annual precipitation totals were generally close to normal, but their uneven seasonal distribution, characterized by summer deficits and intense rainfall episodes, indicated increased variability of the precipitation regime. Continuous monitoring of precipitation, together with the implementation of efficient soil moisture conservation measures, is recommended to mitigate the effects of rainfall irregularity.

Predominantly negative Climatic Water Balance values reflected persistent water deficits and increasing pressure on water

resources. Optimized irrigation scheduling and the use of water-saving technologies are essential to maintain orchard productivity.

The 'De Martonne' Index indicated predominantly arid conditions, especially during summer, highlighting the combined effects of reduced precipitation and elevated temperatures. Adaptation strategies aimed at improving drought resilience and water-use efficiency should be prioritized (e.g., drought-tolerant cultivars, optimized irrigation scheduling, mulching, and soil moisture conservation techniques).

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ANALYSIS OF SOCIAL BEHAVIOUR IN THE CONTEXT OF VALORISATION OF FRUIT RESOURCES IN NATURAL ECOSYSTEMS

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Abstract

Wild fruits are valuable natural resources with significant nutritional, therapeutic, and economic potential; however, their role in modern food systems and local economic development remains insufficiently explored in many regions. This study aimed to analyze public perceptions regarding consumption, their economic valorisation, and their contribution to local community development. Data were collected through a questionnaire examining consumption habits, perceived health benefits, knowledge of traditional products, and attitudes toward economic activities related to wild fruits. The results indicate a moderate level of consumption, although most respondents recognize their health benefits and their role in a balanced diet. Participants also show openness toward the economic use of wild fruits through activities such as harvesting, processing, marketing, and integration into rural tourism. Many respondents believe that establishing collection centres or small processing units could stimulate local economic development. However, the relatively low level of knowledge about traditional products made from wild fruits suggests a decline in transmitting this knowledge between generations. Overall, the findings highlight the potential of wild fruits to support healthy diets, diversify rural economies, and promote the sustainable use of natural resources.

Key words: ecosystem resources, local communities, rural development, rural tourism, traditional knowledge, wild fruits.

INTRODUCTION

Food and nutrition security is a major challenge facing the world today (Duguma, 2020). Over 820 million people worldwide are undernourished, with the highest proportions located in developing countries (Garekae & Shackleton, 2020), while the increased consumption of highly processed foods negatively affects human health (Levy et al., 2024). Wild plants can play an important role as an essential component of people's diets in some regions of the world and can provide greater dietary diversity (Motti, 2022), helping to safeguard food security, nutritional variation, and sustainable incomes (Kidane & Kejela, 2021). The importance and possible contribution of wild fruit trees to reducing food poverty have been recognized, and several researchers have explored the nutritional and economic potential of these plants (Nishimwe et al., 2024). Wild fruits are an important source of nutrition for one in six people worldwide. They provide an important source of nutrition for low-income people, while also representing an important

source of income for improving the living standards of rural communities (Ghanbari et al., 2022). Wild fruits are generally characterized by high medicinal and nutritional values, higher fibre content (Jiru et al., 2023), and richness in antioxidants (Tamayo-Vives et al., 2023) and flavonoids (Mwamatope et al., 2023). In addition to their nutritional perspectives, many of them have shown important beneficial results in the prevention and cure of several chronic diseases, such as age-related disorders (Coppari et al., 2021), cardiac disorders (Wahabi et al., 2025), diabetes (Semwal et al., 2023), and various types of cancer (Ul Abidin et al., 2023). In many indigenous societies, knowledge about wild fruit plants has been passed down from generation to generation, forming an integral part of local ecological and ethnobotanical traditions. However, despite their importance, many of these plants remain underutilized and underexplored, limiting their potential applications in sustainable agriculture, pharmaceuticals, and the food industry (Saensouk et al., 2025). Globally, the commercialization of wild fruits has also

contributed considerably to income generation. Several edible wild fruits are found in local markets and represent an important source of income (Melaku & Ebrahim, 2021). Seeking out untouched rural areas outside cities during the COVID-19 crisis, tourists have rekindled interest in consuming traditional foods based on wild natural products (Luković et al., 2024). The source of income based on wild plants is achievable through their collection and marketing, practical use in gastronomy, tourism, guided tours, natural products, etc. The cultural ecosystem services provided by these plants, as an intangible range of services, include categories related to aesthetics and sense of place, biological and intangible cultural heritage, tourism, and recreational activities, with educational and spiritual functions (Luković et al., 2023). Many communities have developed summer festivals with indigenous wild fruits as a central theme, demonstrating that they are essential for rural tourism and have a great potential for the local economy (Everett, 2007). Thus, wild fruits can contribute to supplementing household income and participating in the substantial development of rural communities that benefit from this natural resource. The purpose of this study is to analyse the perceptions and attitudes of the population towards the consumption and use of wild fruits, both from the perspective of health and socio-economic potential. The study aims to assess the degree of knowledge and use of these fruits and products derived from them, the impact of their commercialization on local households and communities, as well as the tourism potential associated with these natural resources.

MATERIALS AND METHODS

The research method used in this study was that proposed by Melinescu & Cosmulescu (2022) and consisted of developing an online questionnaire using the Google Forms platform, a digital tool that allows the automatic creation, distribution, and collection of responses. The questionnaire included 20 questions aimed at assessing the respondents' perceptions of wild fruits, which were organized into three thematic sections (Table 1). The questionnaire was distributed online through the Google Forms

platform and promoted via the social media networks Facebook and Instagram to facilitate rapid access for respondents. The sample included 100 respondents, Romanian citizens, belonging to different age categories. The questionnaire was structured into three sections. The first section included single-answer questions regarding the socio-demographic characteristics of the respondents (gender, age category, level of education, and occupation). The second section included a multiple-choice question regarding 10 species of wild fruit trees from the spontaneous flora of Romania, to assess the level of knowledge of them, as well as single-choice questions assessed on a Likert scale with five levels of intensity ("to a very great extent" - "not at all"), to which the option "cannot appreciate" was added. This section also included a binary question (yes/no). The third section included questions on perceptions related to the valorisation of wild fruits, formulated on a Likert scale, along with a trichotomous question (yes/no/don't know) and a nominal question.

RESULTS AND DISCUSSIONS

A. Descriptive data of the sample

The analysis of the data provided by the 100 respondents showed that, in terms of the structure of the respondents by gender (Table 2), the sample was composed of 61% women and 39% men, with the majority of female participants being evident. This type of distribution is frequently encountered in research that has, as its main subject, natural products and their consumption (Yobo et al., 2020; Suwardi et al., 2020; Suwardi & Mukhtar, 2023; Gruszecka-Kosowska et al., 2023), where women tend to show increased interest in the consumption of natural foods and products from spontaneous flora. Thus, it can be admitted that the increased participation of women in such studies may be associated with an increased interest in their daily diet and their own health, which is also supported by the study by Bärebring et al. (2020), conducted on a group of individuals in Sweden, where it was found that women pay increased attention to a healthy lifestyle, frequently researching the latest information on nutrition and diet.

Table 1. Questionnaire structure

Question
Section A: Descriptive data of the sample
I. Gender:
II. Age:
III. Latest completed studies:
IV. Occupation:
Section B: Perceptions of wild fruit consumption and use
V. What wild fruits do you know?
VI. To what extent do you consume natural products based on wild fruits (food supplements, teas, syrups, tinctures, creams, etc.)?
VII. Does the family use such fruits or products made from such fruits?
VIII. Do you know people outside the family who consume wild fruits or products obtained from their processing?
IX. Do you think that wild fruits can contribute to maintaining health?
X. Do you think that wild fruits can be integrated into children's diets?
XI. Do you consider that products obtained from wild fruits can constitute food supplements that contribute together with medicines to the treatment of certain diseases?
Section C: Perception of the socio-economic potential of wild fruits
XII. Can the marketing of wild fruits bring additional income to your household?
XIII. Who do you think should be entitled to market them?
XIV. Can the commercialization of wild fruits contribute to the development of the local community?
XV. If you were paid, would you take part in wild fruit picking campaigns?
XVI. Do you think that the presence of a collection centre or a factory that would process wild fruits would contribute to the development of the local community?
XVII. Can picking wild fruits represent an activity that contributes to the development of the local community from a tourism point of view?
XVIII. If rural tourism were practiced in your area, would you agree to take part in various activities or sell products based on wild fruits?
XIX. Do you know of any traditional products that can be obtained from wild fruits?
XX. In your opinion, was this resource better exploited in the past?

For the sample structure by age (Table 2) of the respondents, six age classes were established. The most numerous age classes were 14-25 years (26%) and 26-35 years (20%), totalling 46%, the category of elderly people (over 65 years) was the least represented, comprising only 10% of the respondents. However, the distribution of respondents was balanced, with no classes having a representation of less than 10%. The large number of young people may bring to the fore their perception of the consumption of wild fruits and natural products, given that, as shown by the study by Mothupi & Shackleton (2025), the intake of traditional fruits and vegetables is limited. A contribution to this decrease in consumption may be the erosion of ethnobotanical knowledge about traditional plants, especially among young people; traditional skills and knowledge about the consumption and usefulness of wild fruits tend to be forgotten by new generations. Generational differences were also recorded in the study by Pawera et al. (2020), where data obtained from respondents in Indonesia highlighted a lack of knowledge regarding the taste, preparation, or cultivation of these fruits. Thus, compared to a generation ago, the current generation is facing the loss of traditional knowledge regarding the diversity and uses of spontaneous plants in particular (Ilie &

Cosmulescu, 2023). An important cause of this phenomenon may be the lack of transmission of traditional knowledge regarding spontaneous plants. The level of education of the respondents (Table 2) indicates a sample structure dominated by people who have completed at least the mandatory levels of schooling. The best represented category is that of respondents with higher education, which amounts to 32% of the total participants. However, the highest share is found among people who have completed an average level of education, distributed across two categories: high school graduates (28%) and vocational school graduates (18%). Together, these two categories represent 46% of the sample, exceeding the proportion of respondents with higher education. The least represented categories are those corresponding to low levels of education. Thus, 9% of respondents had only completed primary or secondary school, and 13% stated that they had not completed any education at the time of completing the questionnaire. The structure of the sample highlights the predominance of middle and higher education levels, suggesting the presence of individuals who, through their training, may demonstrate a relatively high level of information on the properties and benefits of wild fruits, as well as the advantages of consuming them.

Table 2. Descriptive data of the sample

I. Gender	Percentage (%)	II. Age	Percentage (%)	III. Latest education	Percentage (%)	IV. Occupation	Percentage (%)
Male	61%	14-25 years	26%	No education	13%	No occupation	18%
		26-35 years	20%	Primary school/gymnasium	9%	Student/University student	19%
		36-45 years	12%	Vocational school	18%	Employed in state organisation	13%
Female	39%	46-55 years	18%			Employed in private sector	37%
		56-65 years	14%	High school	28%		
		over 65 years	10%	Higher education	32%	Retired	12%

However, in terms of traditional knowledge, it is possible that this is not significantly influenced by the level of formal education. For example, the study conducted by Yangdon et al. (2022) did not identify significant differences between the respondents' level of education of the respondents and their degree of knowledge of indigenous information.

On the other hand, the research conducted by Egg et al. (2020) on a sample of adolescents in Austria highlighted that a higher level of education can be associated with a healthier lifestyle and a higher level of nutritional knowledge. This phenomenon is explained by the inclusion, within the educational curriculum, of subjects dedicated to nutrition and health, designed to contribute to the development of nutritional literacy, as well as to the improvement of eating behaviours and lifestyle among children and adolescents. In terms of the occupation of the respondents (Table 2), the largest share is represented by people employed in the private sector (37%). The lowest proportions were recorded among public sector employees and pensioners, with each of these categories representing 13% of the total respondents. The category of pupils and students constituted 19% of the sample, while people without employment represented 18%.

The level of household income, influenced by occupational and socio-economic status, can play an important role in the adoption of behaviors oriented towards maintaining a healthy lifestyle, as well as in the availability to purchase or collect products from spontaneous flora (Stadlmayr et al., 2023). In this context, low-income families may postpone or even avoid the purchase of natural products, prioritizing the purchase of basic foods. However, access to spontaneous plant resources

should not constitute a major difficulty, given their wide spread in the European space (Chrysargyris et al., 2023).

B. Perception of the consumption and use of wild fruits

To determine the familiarity levels (Table 3) of respondents with the most commonly encountered wild fruits in temperate climates, 10 species of wild fruit shrubs were introduced into the questionnaire, tracking whether respondents knew these plants or not. Thus, the most popular fruit shrub was the rosehip (*Rosa canina*) recognized by 83% of individuals, followed by wild strawberries (*Fragaria vesca*) (80% of respondents) and raspberries (*Rubus idaeus*) (80% of respondents). Other fruit shrubs that were recognized by a percentage of over 70% of respondents were: the crab apple (*Malus sylvestris*) (77%), the blackberry (*Rubus fruticosus*) (74%), the cherry plum (*Prunus cerasifera*) (72%) and the wild pear (*Pyrus pyraster*) (71%). The hawthorn (*Crataegus monogyna*) and the blackthorn (*Prunus spinosa*) were recognized by 51% and 53% of respondents, respectively, and the cornelian cherry (*Cornus mas*) was only known by 33% of people who knew it.

Table 3. Level of knowledge of wild fruits

No.	Species	Percentage (%)
1.	Cherry plum (<i>Prunus cerasifera</i>)	72%
2.	Rosehip (<i>Rosa canina</i>)	83%
3.	Hawthorn (<i>Crataegus monogyna</i>)	51%
4.	Blackthorn (<i>Prunus spinosa</i>)	53%
5.	Crab apple (<i>Malus sylvestris</i>)	77%
6.	Wild pear (<i>Pyrus pyraster</i>)	71%
7.	Cornelian cherry (<i>Cornus mas</i>)	33%
8.	Wild strawberries (<i>Fragaria vesca</i>)	81%
9.	Blackberry (<i>Rubus fruticosus</i>)	74%
10.	Raspberry (<i>Rubus idaeus</i>)	80%

These results support the fact that respondents are familiar with fruits found in spontaneous flora, especially with species frequently found in rural landscapes or traditionally used in food, such as rose hips or lingonberries. Differences in recognition between species may reflect both regional distribution and the degree of promotion or use of these fruits in households and in the food industry. The popularity of rose hips can be explained by their ecology, often found in rural landscapes (Stamin & Cosmulescu, 2024a; Stamin & Cosmulescu, 2025), the multitude of beneficial effects already recognized in the specialized literature (Cosmulescu et al., 2017; Kilinc et al., 2020; Khelfi et al., 2024), but also by the high content of bioactive compounds (Peña et al., 2023; Fetni et al., 2020; Alecu et al., 2025; Cardoso-Avila et al., 2021) and especially vitamin C (Arslan et al., 2020; Stamin et al., 2024; Mazzara et al., 2023; Tafreshi et al., 2025), which is why, in Romania, this species is known and often used in teas, tinctures, and various pharmaceutical products. The analysis of responses regarding their own consumption of products obtained from wild fruits (Table 4) indicates a moderate trend of use. Thus, 31% of respondents stated that they use such products "to a small extent", and 28% "to a large extent". Also, 18% reported use "to a very small extent", 13% "to a very large extent",

9% do not consume at all, and 1% could not assess the level of consumption. The distribution of responses suggests occasional rather than regular use of these products. Moderate consumption may also be influenced by the seasonal availability of wild fruits (Ding et al., 2021). However, Hockings et al. (2020) showed that plant density or fruiting period do not significantly influence human selection of fruits, with people being willing to seek the desired resources regardless of their abundance. "" Regarding consumption at the family level (Table 4), the responses follow a similar pattern. Most respondents indicated use "to a small extent" (32%) or "to a large extent" (28%), while the categories "to a very small extent" and "to a very large extent" each recorded 16%. The lowest proportions were for families that do not consume such products at all (7%) or cannot assess the level of consumption (1%). The results indicate that wild fruit products are present in households, but do not represent a constant component of the diet for most respondents. In cases where consumption is more frequent, it may be associated with local traditions and knowledge regarding the use of natural resources, considered important for the adaptation of communities to socio-ecological changes (Punchay et al., 2020).

Table 4. Perception of the consumption and use of wild fruits

Answer options	In very large extent	Largely	In small measure	In very small measure	Not at all	I cannot appreciate
VI. Consumption level of natural products based on wild fruits	13%	28%	31%	18%	9%	1%
VII. Use wild fruit and derived products within the family	16%	28%	32%	16%	7%	1%
VIII. Knowledge of other people who consume wild fruits or derivative products	Yes – 71%			No – 29%		
IX. Perception regarding the contribution of wild fruits to maintaining health	34%	41%	14%	5%	4%	2%
X. Perception regarding the integration of wild fruits into children's diets	30%	33%	34%	5%	4%	4%
XI. Perception regarding the role of wild fruit products as adjuvant dietary supplements in the treatment of diseases	36%	36%	16%	7%	1%	4%

The analysis of extra-family interactions (Table 4) shows that 71% of respondents know people outside the household who consume wild fruits or derived products, while 29% stated the opposite. This result suggests that the use of these products is perceived as being present at the community level, not just within the family. Consumption observed in the social environment can contribute to the consolidation and

transmission of food practices through the exchange of information and the adoption of common habits. In the context of the transformation of food environments, urbanization and modernization determine significant changes in lifestyle and food practices, including the replacement of traditional markets with modern stores and the change of cultural meanings associated with food consumption (Wertheim-

Heck & Raneri, 2020). The perception of the benefits of consuming wild fruits (Table 4) is predominantly positive. The majority of respondents (76%) consider these products to be beneficial for health, of which 41% appreciated the benefits "to a great extent" and 34% "to a very great extent". Lower percentages were recorded for the categories "to a small extent" (14%), "to a very small extent" (5%), "not at all" (4%) and "cannot appreciate" (2%). These results indicate that wild fruits are associated with favorable health effects, a perception that can be correlated with their high content of vitamins, antioxidants, and bioactive compounds, frequently highlighted in the specialized literature (Čulum et al., 2025; Moldovan et al., 2021; Kotsou et al., 2024; Wilk et al., 2025; Bei et al., 2023; Mitea et al., 2025; Radbeh et al., 2020; Demir et al., 2020; Moldovan et al., 2022; Stamin & Cosmulescu, 2024b). The respondents' opinion regarding the integration of wild fruits into children's diets (Table 4) is generally favorable. Thus, 33% of respondents assessed this possibility as "to a large extent" and 30% as "to a very large extent". However, 24% indicated the category "to a small extent", suggesting a certain reservation regarding the inclusion of these products in children's diets. Negative perceptions were less frequent: 5% chose the option "to a very small extent", and the categories "not at all" and "cannot appreciate" recorded 4% each. These reservations can be associated with concerns about food safety, possible allergies, or low familiarity with such products. At the same time, the issue of child nutrition remains relevant globally, in the context of which undernutrition affects approximately 162 million children under five years of age, while overweight and obesity are increasing even in low-income countries (Sahoo et al., 2021). The role of wild fruit products as adjuvant food supplements in the treatment of diseases (Table 4) was positively assessed by the majority of respondents. A total of 72% believe that they can be useful in combination with drug treatments, with the categories "to a very large extent" and "to a large extent" each recording 36%. Lower percentages were observed for "to a small extent" (16%), "to a very small extent" (7%), "not at all" (1%) and "cannot assess" (4%). The results indicate a favorable perception of the complementary therapeutic potential of wild

fruits, also supported by the specialized literature (Basavarajappa et al., 2023; Ez-Zahra et al., 2024; Goudjil et al., 2024; Ousaaid et al., 2025; Khelfi et al., 2024; Tiboni et al., 2020; Coppari et al., 2021; Condello et al., 2021; Nawrot et al., 2022; Badoni et al., 2024), although the existence of some reserved opinions suggests a certain caution related to clinical efficacy or the level of information of the population.

C. Perception on social-economic potential of wild fruits

Regarding the potential for generating income through the marketing of wild fruits (Table 5), the opinions of the respondents are relatively varied. The largest share (31%) was recorded in the category of those who consider "to a large extent" that these products can generate income. However, a significant part of the respondents expressed reservations: 25% appreciated this possibility "to a small extent", 8% "to a very small extent", and 15% considered that this potential does not exist at all. A similar percentage (15%) assessed the idea "to a very large extent", and 6% could not appreciate it. The observed reluctance may be associated with perceptions regarding the demand or market value of these products, although the literature highlights a significant economic potential of wild fruits in various regions, such as Iran (Ghanbari et al., 2022), South Africa (Salami et al., 2022) or Sudan (Musa et al., 2023). At the European level, the annual economic value of products from wild flora is estimated at approximately €23.3 billion, with the highest revenues reported in Russia (€3.7 billion), France (€3.4 billion), Germany (€3.2 billion) and Turkey (€2.5 billion). In contrast, lower values were estimated for Ireland (€37.7 million), the Netherlands (€65.9 million) and Estonia (€89.5 million), while Romania ranks ninth, with approximately €0.6 billion annually (Lovrić et al., 2020). The analysis of the perception of the economic actors that should market wild fruits (Table 5) shows a relatively diverse distribution of options. The most frequently mentioned categories were individuals (29%), the local community (22%) and the Forestry District (20%). Private companies or organizations and city halls were each selected by 13% of respondents, while other state institutions registered only 3%.

Table 5. Perception on social-economic potential of wild fruits

Answer options	In very large extent	Largely	In small measure	In very small measure	Not at all	I cannot appreciate
XII. Perception regarding income potential associate marketing fruit savage	15%	31%	25%	8%	15%	6%
XIII. Perception regarding POTENTIAL PLAYERS justified saddle commercialize fruits savage	Local city hall – 13%	Detour in the area – 20%	People physical – 29%	Community local – 22%	Other state institutions – 3%	Private companies/organizations – 13%
XIV. Perception regarding the contribution of wild fruit trading to local community development	27%	31%	27%	7%	5%	3%
XV. Respondents' willingness to participate in paid wild fruit picking campaigns	26%	35%	23%	6%	8%	2%
XVI. Perception of the impact of a collection center or processing unit on local community development	35%	36%	19%	6%	4%	0%
XVII. Perception of the potential of wild fruit picking as a local tourism development activity	26%	35%	21%	12%	5%	1%
XVIII. Willingness to participate in activities or to market wild fruit products in the context of rural tourism	31%	39%	16%	8%	4%	2%
XIX. Knowledge of traditional products made from wild fruits	Yes – 48%			No – 52%		
XX. Perception regarding level of fruit exploitation savage in last	Yes – 61%		No – 12%		Don't know – 27%	

The results suggest that respondents perceive the valorization of wild fruits as a responsibility shared between the individual, community, and institutional sectors. The lack of a clear consensus indicates the possibility of collaboration models between local communities and public institutions, oriented towards the sustainable exploitation of these resources without affecting ecosystems. Relevant examples in this regard come from countries such as Brazil, Kenya, Guatemala, India, Mali, Sri Lanka and Turkey, which have integrated edible wild species into national food system programs and policies. These initiatives aim to strengthen the evidence on the nutritional and biocultural value of these resources, integrate them into food security strategies, and raise consumer awareness, while benefiting from financial support from international bodies such as the Global Environment Facility (GEF), the International Fund for Agricultural Development (IFAD) or the European Commission (Borelli et al., 2020). The responses on the perception of the contribution of wild fruit marketing to local community development (Table 5) indicate a predominantly positive assessment. Thus, 31% of respondents consider the impact to be “to a large extent”, and 27% “to a very large extent”. At the same time, 27% appreciate the contribution “to a small

extent”, while negative assessments are lower: 7% “to a very small extent”, 5% “not at all”, and 3% could not appreciate. The results suggest that respondents perceive the valorization of wild fruits as having economic and social potential for local communities, including through the generation of income and jobs. Similarly, a study conducted in Indonesia showed that these resources constitute an important source of food and income, being harvested by approximately 86% of respondents and contributing about 43% to the annual income of households (Navia et al., 2020). Regarding participation in paid wild fruit picking campaigns (Table 5), the results indicate a high willingness: 35% of respondents would be involved “to a large extent”, and 26% “to a very large extent”. Another 23% of people indicated a “small” willingness, while only 6% declared themselves very unwilling, 8% would not participate at all, and 2% could not assess. These results suggest a considerable interest in such activities, especially if they are associated with economic benefits. In the literature, the collection of wild plants and fruits is often associated with cultural or recreational activities, while also contributing to the provision of ecosystem services in urban and peri-urban environments (Fischer & Kowarik, 2020; Sardeshpande & Shackleton, 2023).

The perception of the impact of establishing collection centres or processing units (Table 5) is also favourable. The majority of respondents consider this initiative beneficial for the community, with 36% evaluating it “to a very great extent” and 35% “to a great extent”. Another 19% of the responses indicate “to a small extent”, while negative assessments are limited (6% “to a very small extent” and 4% “not at all”). These results reflect the perception that such infrastructures can contribute to the development of the local economy, the efficient use of natural resources, and the creation of jobs. Regarding the potential of wild fruit collection as a tourist activity (Table 5), the majority of respondents believe that it could contribute to the development of local tourism: 35% evaluated the impact “to a very great extent”, and 26% “to a great extent”. More reserved assessments include 21% “to a small extent” and 12% “to a very small extent”. The results suggest the opportunity to integrate this activity into the local tourism offer through the development of thematic routes, gastronomic experiences, or seasonal events. A relevant example is the Catalonia region of Spain, where wild food gathering represents a cultural ecosystem service integrated into rural and agrotourism, a phenomenon known as “fruit tourism” (Fusté-Forné, 2022; Crespi-Vallbona et al., 2023; Ginés-Ariza et al., 2023). Respondents’ assessments of the potential participation in activities or marketing of wild fruit products in the context of rural tourism (Table 5) indicate a high level of availability. In total, 70% of respondents assessed this possibility favourably, of which 39% “to a large extent” and 31% “to a very large extent”. Another 16% of the responses indicated “to a small extent”, while the proportions of those who were reluctant were reduced (8% “to a very small extent” and 4% “not at all”), and 2% could not assess. The results suggest that the integration of activities of collecting, processing, or marketing wild fruit products into rural tourism is perceived as an opportunity to diversify income and capitalize on local traditions, thus contributing to rural development (Mahapatra & Panda, 2012). Regarding the knowledge of traditional products made from wild fruits (Table 5), the results indicate a relatively balanced situation: 47% of

the respondents declared that they are familiar with such products, and 53% stated that they do not know them. These data may reflect a moderate visibility of these products in the communities analysed, possibly influenced by urbanization, the orientation towards modern foods and medicines, and the limited transmission of knowledge between generations (Punchay et al., 2020; Faruk et al., 2024). The perception regarding the level of exploitation of wild fruits in the past (Table 5) shows that the majority of respondents (61%) believe that these resources were exploited more intensively than at present, while 12% indicated the opposite, and 27% could not assess. This perception can be explained by the existence, during the communist period, of organized programs for the collection of wild fruits coordinated by state institutions, which encouraged the participation of the population and the systematic exploitation of these resources, a situation similar to that observed in the Soviet Union (Bexultanova et al., 2022; Faruk et al., 2024).

CONCLUSIONS

The results of the study indicate that wild fruits are perceived by respondents as valuable resources from a nutritional, therapeutic, and economic point of view, but their consumption generally remains moderate. Most participants recognize the health benefits of these products and consider that they can be part of a healthy diet, although their use is rather occasional. Respondents also show significant openness to the economic exploitation of wild fruits. A significant proportion believes that their commercialization can contribute to the development of local communities and the generation of additional income. In this context, there is interest in participating in activities such as paid collection, processing, or marketing of products within rural tourism. Also, most respondents positively perceive initiatives regarding the establishment of collection centres or processing units, considered opportunities for stimulating the local economy and the sustainable exploitation of natural resources. At the same time, the moderate level of knowledge of traditional products suggests a decrease in the intergenerational transfer of knowledge related to the use of wild fruits. Overall, the results

highlight the potential of these resources for rural development, provided that strategies are implemented to support their sustainable valorisation and the promotion of local traditions.

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RELATIONSHIPS BETWEEN THE MINERAL COMPOSITION OF FLOWERS AND LEAVES IN THREE PEACH CULTIVARS

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Abstract

Peach leaves function as the main organ for mineral accumulation and redistribution, reflecting soil conditions and cultivar characteristics, while the mineral composition of flowers provides an earlier indication of the tree's nutritional status. The present study examines the cultivars Cresthaven, Glohaven, and Redhaven using data on essential nutrients (N, P, K, Ca, Mg, and Fe) in flowers and leaves. Calculated elemental ratios reveal differences in nutritional balance and potential productivity among the cultivars. Principal Component Analysis (PCA) was performed, identifying variable correlation relationships between nutrient contents in flowers and leaves. Moderate to strong correlations were observed at 60 DAFB, particularly for P, Mg, Ca, and Fe, indicating coordinated physiological roles during early vegetative growth. The study provides a scientifically grounded basis for improving nutrient management strategies based on the individual needs of plants rather than a uniform approach.

Key words: peach, nutrient management strategies.

INTRODUCTION

Plant tissue analysis is a fundamental tool for diagnosing nutritional status, with leaf analysis traditionally considered the standard method in fruit-growing practice. In recent years, the classical diagnostic approach has expanded through the inclusion of different plant organs and the monitoring of nutrient dynamics during individual phenological stages. It has been established that flower analysis can serve as a complementary method to leaf analysis, particularly for early diagnosis at the beginning of the growing season (Gui et al., 2014; Martinez et al., 2003). Under conditions of pronounced nutrient deficiency or excess, this approach allows timely correction of fertilization before irreversible losses in yield and quality occur.

Studies on apple ('Gala') have demonstrated significant correlations between nutrient contents in buds, flowers, and leaves, creating opportunities for early assessment of nutritional status and optimization of fertilization practices (Mota et al., 2022). Similarly, in almond, flower analysis at the beginning of the growing season has been shown to predict the dynamics of macro- and micronutrients in leaves and fruits throughout the season (Bouranis et al., 2001).

Contemporary research emphasizes the importance of phenology-oriented diagnostics and integrated nutrient management strategies. Monitoring the seasonal dynamics of individual elements, including micronutrients, reveals pronounced variations associated with growth intensity and reproductive development (Kozin et al., 2025). Review publications highlight the necessity of applying the "4R" principles (right source, right rate, right time, and right place) and combining different diagnostic methods to increase the precision and sustainability of nutrient management (Muneer et al., 2024). The practical effectiveness of this approach has been confirmed in various fruit crops, where optimized fertilization rates significantly influence the mineral composition of leaves, as well as yield and fruit quality (Al-Saif, 2026; Ferreira et al., 2025; Wang et al., 2025).

Research in this field also emphasizes the integration of physiological, biochemical, and statistical approaches for a better understanding of growth, adaptability, and productivity in plant species. Methods such as Principal Component Analysis (PCA) are widely used to reveal complex relationships among studied traits, both in the context of physiological and nutritional analyses (Bolat et al., 2026) and in the synthesis of phenological and genetic variation (Nicolás-

Almansa et al., 2026; Osorio et al., 2026). The present study fits within this contemporary scientific framework by applying PCA for an integrated evaluation of mineral nutrition and physiological responses with an applied nutritional and diagnostic perspective.

The study aims to analyze the relationships between the contents of chemical elements in flowers and leaves of three peach cultivars in order to provide scientifically justified recommendations for nutrient application in peach production and to optimize production outcomes.

MATERIALS AND METHODS

The experiment was conducted during the period 2023-2024 in a fruit-bearing peach orchard including the cultivars *Cresthaven*, *Glohaven*, and *Redhaven*. Samples for mineral composition analysis were collected from 15 trees of each cultivar, with each tree considered as a separate replication. Flower samples were collected at the phenological stage “Full Bloom” (FB - more than 75% of flowers open), with 110 whole flowers taken from each tree. Leaf samples were collected at 60 and 120 days after full bloom (DAFB) from the middle part of one-year-old shoots.

The content of the main nutrient elements in flowers and leaves was determined as follows: nitrogen (N) by the Kjeldahl method; potassium (K) by flame photometry; phosphorus (P) and iron (Fe) by spectrophotometry; and calcium (Ca) and magnesium (Mg) by complexometric titration.

Principal Component Analysis (PCA) was applied in the study to examine the correlation relationships between chemical elements in flowers and leaves of the three cultivars. The results of the analysis indicate which elements have the greatest significance for the overall mineral pattern of the plants in the experiment. Principal Component Analysis is widely used in economic and agro-economic research (Keranova et al., 2022; Masheva & Uzunhalieva, 2025; Sharma et al., 2025; Amir et al., 2026; Khadici et al., 2026).

To evaluate the relationships between the contents of chemical elements in flowers and leaves of the studied peach cultivars, Spearman's rank correlation coefficient (ρ) was used. A standardized interpretation scale was

applied: $\rho = 0.00-0.19$ (very weak correlation, indicating no relationship between variables); $\rho = 0.20-0.39$ (weak correlation); $\rho = 0.40-0.59$ (moderate correlation); $\rho = 0.60-0.79$ (strong correlation); and $\rho = 0.80-1.00$ (very strong correlation). The correlation analysis was applied in a cross-comparison between the element contents in flowers and the different elements in leaves analyzed on the 60th and 120th day after flowering, in order to identify potential physiological and nutritional interactions relevant to mineral nutrition management in peach. Principal Component Analysis was calculated using the following formula: $PC1 = a_1 \cdot X_1 + a_2 \cdot X_2 + a_3 \cdot X_3 + a_4 \cdot X_4$, where a_1 , a_2 , a_3 , and a_4 represent the factor loadings (the importance of the indicators), and X_1 , X_2 , X_3 , and X_4 are the standardized variables (the chemical elements in the present study). The principal components are arranged according to the explained variance - PC1 explains the largest proportion, PC2 the next most important, and so on. In physiological analysis, the variance aims to demonstrate the importance of the chemical elements for the overall plant model.

RESULTS AND DISCUSSIONS

The obtained results indicate that mineral nutrition in peach is a dynamic process strongly dependent on the phenological stage and the agroecological conditions during the respective year. Interactions between nutrient elements change both over time (FB, 60 and 120 DAFB) and between years, which corresponds to contemporary concepts of variable nutritional status in fruit crops under the influence of climatic factors, agricultural practices, and physiological activity (Cai et al., 2023).

In 2023, the correlation analysis between elements in flowers and those in leaves at 60 DAFB for the cultivar *Cresthaven* demonstrated the presence of several moderate and strong relationships between elements in flowers and leaves, indicating that flower analysis may serve as an early indicator of the subsequent mineral status of vegetative organs (Figure 1). The most pronounced relationships were observed for the following element pairs: Ca (Flowers) - K (Leaves); P (Flowers) - P (Leaves); P (Flowers) - Mg (Leaves); Fe (Flowers) - Fe (Leaves). At

60 DAFB, a strong synergistic interaction between P–Fe–Mg was observed, associated with intensive growth and high photosynthetic activity, while K showed antagonistic relationships with micronutrients.

At the later stage (120 DAFB), the synergism between P–Ca–N increased, which is associated

with fruit formation and ripening, while Fe showed a clearly expressed antagonism toward the main macronutrients. The interactions among the elements became more structured, indicating a redistribution of nutrients toward the fruits (Figure 2).

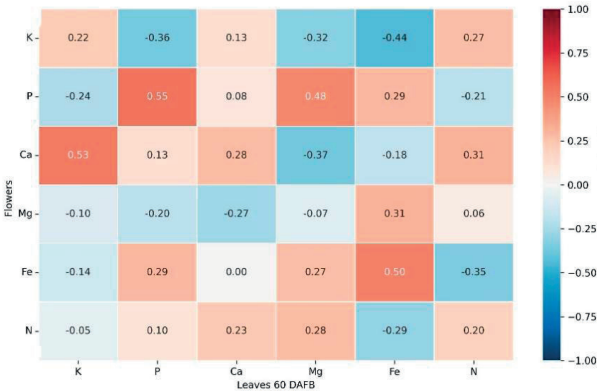


Figure 1. Correlation matrices showing the relationships between chemical elements in flowers and leaves at 60 DAFB in the cultivar ‘Cresthaven’ in 2023

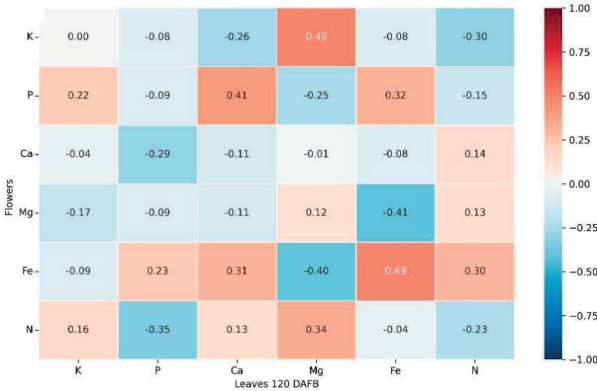


Figure 2. Correlation matrices showing the relationships between chemical elements in flowers and leaves at 120 DAFB in the cultivar ‘Cresthaven’ in 2023

In 2024 (60 DAFB), a strong positive correlation was established between P (Flowers) – Mg (Leaves) and N (Flowers) – Mg (Leaves), as well as a moderate relationship between Fe (Flowers) – N (Leaves), highlighting the role of magnesium in early physiological processes. A pronounced negative relationship was also recorded between Ca (Leaves) and Mg (Flowers), indicating antagonism between the two elements (Figure 3). These interactions

suggest an active exchange among macroelements at the early stage of development. At 120 DAFB, the correlation relationships weaken, with only a moderate dependence between Fe (Flowers) – N (Leaves) remaining (Figure 4). This confirms the dynamic nature of the mineral balance during different phenological phases.

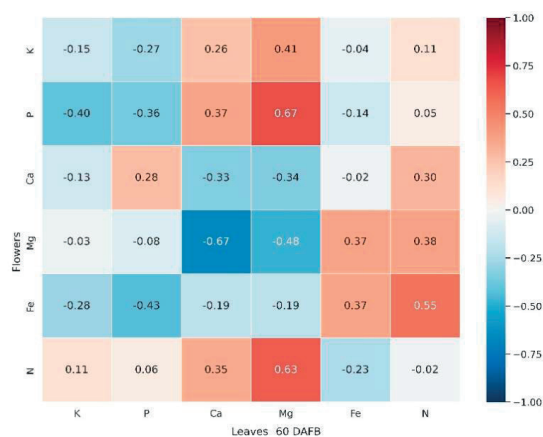


Figure 3. Correlation matrices of the relationships between chemical elements in flowers and leaves at 60 DAFB of the ‘Cresthaven’ cultivar in 2024

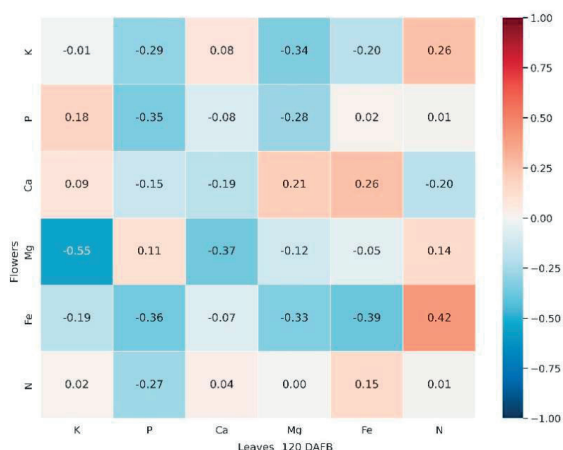


Figure 4. Correlation matrices of the relationships between chemical elements in flowers and leaves at 120 DAFB of the ‘Cresthaven’ cultivar in 2024

The application of a comprehensive PCA identified the leading chemical elements that describe the mineral balance of the plant.

The results are presented through a Scree plot figure, which facilitates the visualization of the significant components, and through a table describing the component loadings of the chemical elements, where a higher value indicates a greater physiological contribution (Figure 5).

The PCA results reveal a clearly structured mineral balance, in which the first two principal components (PC1 and PC2) explain the major part of the variation. Of particular interest is the fact that the leading elements change between 2023 and 2024 and also between the cultivars.

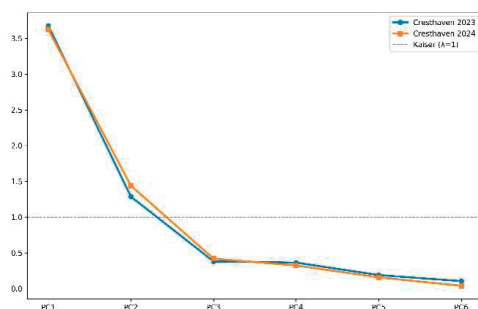


Figure 5. Eigenvalues of the principal components for 2023 and 2024

In 2023, PC1 was dominated by Fe, P, Mg, and K, whereas in 2024 a rearrangement was

observed with a stronger contribution from Ca and Mg (Table 1). In 2024, Fe showed a strong loading in the second component (PC2), indicating a change in its role within the overall elemental structure.

This reorganization of the component loadings reflects physiologically driven changes in nutrient priorities, likely influenced by climatic conditions and yield load. These two factors represent a prerequisite for strengthening the robustness of the study through the accumulation of a longer statistical data series.

Table 1. Component loadings of chemical elements for the ‘Cresthaven’ cultivar in 2023 and 2024

Chemical elements	2023 PC1 (%)	2023 PC2 (%)	2024 PC1 (%)	2024 PC2 (%)
K	19.32	5.18	23.26	2.43
P	19.88	2.75	19.52	5.72
Ca	18.60	16.24	23.16	4.52
Mg	19.46	7.86	23.98	0.36
Fe	20.63	0.91	1.35	54.67
N	2.12	67.11	8.75	32.23

Based on the conducted study, it was established that the application of combined fertilizers should not follow a uniform scheme throughout the entire growing season, but should instead be based on a stage-specific analysis of plant material. Flower analysis is used for early diagnosis of the mineral status and for guidance regarding initial corrective measures. Around the 60th day after full bloom (DAFB), a leaf

analysis is performed, allowing targeted fertilization - either jointly in the presence of synergistic relationships between elements or sequentially in the case of antagonistic interactions.

By the 120th day after full bloom, when the correlation relationships weaken and become restructured, fertilization should be individualized and limited to the correction of specific deficiencies, with the aim of maintaining the mineral balance, rather than through general combined applications.

The correlation relationships between elements in flowers and leaves in the ‘Glohaven’ cultivar do not follow a common pattern but vary over time. In 2023, the correlation analysis at 60 DAFB revealed moderate relationships between K (Flowers) – Mg (Leaves), P (Flowers) – Fe (Leaves), and N (Flowers) – N (Leaves) (Figures 6 and 7).

In 2024, at 60 DAFB, moderate positive correlations were observed between Ca (Flowers) and Ca (Leaves), as well as between N (Leaves) and Mg (Flowers), while Fe (Leaves) showed a strong negative relationship with K (Flowers). At the second sampling date (120 DAFB), the correlations weakened, with Ca showing more pronounced negative relationships with N and P (Figures 8 and 9). This suggests that as leaf development progresses, the interactions among nutrient elements change.

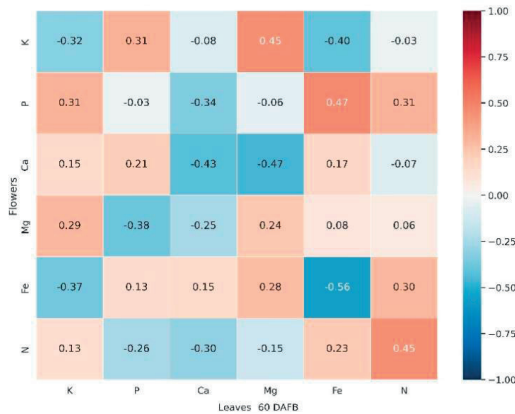


Figure 6. Correlation matrices of the relationships between chemical elements in flowers and leaves at 60 DAFB for the ‘Glohaven’ cultivar in 2023



Figure 7. Correlation matrices of the relationships between chemical elements in flowers and leaves at 120 DAFB for the 'Glohaven' cultivar in 2023

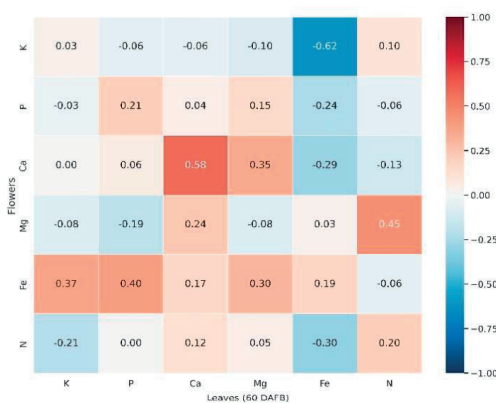


Figure 8. Correlation matrices of the relationships between chemical elements in flowers and leaves at 60 DAFB for the 'Glohaven' cultivar in 2024



Figure 9. Correlation matrices of the relationships between chemical elements in flowers and leaves at 120 DAFB for the 'Glohaven' cultivar in 2024

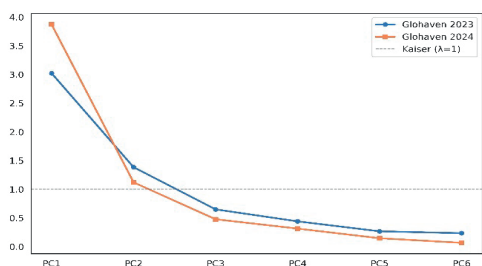


Figure 10. Eigenvalues of the principal components for 2023 and 2024

The PCA results show that in the ‘Glohaven’ cultivar there is a clearly expressed structural organization of the mineral balance, with the main variation explained by a limited number of leading elements (Table 2, Figure 10). A change in the leading elements and their significance is observed between the individual years, indicating that the mineral balance in this cultivar is also dynamic. The PCA results further demonstrate that the mineral balance in ‘Glohaven’ is determined by a small number of key elements, although their relative importance varies between years.

In 2023, Fe and Mg showed high loadings in PC1, whereas in 2024 Fe strongly dominated in the second component (PC2). This confirms the need for an adaptive rather than a uniform approach in the management of mineral nutrition.

Table 2. Component loadings of chemical elements for the ‘Glohaven’ cultivar in 2023 and 2024

Chemical elements	2023 PC1 (%)	2023 PC2 (%)	2024 PC1 (%)	2024 PC2 (%)
K	0.32	57.84	20.40	8.08
P	21.00	2.21	23.08	3.19
Ca	14.32	22.24	16.28	0.98
Mg	22.62	0.02	17.63	7.78
Fe	25.74	0.84	1.39	78.90
N	16.01	16.86	21.22	1.07

For the ‘Redhaven’ cultivar, the applied correlation analysis between elements in flowers and those in leaves in 2023 revealed a strong correlation between Ca (Flowers) – Mg (Leaves) at 60 DAFB, which was not maintained during the second sampling and analysis period of leaf samples (120 DAFB) (Figures 11 and 12).

At 120 DAFB, strong positive correlations were observed between P and Mg, as well as between Fe and Mg, indicating a synchronization in the accumulation of these elements in the leaves. At the same time, Ca exhibited clearly negative relationships with K and N, which may suggest antagonistic interactions. Overall, a clear separation was observed between groups of elements with positive internal relationships and those showing opposing trends (Figure 12).

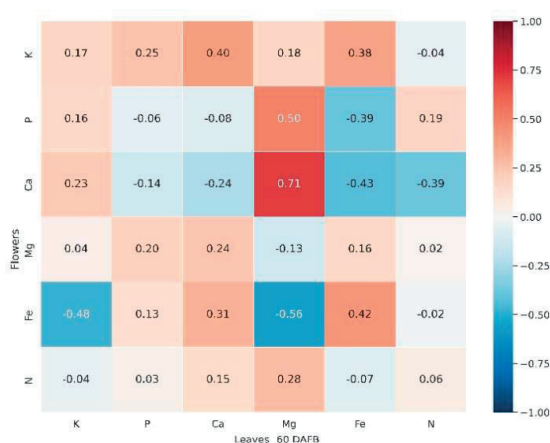


Figure 11. Correlation matrices of the relationships between chemical elements in flowers and leaves at 60 DAFB for the ‘Redhaven’ cultivar in 2023.

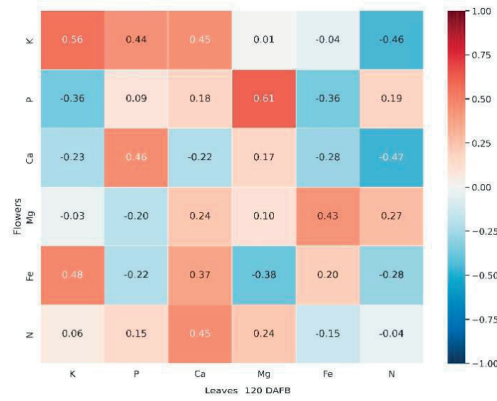


Figure 12. Correlation matrices of the relationships between chemical elements in flowers and leaves at 120 DAFB for the 'Redhaven' cultivar in 2023

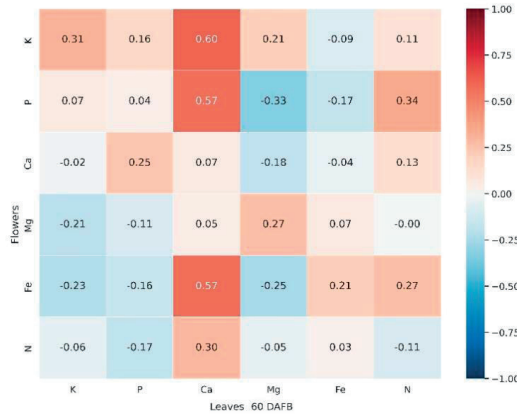


Figure 13. Correlation matrices of the relationships between chemical elements in flowers and leaves at 60 DAFB for the 'Redhaven' cultivar in 2024.

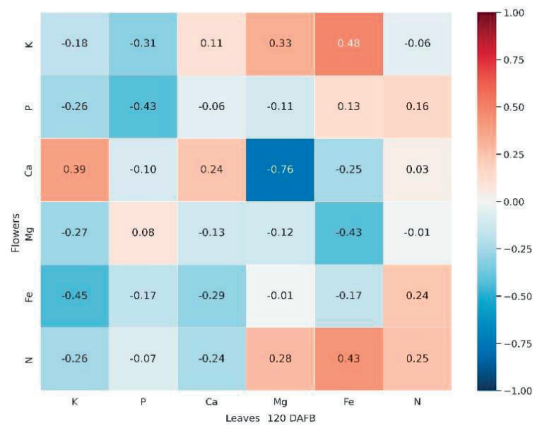


Figure 14. Correlation matrices of the relationships between chemical elements in flowers and leaves at 120 DAFB for the 'Redhaven' cultivar in 2024

For 2024, strong correlation relationships were established between K (flowers) and Ca (leaves), as well as moderate correlations between Ca (leaves) and P (flowers) and between Ca (leaves) and Fe (flowers) (Figures 13 and 14). The lack of stable relationships during the second sampling period confirms that element interactions are temporally variable and cultivar-specific.

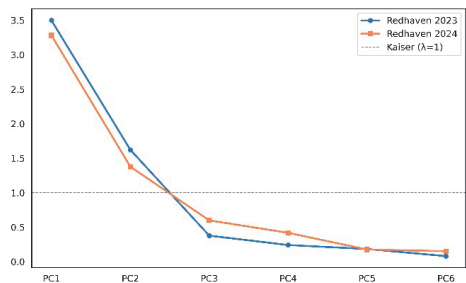


Figure 15. Eigenvalues of the principal components for 2023 and 2024

The eigenvalues and component loadings, similarly to the first two studied cultivars, do not follow a common pattern but develop dynamically, which confirms, on the one hand, the non-uniform interaction among chemical elements and, on the other, the need for an individual approach to fertilization (Figure 15). In 2024, Ca and Mg dominated in PC1, while Fe showed a high loading in PC2, indicating a functional redistribution of the role of microelements in the overall mineral structure (Table 3).

Table 3. Component loadings of chemical elements for the ‘Redhaven’ cultivar in 2023 and 2024

Chemical elements	2023 PC1 (%)	2023 PC2 (%)	2024 PC1 (%)	2024 PC2 (%)
K	2.09	53.68	19.15	1.79
P	21.36	0.60	14.25	29.76
Ca	18.47	13.86	25.28	0.06
Mg	22.19	3.34	25.51	0.61
Fe	16.32	17.22	6.64	46.09
N	19.55	11.29	9.17	21.70

The obtained data have direct practical significance. Early flower analysis provides an opportunity to predict potential deficiencies and to implement early corrective measures, particularly with respect to P, Mg and Fe. Leaf analysis at 60 DAFB allows more precise

adjustment of fertilization during the period of active growth and yield formation.

By 120 DAFB, when correlation relationships weaken, the approach should be strictly individualized and based on specific deficiencies, rather than on combined fertilizer formulations. This corresponds to the modern principles of precision agriculture and sustainable nutrient management, where the objective is to optimize yield and fruit quality while minimizing environmental risk.

Maatallah et al. (2024) and Dascalu et al. (2024), reported results on the role of nitrogen and potassium as leading factors in individual PCA components in experimental studies on peach, demonstrating that optimal levels of N and K during fruit development and ripening increase yield and improve quality parameters (fruit mass, soluble solids content, and coloration). At the same time, excessive nitrogen application does not lead to further yield increases, highlighting the need for precise diagnostics and balanced fertilization. This corresponds with the conclusion of the present study that fertilization at 120 DAFB should be limited to the correction of specific deficiencies rather than using general combined applications.

The established relationship between Fe and N content in different years is consistent with contemporary research on leaf diagnostics applying correlation and multivariate analysis. Mertoğlu & Kırca (2025) identified significant relationships between macro- and microelements (including Fe) in leaves and fruits of apple. Modern chemometric models confirm that leaf nitrogen is a reliable indicator of the physiological and nutritional status of apple (Hou et al., 2024; Zhao et al., 2024).

The PCA analysis in the present study demonstrates a clearly structured mineral balance, with the first two principal components explaining a substantial proportion of the total variation. A similar approach is widely used in the evaluation of complex biochemical and nutritional indicators in peach and other fruit crops for the identification of leading factors and grouping of cultivars according to similarity (Nowicka et al., 2019). In this context, the changes in component loadings between 2023 and 2024 reflect not random variability but a physiologically driven reorganization of elemental interactions.

CONCLUSIONS

The mineral balance in peach is a dynamic, phenologically determined, and cultivar-specific process. The leading nutrient elements and their relative importance change between developmental stages and between years.

Early flower analysis (during flowering) may serve as a tool for predicting potential imbalances. Leaf analysis at 60 DAFB enables refinement of fertilization during the period of active growth, while at 120 DAFB the approach should be strictly individualized and focused on specific deficiencies.

The obtained results support the need for an adaptive, phenology-based management of mineral nutrition, rather than the application of uniform fertilization schemes.

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FURTHER STUDIES ON THE DAMAGES ON *FRAGARIA ANANASSA* VAR. 'SNOW WHITE' CAUSED BY *ERWINIA AMYLOVORA* AND APPLICATION OF *CHAENOMELES* PLANT EXTRACT FOR REDUCTION OF SYMPTOMS

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Abstract

Erwinia amylovora causes the well-known disease "fire blight", which affects a large number of plants from the Rosaceae family. Strawberries are also among the pathogen's hosts. The present study aimed to further investigate the damages that *E. amylovora* induces in greenhouse-grown strawberries (*Fragaria ananassa*) var. "Snow White" in Bulgaria and the potential of a plant-derived extract from fruits of *Chaenomeles* sp. to reduce the symptoms for another vegetation period following early infection. The experiment includes four irrigation and fertilization levels, as well as a comparison between the symptoms of the disease in the greenhouse and those induced in optimal laboratory conditions. The results showed greater leaf and flower losses in the diseased plants compared to the healthy plants. Treatments with the plant extract reduced the manifestation of symptoms below the levels observed in the diseased plants.

Key words: *Erwinia amylovora*, *Fragaria ananassa*, disease control, *Chaenomeles* sp., plant extract.

INTRODUCTION

Erwinia amylovora is a pathogen affecting numerous crops and other plants belonging to the family Rosaceae, thus posing a serious challenge for agricultural producers. The pathogen is difficult to control, and copper-based products are still extensively used, resulting in their accumulation in the environment. In research for safer alternatives, scientists have investigated the potential of botanical extracts as sources of antimicrobial compounds. Although such studies have been extensively conducted over the years, most have focused on applications in medicine, pharmaceuticals, food technology, and cosmetology, whereas investigations in plant pathology remain scarce (Jantova et al., 2000; Borges et al., 2018; Gutiérrez-Pacheco et al., 2019). Only a limited number of plant-derived products have been examined for their activity against plant pathogenic bacteria (Stangarlin et al., 1999; Schwan-Estrada et al., 2005; Mohamed, 2010; Bhagwat & Datar, 2014; Stoyanova & Valkova, 2014, 2015a, 2015b;

Ganiyu et al., 2017). This is largely due to the enormous diversity of plant species as well as the wide range of phytopathogenic microorganisms. Additional factors, including strain differences, extraction procedures, application methods, and host specificities, further contribute to the global pace of research and the still limited availability of data.

Several plant extracts demonstrated antibacterial activity against *E. amylovora* *in vitro* (Mosch et al., 1990; Mosch & Zeller, 1996; Chiriac & Ulea, 2012; Stoyanova & Valkova, 2014; Fontana et al., 2022; Stoyanova et al., 2024). A recent *in vivo* study revealed a promising potential of a methanol extract from *Chaenomeles* sp. fruits to control the pathogen when applied as preventive sprays under greenhouse conditions (Stoyanova et al., 2025). *Chaenomeles* is widely distributed throughout Asia and Europe and exhibits considerable genetic diversity. Nevertheless, current evidence suggests that the antibacterial activity against *E. amylovora* is not restricted to certain genotypes (Stoyanova et al., 2024).

The aim of the present study was to further investigate the damages induced by *E. amylovora* in greenhouse-grown strawberries (*Fragaria ananassa*) var. “Snow White” in Bulgaria and the potential of a plant-derived extract from fruits of *Chaenomeles* sp. to reduce the symptoms during the next vegetative period under conditions of early infection.

MATERIALS AND METHODS

Plant extract. The extract was derived from frozen fruits of *Chaenomeles* sp., genotype 29, originating from the field of the Research Institute of Mountain Stockbreeding and Agriculture, Agricultural Academy, Troyan, by Soxhlet extraction in methanol (HPLC grade) at 80°C for 5 hours. The solvent was recovered in a vacuum vaporizer (model Hei VAP Precision, Heidolph Inst.) at 55°C, 30 000 Pa, and the extracts were concentrated at decreasing pressure down to 7 200 Pa and a temperature of 65°C. The extract was stored at 18°C, in the dark until use and applied as a freshly prepared 2% (w/v) aqueous solution by spraying, based on previous studies (Stoyanova et al., 2024, 2025).

Bacterial strain. The test strain *Erwinia amylovora* NBIMCC 2331 (=ATCC 15580, type strain, isolated from pear) was applied by spraying as water suspensions with 75% transmittance (measured with a turbidimeter Biolog Inc.) for plant inoculation.

Vacuum infiltration. Young plants were inoculated following the method of Jones et al. (1981). Plants were immersed in a bacterial suspension at 85% transmittance and subjected to vacuum in a desiccator generated by a water aspirator with a flow rate of 10 L/min, two consecutive 3-minute exposures separated by a 5-minute interval, in five replicates. Control plants were infiltrated with sterile distilled water. The experiment was performed twice, using plants from varieties “Murano” and “Snow White” in 2024 and 2025, respectively.

Plants. A total of 160 strawberry plants *Fragaria ananassa* variety “Snow White” were used for the main study. The plants were grown in a non-heated tunnel greenhouse, covered with a 150 µm five-layer UV+EVA+IR+AD+dif polyethylene film, and located at the Experimental Base “Chelopechene” of the Institute of Soil Science, Agrotechnologies and

Plant Protection “Nikola Pushkarov”, Agricultural Academy, Sofia, Bulgaria (42°44'22.8"N, 23°28'3.7"E). The plants were mulched with 30 µm silver-black UV polyethylene film and cultivated using drip irrigation and fertilization.

Variants of Irrigation and Fertilization (VIF). We used four VIFs: 100% irrigation (I0F0), 75% irrigation and 100% fertilization (I1F1), 75% irrigation and 75% fertilization (I1F2), 50% irrigation and 75% fertilization (I2F2).

Experimental design. For each VIF, the plants were divided into four groups: healthy (negative control, *H*), treated with extract (second negative control, *T*), experimental (treated with extract and inoculated with pathogen, *E*) and infected (inoculated with pathogen, *I*). Each group included 10±1 plants. The withered leaves and flowers of each plant were recorded weekly and removed from the plants prior to treatment to avoid recounting. Treatments were applied once per week for eight weeks during the period April-June 2025. Inoculations were conducted in the first and fourth weeks, one hour after treatment, to compensate for the reduced disease pressure due to the manual removal of diseased plant parts.

Statistics. The data were statistically analyzed by Duncan's Multiple Range test at significance levels of $p < 0.05$ using STATISTICA 8.0 software.

RESULTS AND DISCUSSIONS

The progression of disease symptoms following infection with *E. amylovora* was monitored after vacuum infiltration of strawberry seedlings. The objective was to compare symptom development under optimal conditions for pathogen growth with the symptoms induced on infected white strawberry plants under greenhouse conditions.

Seedlings subjected to vacuum infiltration predominantly developed marginal or diffuse necrosis of the leaves, which rapidly spread across the entire leaf surface, accompanied by necrosis of the petioles. In a smaller proportion of leaves, necrotic lesions were associated with transient chlorosis. The young plants became completely desiccated within approximately three weeks. Both strawberry varieties exhibited similar symptoms, severity, and progression

rates. Similar symptom patterns were observed in the main greenhouse experiment. Infection of leaves in the greenhouse most commonly began as marginal necrosis, which subsequently progressed to involve the entire leaf petiole, with the young leaves being more susceptible. Less frequently, diffuse necrosis could also be observed. Preceding chlorosis was

observed only rarely. Symptoms on flowers included necrosis and chlorosis on sepals (Figure 1). Necrosis of the petioles or peduncles could also develop. At the final stage of infection, complete withering of the infected leaves and flowers was observed regardless of the inoculation method, with the affected organs remaining attached to the plants.

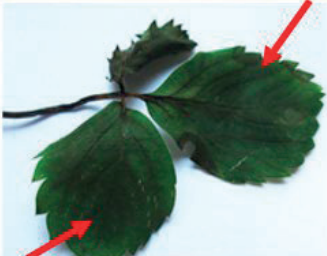
Frequency of symptoms	Symptoms of infection after inoculation with <i>E. amylovora</i> by vacuum infiltration	Symptoms of infection after inoculation with <i>E. amylovora</i> by spraying	Frequency of symptoms
Common (marginal necrosis)			Most common (marginal necrosis)
Common (diffuse necrosis)			Common (diffuse necrosis)
Rare (chlorosis and necrosis)			Rare (chlorosis and necrosis)
Rare (chlorosis)			Very rare (chlorosis)

Figure 1. Symptoms of infection with *E. amylovora* on leaves and flowers of strawberry plants using two methods of inoculation - vacuum infiltration in laboratory conditions and spraying in greenhouse conditions

Frequency of symptoms	Symptoms of infection after inoculation with <i>E. amylovora</i> by vacuum infiltration	Symptoms of infection after inoculation with <i>E. amylovora</i> by spraying	Frequency of symptoms
Common (necrosis of petioles)			Common (necrosis of petioles)
Final stage of infection (withering)			Final stage of infection (withering)

Figure 1. Symptoms of infection with *E. amylovora* on leaves and flowers of strawberry plants using two methods of inoculation - vacuum infiltration in laboratory conditions and spraying in greenhouse conditions (*continued*)

The types of symptoms of infection in greenhouse conditions during 2025 were observed at approximately the same frequency like in 2024.

In the main greenhouse experiment, infected, non-treated plants (positive control) exhibited the greatest loss of leaves and flowers compared to all other groups. The number of withered leaves in the positive control plants was 2.7-fold higher than in the negative control (non-inoculated, non-treated plants), which primarily

reflects physiological senescence. Compared to treated (non-inoculated, extract-applied) and experimental (inoculated, extract-applied) plants, the positive controls displayed 3.7- and 3.6-fold greater leaf loss, respectively (normalized per plant) (Figure 2). A similar pattern was observed for flowers. Infected plants lost 14.8-, 8-, and 10.3-fold more flowers than the healthy, treated, and experimental plants, respectively (normalized per plant) (Figure 2).

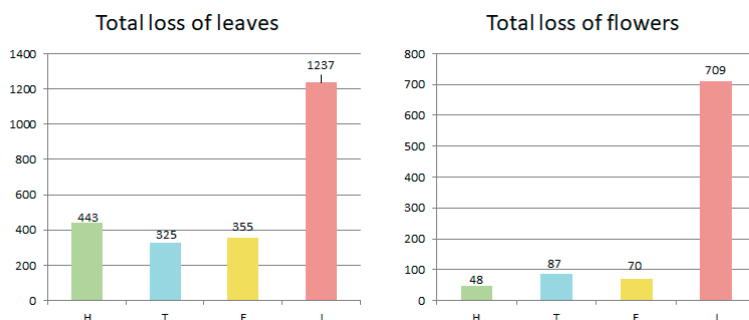


Figure 2. Loss of leaves and flowers of healthy (H), treated (T), experimental (E) and infected (I) plants for a period of eight weeks

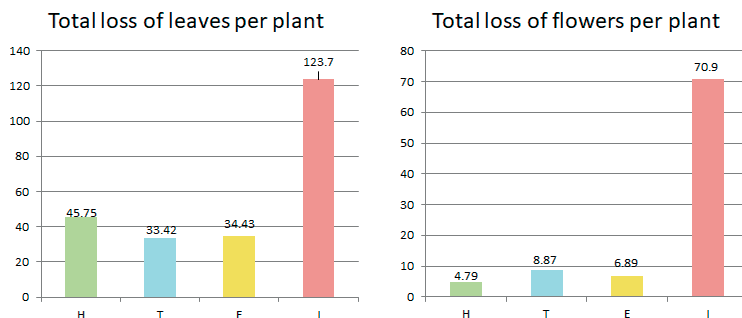


Figure 2. Loss of leaves and flowers of healthy (H), treated (T), experimental (E) and infected (I) plants for a period of eight weeks (*continued*)

Leaf and flower losses were normalized relative to the healthy control (H), yielding approximate ratios for each treatment group. According to the data, the ratios of the infected group are substantially higher compared to the ones of the treated and experimental plants (Table 1).

Table 1. Approximate ratios of leaf and flower loss calculated towards the healthy plants (values are rounded)

H : T : E : I	
leaves	flowers
1 : 0.7 : 0.8 : 2.7	1 : 1.9 : 1.4 : 14.8

Statistical analysis confirmed that the differences observed in the infected plants were significant ($p < 0.05$), while the losses of the healthy, treated and experimental groups were not statistically different (Table 2). These results further indicate that the extract had no detrimental effect on strawberry plants.

Table 2. Average loss of leaves and flowers of the groups: healthy (H), treated (T), experimental (E) and infected (I)

	H	T	E	I
leaves	5.64a	4.18a	4.3a	15.46b
flowers	0.6a	1.11a	0.86a	8.85b

Values with different lowercase letter for the same parameter were statistically different.

Analysis of the recorded data with respect of the different variants of irrigation and fertilization (VIFs) distributed the leaf and flower losses in a tendency following the global one: in every variant, the plants that lost the most leaves and flowers were the infected ones. The experimental plants suffered lower leaf loss than

the healthy ones in I0F0, I1F2 and I2F2, and the loss value of I1F1 leaves was closer to the healthy plants compared to the diseased ones (Figure 3). The experimental plants suffered lower flower losses than the healthy ones in I1F2, and the flower loss values of I0F0, I1F1 and I2F2 were closer to the healthy plants compared to the diseased ones (Figure 3).

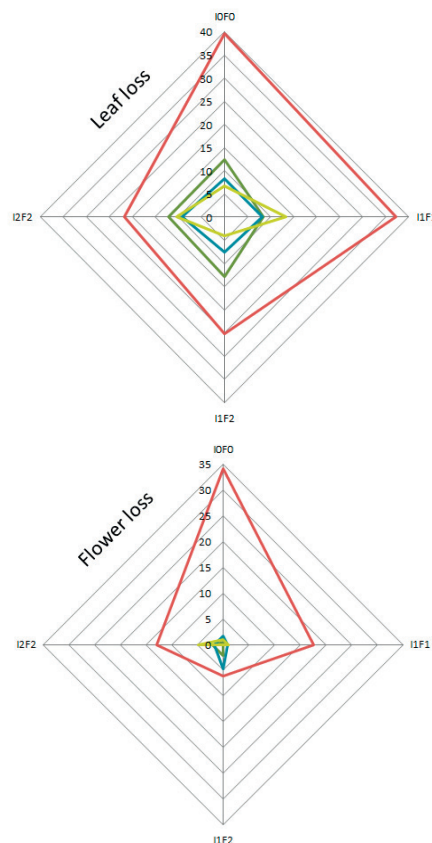


Figure 3. Leaf and flower loss per plant by VIFs

However, some differences among the groups could be observed. Bigger gaps of leaf and flower losses between the infected and the other three groups (H, T and E) were observed for variants I0F0 and I1F1. The least differences were recorded for flower loss of variant I1F2. Through the vegetation period of 2025, the lack of fertilization, in combination with optimal irrigation, favored the most the development of the disease in terms of both leaf and flower loss. When compared to the previous year, the counts of lost leaves per plant for 8 weeks are not very different as values for variants I1F1, I1F2 and I2F2, but the losses for I0F0 are much higher (Table 3). This could be explained by the continuous exhausting of the plants due to lowering of the soil nutrients.

Statistical analysis of the data on leaf loss with respect to the VIFs revealed significant differences among the groups. In all four variants, the infected plants were clearly separated from the healthy, treated, and experimental plants (Table 4).

Statistical analysis of flower loss with respect to the VIFs indicated a significant separation only between the infected plants and the I0F0. All other plants were grouped within the same statistical category, which may be attributed to the relatively low number of flowers recorded (Table 5).

Compared to the results received in 2024, the losses of leaves per plant for 8 weeks are not very different as values for three of the variants. However, the loss of flowers is significantly lower, which mainly corresponds to the significantly lower rate of flowering (Table 3). A main reason for this could be the age of the strawberry plants. Differences in temperatures could also add to these results. During 2025, the experimental period started 4 weeks earlier

which was related to lower daily temperatures (Table 6). In addition, the last 4 weeks were colder compared to the same period in 2024 (Stoyanova et al., 2025) both in means of minimal and maximal values with differences up to 7 °C in the absolute values.

Table 3. Approximate ratios of leaf and flower loss of infected plants for years 2025/2024 (values are rounded)

VIFs	leaves	flowers
I0F0	1.6	0.7
I1F1	0.9	0.2
I1F2	0.8	0.1
I2F2	0.9	0.2

Values closer to 1 refer to closer count numbers for the two consecutive years.

Table 4. Average loss of leaves of healthy (H), treated (T), experimental (E) and infected (I) plants with respect to VIFs

leaves	H	T	E	I
I0F0	6.2a	4.2a	3.36a	19.85d
I1F1	4.17a	4.15a	6.7ab	18.55d
I1F2	6.09a	3.75a	2a	12.6c
I2F2	6.11a	4.61a	5.15a	10.85bc

Values with same lowercase letter for the same parameter were not statistically different.

Table 5. Average loss of flowers of healthy (H), treated (T), experimental (E) and infected (I) plants with respect to VIFs

flowers	H	T	E	I
I0F0	0.25a	0.85a	0.5a	17.05b
I1F1	0a	0.4a	0.5a	8.8a
I1F2	1.09a	2.35a	0.05a	3.05a
I2F2	1.06a	0.83a	2.4a	6.5a

Values with same lowercase letter for the same parameter were not statistically different.

Table 6. Temperature data during the experimental period (values based on 48 measurements per 24 hours, °C)

week	1st	2nd	3rd	4th	5th	6th	7th	8th
period	10.04-16.04	17.04-23.04	24.04-30.04	01.05-07.05	08.05-14.05	15.05-21.05	22.05-28.05	29.05-04.06
Absolute Min	-3.4	6.0	1.2	3.8	3.1	2.2	6.8	7.2
Daily Min	2.9±4.2	8.4±4.0	5.8±3.4	8.1±3.1	7.0±2.2	5.4±1.7	9.1±1.6	9.0±1.9
Absolute Max	35.5	42.7	40.8	43.6	39.0	39.1	41.8	46.5
Daily Max	27.8±5.3	38.4±6.0	33.7±5.5	39.4±2.9	33.2±3.8	34.2±7.9	28.5±9.3	39.2±6.5
Average	13.0±2.2	19.3±3.0	16.8±1.8	21.3±1.4	18.0±1.3	18.2±2.6	17.2±4.4	21.8±4.0

Infection with *E. amylovora* consistently induces premature leaf death in strawberries which can potentially affect plant development and physiological status. The disease also causes high flower loss, potentially affecting yield. The application of aqueous solution of the methanol extract from *Chaenomeles* sp. fruits in concentration of 2% (w/v) does not exert any negative visual effects on the plants and can significantly reduce the symptoms to levels that are characteristic to the healthy plants.

CONCLUSIONS

The study revealed that fire blight of strawberries var. "Snow White" manifests as leaf and flower loss, which may adversely affect overall plant development. The extract prepared from chaenomeles demonstrated a protective effect by limiting the development of the disease in the plants.

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GENETIC DIVERSITY, HERITABILITY AND HETEROSIS ESTIMATION IN F1 STRAWBERRY HYBRIDS

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Abstract

Strawberry (Fragaria × ananassa Duch.) is cultivated and enjoyed globally for early ripening of the fruits, high yields, and special nutritional qualities (high content of minerals and vitamins). In strawberry breeding, agronomic performance and fruit quality attributes highlight the success of strawberry breeding in recent decades. The present study evaluates the heritability of some biometric and biochemical parameters in hybrids derived from intraspecific crosses and their inheritance patterns. Broad sense heritability calculated over the entire experiment with hybrid combinations for most traits indicated a high degree of genotypic control, ranging between 0.59 (hue angle) and 0.91 (yield and chroma index). The values indicate a high proportion of total genetic variance to phenotypic variance, which indicates that hereditary endowment plays a significant role in the expression of traits. Narrow sense heritability estimates were mainly high for yield, fruit size, and fruit colour and low for fruit firmness, total soluble solids, and pH, indicating that, for half of the traits, more than 50% of the total genetic variation was the result of non-additive genetic effects and that the breeding process was quick. The results can use breeders in selecting parents for cross combinations and in maximizing genetic gain, ultimately for creating varieties that support the advancement of commercial strawberry production.

Key words: *Fragaria × ananassa, breeding, cross combinations, genetic gain, fruit quality.*

INTRODUCTION

Strawberry (*Fragaria × ananassa* Duch.) is cultivated and enjoyed globally for early ripening of the fruits, high yields, and special nutritional qualities (high content of minerals and vitamins). Research has demonstrated that consuming berries on a regular basis can help reduce the risk of cancer, cardiovascular disease, and neurodegenerative diseases (Hannum, 2004; Giampieri et al., 2015). Strawberries have a high antioxidant level that can help reduce oxidative stress when consumed fresh (Giampieri et al., 2013; Newerli-Guz et al., 2023). In addition, adding freeze-dried strawberry popping powder to the diets of individuals who are overweight or obese has been shown to reduce cholesterol levels (Richter et al., 2023). Strawberries are among the most recent crops, and they have only been cultivated for the past 275 years after the initial hybridization event in a French botanical garden (Wilhelm, 1974; Nellist, 2018). In 1817, Thomas A. Knight made history by creating the first official strawberry breeding program (Wilhelm, 1974; Hancock et al., 2005; Hummer & Hancock, 2009). The strawberry has undergone rapid evolution from a small, soft, short-season

fruit to the large, firm, long-season fruit (Simpson, 2018) through breeding programs. Strawberry breeders have been enhancing strawberry marketable yield and fruit quality (Zorrilla-Fontanesi et al., 2011; Klee and Tieman, 2018; Mezzetti et al., 2018). Fruit quality is a complex trait that includes multiple visual factors (uniformity, colour), organoleptic factors (flavour, texture), and sensory factors (firmness) Cardello (1995); Song et al. (2023); Wang et al. (2025). Environmental influences continue to cause significant variations in strawberry fruit quality and consistency, even though there have been extensive improvements over recent centuries (Schwieterman et al., 2014; Lado et al., 2019). Accurate selection can capture this variation with robust phenotyping protocols, resulting in maximum genetic gain and improved desirable traits. The new objective of strawberry breeding is to create fruit varieties that deliver a satisfying multi-sensory eating experience. Breeding programs require stages of selection for parents and progenies. The selection criteria for elite plants and progenies are determined by the variability in the base population and the relative magnitude of the genes that influence the phenotypic expression of traits. Information

about heritability, gene action, and correlation among traits is crucial in determining parents and selecting progeny. Selection of individual plants can only be successful if the variables being selected have high heritability values. Genetic parameters for plant and fruit traits of strawberries have been reported in previous studies, including Galleta and Maas (1990), Shaw (1993), Pringle and Shaw (1998), Fort and Shaw (2000), Hancock et al. (2008), Whitaker et al. (2012), Whitaker et al. (2020), Cockerton et al. (2021) and Zhongchi et al. (2023). Broad-sense heritability estimates were presented in the strawberry by Whitaker et al. (2012) and Cockerton et al. (2021) for most traits, which indicate that gains are possible for several economically important characters in the strawberry breeding population in terms of clonal deployment. Narrow-sense heritability estimates based on the means of strawberry families, such as fruit chemical traits, fruit traits including average fruit weight, and plant diameter, have been low to moderate, with little contribution of non-additive variance (Shaw, 1993; Fort and Shaw, 2000; Whitaker et al., 2012; Cockerton et al., 2021). Genetic parameters such as heritability and heterosis estimation for the Romanian strawberry breeding population have not been published until recently, which would be beneficial for shaping breeding and selection strategies (Sturzeanu & Hera, 2019). The effects of trait selection on the long-term and the behaviour of correlated traits, which, if negative, may hinder breeding progress if ignored during selection, are explained in this article. The present study evaluates the genetic diversity, heritability, and heterosis estimation of some biometric and biochemical parameters in hybrids derived from intraspecific crosses and their inheritance patterns. The results can use breeders in selecting parents for cross combinations and in maximizing genetic gain, ultimately for creating varieties that support the advancement of commercial strawberry production.

MATERIALS AND METHODS

Location and Conditions of the Study

The research was carried out at the Research Institute for Fruit Growing Pitesti (RIFG) located in Maracineni village (44°54'12" Northern latitude, and 24°52'18" Eastern

longitude, 284 m altitude) within the Department of Small Fruits and Strawberries. The institute was founded in 1967 and has since developed seven strawberry cultivars. The main strawberry breeding objectives were high productivity, high-quality fruit for fresh consumption and processing, large size fruit, color and shape of fruit, good shelf life, a wide early-, medium-, and late-ripening season, and resistance to diseases. The strawberry breeding sector produced 2,000-3,000 hybrids annually and followed selection procedures in the field. Soil type prevailing in the strawberry hybrid fields is the medium-textured and heavy-clay soils: clayey-illuvial luvisols, all showing medium and low humus content. The average annual temperature is 10.1° C and the average annual precipitation is 670.6 mm.

Plant Material and Determination

The experimental material studied comprised of 5 hybrid combinations ('Onebor × Fortuna', 'Onebor × Tecla', 'Elsanta × Onebor', 'Benicia × Sugar Lia', 'Nora × Benicia') and their parents. The optimal time to harvest strawberries was when they were completely red (Voca et al., 2008). After harvesting the strawberry fruit, the fruit samples were immediately analysed. Sample of 20 strawberry fruits was analysed for the indicators studied. To determine fruit production, the weight of ripened fruits at each harvest was weighed, and summing all harvests was done. The weight of each fruit was determined by using an HL-400 digital balance to weigh them individually. The fruit was measured using a digital calliper to determine its length and diameter. The shape index (SI) of the fruit was calculated as the ratio of these two dimensions (Tudor et al., 2014; Jamieson, 2016; Cockerton et al., 2021). The fruit firmness was measured for each sample using a non-destructive penetrometer with a 0.50 cm² measurement surface. The content of soluble solids (SSC) was estimated by means of a digital refractometer PR Series. The pH values of strawberry fresh juice were measured with the aid of a pH meter (ISFET pH Meter, IQ 125, Japan), Song et al. 2018. The external fruit colour was determined with a colorimeter Konica Minolta CR 400, based on system Huntel L*, a*, b* on both sides of the fruit (L* corresponds to brightness, a* and b*

chromaticity coordinates from green to red and from blue to yellow, respectively) (Lerceteau-Köhler et al., 2012; Alarfaj et al., 2021). According to McGuire (1992), the chroma index is determined by calculating $C = (a^{*2} + b^{*2})^{1/2}$ and the hue angle of $h^0 = \arctangent(b^*/a^*)$, with 0^0 representing red-purple, 90^0 representing yellow, 180^0 representing bluish-green, and 270^0 representing blue.

Statistical Analysis

The basic data were organized, calculated, and plotted using Excel 2019. On this basis, one-way ANOVA was conducted using SPSS 26.0. We also introduced the LSD method to compare the differences among data groups, with the significance level set as $\alpha=0.05$. Broad-sense heritability (H^2) was calculated using the following formula: $H^2 = VG/VP$ (VG = genotypic variation and VP = phenotypic variance) and narrow-sense heritability was calculated according to the formula $h^2 = VGA/VP$ (VGA = additive genetic variance and VP = phenotypic variance) Falconer and Mackay (1996), Titirică et al. (2023). The coefficient of variability/variation ($CV\%$) was calculated using the following formula: $CV\% = \sigma / \bar{x}$ (σ - square root of the total variance among families; $\bar{x}$ - mean of trait), Dan et al. (2010). The procedures outlined by Bakhsh et al. (2007)

and Murti et al. (2012) were used to calculate the heterosis percentages relative to the mid-parents for the studied characters, as follows: Mid-parent heterosis (%) = $((F1-MP) \times 100)/MP$. The dominance estimated was calculated "potence ratio" (hp) using the methods Petr and Frey (1966), Murti et al. (2012). $hp = ((F1-MP)/(BP-MP))$. Where; $F1$ = mean value of the particular hybrid population, $M.P.$ = mean value of the two parents for that hybrid $(P1 + P2)/2$, and BP = better parent. Complete dominance was indicated when $P = +1$; while partial dominance is indicated when " P " is between $(-1$ and $+1)$, except the value zero, which indicates absence of dominance. Over dominance was considered when potence ratio exceeds ± 1 . The positive and negative signs indicate the direction of dominance of either parent.

RESULTS AND DISCUSSIONS

The variation in biometric parameters among different F1 strawberry hybrid combinations is shown in Table 1, demonstrating how parental genotypes have an impact on these key fruit size characteristics. Variations in production were observed in the strawberry hybrid combinations that were studied. The 'Nora $\times$ Benicia' hybrid combination (556.82 g/plant) was found to yield the highest production, as indicated in Table 1.

Table 1. Productivity and fruit size characteristics of strawberry hybrid combination

Hybrid Combination (Parent $\sigma \times \sigma$)	Yield (g/plant ⁻¹)		Fruit weight (g/fruit ⁻¹)		Shape index	
	Mean $\pm$ SD	CV%	Mean $\pm$ SD	CV%	Mean $\pm$ SD	CV%
Onebor $\times$ Fortuna	337.70 $\pm$ 6.74 ^b	15.40	19.13 $\pm$ 0.90 ^b	14.50	1.53 $\pm$ 0.58 ^a	12.20
Onebor $\times$ Tecla	355.02 $\pm$ 1.75 ^b	14.40	19.81 $\pm$ 0.72 ^{a^b}	12.70	1.17 $\pm$ 0.14 ^a	9.40
Elsanta $\times$ Onebor	556.82 $\pm$ 4.79 ^a	14.01	27.55 $\pm$ 1.12 ^a	9.90	1.19 $\pm$ 0.55 ^a	10.20
Benicia $\times$ Sugar Lia	430.93 $\pm$ 8.47 ^{ab}	13.50	26.35 $\pm$ 6.45 ^{ab}	15.30	1.23 $\pm$ 0.11 ^a	8.20
Nora $\times$ Benicia	332.09 $\pm$ 1.95 ^b	15.40	21.19 $\pm$ 5.99 ^{ab}	13.20	1.26 $\pm$ 0.55 ^a	6.50
Mean	402.51 $\pm$ 6.68	-	22.81 $\pm$ 4.93		1.20 $\pm$ 0.08	

*The values in the table that do not have common letters differ significantly for a statistical assurance level of 5% ($P \leq 0.05$).

The differences in productivity among the hybrid combinations studied were large, with a variation in production from 332.09 g/plant⁻¹ ('Onebor $\times$ Fortuna') to 556.82 g/plant⁻¹ ('Nora $\times$ Benicia') being significantly different. In 2025, the average marketable yield per plant was between 176 g/plant⁻¹ and 331 g/plant⁻¹, according to Menzel, (2025). The size of the fruit is influenced by the genotype, ecological factors, and cropping system. The size of fruits is expressed through their average weight, which is highly commercially important for

fresh consumption. In our study, we observed a significant impact of hybrid combinations on fruit weight, particularly in the 'Nora $\times$ Benicia' hybrid combination (27.55 g), which had a significantly higher fruit weight than the 'Onebor $\times$ Tecla' hybrid combination (19.13 g) (Table 1). The average fruit weight recorded in the present study was similar to the findings of Menzel (2025), which showed that strawberry cultivars produced fruits ranged of 18.20 g to 24.30 g. To evaluate strawberry shape as an ordinal trait, a quantitative measure was used to

measure the length and diameter of each berry. The length and diameter ratio is a trait that is continuous and can be used for genetic analysis with data from across the population Cockerton et al. (2021). The fruit shape index did not show any significant differences between hybrid combinations during this study. It varied from 1.15 ('Onebor × Tecla') to 1.26 ('Onebor ×

Fortuna') (Table 1). The results of fruit shape recorded in the present study were similar to the findings of Tudor et al. (2014), which showed that 'San Andreas's fruit is conical, with a shape index of 1.21, 'Albion' cultivar develops long conical fruits with a shape index of 1.26, and 'Benicia' cultivar has long, conical fruit with a shape index of 1.32.

Table 2. Firmness, total soluble solid, and pH characteristics of strawberry hybrid combinations

Hybrid Combination (Parent♀×♂)	Fruit firmness (Ncm ⁻²)		Total soluble solid (°Brix)		pH	
	Mean ±SD	CV%	Mean ±SD	CV%	Mean ±SD	CV%
Onebor × Fortuna	40.62±2.76 ^a	30.90	8.11±0.60 ^a	10.10	3.84±0.04 ^a	6.70
Onebor × Tecla	45.74±5.34 ^a	23.40	8.83±0.35 ^a	12.90	3.90±0.04 ^a	4.60
Elsanta × Onebor	38.76±3.72 ^a	17.10	7.91±0.99 ^a	8.90	3.70±0.08 ^a	4.40
Benicia × Sugar Lia	39.48±10.34 ^a	25.20	8.31±1.23 ^a	10.80	3.76±0.29 ^a	4.70
Nora × Benicia	40.08±6.48 ^a	20.70	8.56±2.23 ^a	13.40	3.86±0.11 ^a	5.90
Mean	40.94±5.91	-	8.34±1.12	-	3.81±0.14	-

*The values in the table that do not have common letters differ significantly for a statistical assurance level of 5% (P≤0.05).

The fruits were found to be more resilient, with a higher resistance value for the hybrid combination 'Elsanta × Onebor' (45.47 N) than for 'Nora x Benicia' (38.78 N) (Table 2). Genetic factors, fruit ripeness, climate, and other factors can affect the quantitative variation of the SSC. In our study, we recorded a significant influence of the hybrid combination on the SSC in the 'Elsanta × Onebor' hybrid combination (8.83), which had the highest value of this parameter among the studied hybrid combinations. Sehrish et al. (2021) discovered that fruit SSC, in a similar manner to our research, with a range of 7.21 °Brix in the 'Everly' cv. to 12.63 °Brix in the 'Brighton' cv. (Table 2). The results of the study indicated that the 'Elsanta × Onebor' hybrid combination had the highest pH level, measuring 3.90, whereas 'Nora x Benicia' showed the lowest values of 3.70 (Table 2). The study by Ozkurt and Altuntas (2018) provides information about fruit acidity. It suggests that,

in general, their acidity ranges between 3.36 and 3.39. The study by Ozkurt and Altuntas (2018) provides information about fruit acidity. It suggests that, in general, their acidity ranges between 3.36 and 3.30. Strawberries are a popular fruit worldwide, and their colour has a major impact on their appeal. The ripeness and sweetness of strawberries are both visually appealing and are indicated by their bright red colour. The colour of strawberries may change depending on various factors, such as the variety, the stage of ripeness, and the growing conditions, and it is not always consistent. The presence of different pigments, such as anthocyanins, carotenoids, and chlorophyll, determines the colour of strawberries (Denoyes et al., 2023; Zhongchi et al., 2023; Yue et al., 2024). Anthocyanins play a role in the red, purple, and blue hues of many fruits and vegetables, including strawberries.

Table 3. Fruit colour characteristics of strawberry hybrid combinations

Hybrid Combination (Parent♀×♂)	Brightness (L*)		Chroma Index (C*)		The angle (h °)	
	Mean ±SD	CV%	Mean ±SD	CV%	Mean ±SD	CV%
Onebor × Fortuna	30.20±0.80 ^a	5.70	28.92±1.76 ^c	6.60	23.16±0.69 ^a	10.50
Onebor × Tecla	29.72±0.93 ^a	7.10	30.29±0.32 ^{abc}	8.40	22.98±0.44 ^a	9.30
Elsanta × Onebor	31.61±1.40 ^a	5.70	32.58±1.43 ^a	7.30	23.57±0.74 ^a	7.80
Benicia × Sugar Lia	30.54±1.08 ^a	6.00	29.05±1.53 ^{bc}	2.90	25.81±2.12 ^a	7.00
Nora × Benicia	31.47±1.74 ^a	7.50	31.42±0.66 ^{ab}	11.90	25.60±2.81 ^a	10.30
Mean	30.71±1.29	-	30.45±1.80	-	24.35±1.82	-

* The values in the table that do not have common letters differ significantly for a statistical assurance level of 5% (P≤0.05)

These pigments are strong antioxidants that aid in defending the fruit against the harm caused by

free radicals (Carbone et al., 2009). Strawberry is also a source of carotenoids, which are

pigments that are yellow, orange, and brown. Strawberry contains chlorophyll, a green pigment found in plants, but it is not as obvious as the other pigments. There was no significant difference in the L* and h values of the strawberry hybrid combinations, but there was a significant difference in the C* values. L* values increased from 29.72 ('Onebor × Tecla') to 31.47 ('Nora × Benicia'), C* from 28.92 ('Onebor × Fortuna') to 31.42 ('Nora × Benicia') and h° from 22.98 ('Onebor × Tecla') to 25.81 ('Benicia × Sugar Lia') (Table 3). The lower L* and h° values in the colour sphere indicate the darker sample colour with a dark red, while the higher values indicate the lighter sample with a red colour. It can be concluded that the 'Onebor × Tecla' hybrid combination was the dark-red genotype and 'Nora × Benicia' hybrid combination light-red colour, as stated by the results described above. Present findings are also confirmed by Henschel et al. (2017): L* values ranged from 32.12 to 33.61, C* from 27.19 to 31.26, and h° from 20.51 to 27.48. The coefficient of variation (CV%) is a significant indicator of the stability and consistency of the traits that were evaluated. Low CV% is associated with low variability and stable performance, making it an ideal choice for large-scale production and enhancing fruit quality predictability. In contrast, a high CV% suggests more variation, which can result in fruit characteristics that are unpredictable and may be less favourable for selecting hybrids for commercial production. The coefficient of variation in biometric parameters among F1 strawberry hybrid combinations showed moderate variability for yield, fruit weight, and

shape index. Among these, they are excepted 'Elsanta × Onebor' for fruit weight, whose CV% value was 9.90 and 'Onebor × Tecla' for shape index, with CV% value of 9.40, which indicates low variability. The coefficient of variation for fruit firmness showed a value over 20.00 which indicates high variability, except for 'Elsanta × Onebor', which showed moderate variability (CV% = 17.10) (Table 2). The coefficient of variation for fruit taste among the analysed families was low to moderate, consistent with the results of Simkova et al. (2023) (Table 2). Also, the coefficient of variation in colour parameters among F1 strawberry hybrid combinations showed low variability and stable performance, making it an ideal choice for selection (Table 3). Overall, the results obtained for the analysed hybrids that were analysed are similar to those found in the published literature regarding parent cultivars and other strawberry varieties (Cervantes et al., 2020; Simkova et al., 2023). The developed hybrids' quality, agronomic potential, and commercial viability are confirmed. Broad sense heritability calculated over the entire experiment with the hybrid combinations for most traits indicated a high degree of genotypic control, ranging between 0.59 (hue angle) and 0.91 (yield and chroma index) (Tables 4, 5, 6). In addition to the lowest heritability value observed for hue angle, low broad-sense heritability was also recorded for brightness (0.62) and fruit weight (0.66). The values indicate a high proportion of total genetic variance to phenotypic variance, which indicates that hereditary endowment plays a significant role in the expression of traits.

Table 4. Statistical analysis of productivity and fruit size characteristics in strawberry hybrid combination

Hybrid Combination (Parent♀×♂)	Yield (g/plant ⁻¹)				Fruit weight (g/fruit ⁻¹)				Shape index			
	H ²	h ²	MPH	hp	H ²	h ²	MPH	hp	H ²	h ²	MPH	hp
Onebor × Fortuna	0.91	0.76	-0.45	-12.08	0.66	0.25	0.05	1.54	0.69	0.29	0.11	0.80
Onebor × Tecla	0.91	0.77	0.08	0.32	0.90	0.74	-0.10	-0.74	0.89	0.71	0.13	3.07
Elsanta × Onebor	0.90	0.74	0.33	2.79	0.94	0.84	0.32	3.55	0.67	0.27	0.25	5.97
Benicia × Sugar Lia	0.88	0.68	0.34	3.14	0.85	0.61	0.32	4.33	0.76	0.43	1.01	2.15
Nora × Benicia	0.94	0.84	0.28	-2.99	0.90	0.74	0.02	-0.23	0.86	0.64	0.07	0.56

H² = heritability in broad sense; h² = heritability in narrow sense; MPH = Mid parent heterosis; hp = potency ratio

Narrow sense heritability estimates were mainly high for yield, fruit size, and fruit colour, and low for fruit firmness, total soluble solids, and pH, indicating that, for half of the traits, more than 50% of the total genetic variation was the result of non-additive genetic effects, and the

breeding process was quick. The yield and size of the fruit are the most desired traits in strawberry breeding, as they have a direct impact on market acceptance and consumer preference. Table 4 displays a range of genetic responses dependent on the specific parent

combinations, emphasizing the potential for cultivar development. The success of strawberry breeding can be achieved through judicious parent choices, as demonstrated by the heterosis, dominance, broad-sense, and narrow-sense heritability values of certain traits. The yield in 'Nora × Benicia' hybrid combination is mostly influenced by additive genetic factors, as evidenced by the exceptional broad-sense heritability of 0.94 and the narrow-sense heritability of 0.84 (Table 4). Heterosis, also known as hybrid vigour, is the definition of an improvement in performance compared with the parental varieties. The heterosis values for yield were predominantly positive for all hybrid combinations, ranging 0.08 ('Onebor × Tecla') and 0.34 ('Benicia × Sugar Lia'), excepted 'Onebor × Fortuna' hybrid combination, negative (-0.45). In F1 hybrids for yield, the potence ratio ranged from -12.08 ('Onebor × Fortuna') to 3.14 ('Benicia × Sugar Lia') with two hybrid combinations indicating over dominance positive ($>\pm 1$), and three combinations exhibiting partial dominance (-1 to +1). The high broad-sense and narrow-sense heritability values for fruit weight, specifically in 'Elsanta × Onebor', show that genetic variation plays a significant role in this trait. Thus, selecting fruits for weight in early generations can be a highly successful approach for these crosses. Also, heterosis values were predominantly positive in all combinations: 'Nora × Benicia' (0.02) and 'Elsanta × Onebor' respectively 'Benicia × Sugar Lia' (0.32), indicating that the performance of hybrid combinations was often higher than the average

of the two parents. The exception is 'Onebor × Tecla' (-0.10), performance of hybrid combinations a lower compared to the parental mean. For fruit weight, the potence ratio ranged from -0.23 ('Nora × Benicia') to 4.33 ('Benicia × Sugar Lia') with three hybrid combinations indicating overdominance ($>\pm 1$), and two combinations exhibiting partial dominance (-1 to +1). The high broad-sense heritability (0.89), and narrow-sense heritability (0.71) values for the shape index, particularly in 'Onebor × Tecla', indicate that a substantial proportion of the phenotypic variation in this trait is genetically determined. However, the low values of narrow-sense heritability for the other hybrid combinations indicated that the character is principally influenced by the environment. Heterosis values were predominantly positive in all combinations, ranging from 0.07 ('Nora × Benicia') to 1.01 ('Benicia × Sugar Lia'), indicating that the performance of hybrid combinations was greater than the average of the two parents. In F1 for shape index trait, the potence ratios ranged from 0.56 ('Nora × Benicia') to 5.97 ('Elsanta × Onebor'), with three crosses indicated over dominance ($>\pm 1$) and two indicating partial dominance (-1 to +1). The firmness of the fruit is influenced by its size and shape, ripening stage, season, and year (Moore, 2001; Rasing et al., 2003). According to Santiago-Domenech et al. (2008), firmness during fruit ripening was related to a decrease in pectin level, a slight decrease in pectin covalently bound to cell walls, and a decrease in pectin depolymerization.

Table 5. Statistical analysis of firmness, total soluble solids, and pH characteristics in strawberry hybrid combinations

Hybrid Combination (Parent ♀ × ♂)	Fruit firmness (Ncm ⁻²)				Total soluble solid (°Brix)				pH			
	H ²	h ²	MP H	hp	H ²	h ²	MPH	hp	H ²	h ²	MPH	hp
Onebor × Fortuna	0.79	0.49	0.41	8.50	0.73	0.37	0.00	0.02	0.84	0.60	0.10	7.16
Onebor × Tecla	0.77	0.43	0.43	3.07	0.73	0.37	0.05	2.16	0.76	0.42	0.17	4.16
Elsanta × Onebor	0.86	0.63	0.48	11.86	0.82	0.54	-0.06	-6.00	0.66	0.24	0.02	0.41
Benicia × Sugar Lia	0.62	0.17	0.17	1.64	0.83	0.56	0.07	0.78	0.83	0.57	0.10	1.69
Nora x Benicia	0.80	0.51	0.37	8.34	0.80	0.50	0.19	3.33	0.68	0.28	0.15	6.67

H² = heritability in broad sense; h² = heritability in narrow sense; MPH = Mid parent heterosis; hp = potence ratio

Heritability estimates of 0.62-0.80 (H2) and 0.68-0.84 (h2) for firmness suggest a strong genetic basis for selection. Table 5. The results of Whitaker et al., 2012, Cockerton et al., 2021, and Hera et al., 2025 back these estimations.

Heritability estimates increased by 0.542 and 0.85 from Coman and Popescu (1997) and Ukalska et al. (2006). Parent firmness appears to have had an impact on the heterosis and potence ratio values of F1 fruit firmness. All crossing

combinations resulted in F1 progenies having positive heterosis and potence ratio values (Table 5). The combination of genotypes determines how genes interact to control the firmness of strawberries, making it a complex process. As Ohtsuka et al. (2004) and Murti et al. (2013) noted, it meant that the octoploid plants were not pure lines. The accumulation of genes that are favourable for firmness could be expected to have a highly additive genetic effect (Mori, 2000; Murti et al. 2012). According to estimates of broad and narrow-sense heritability for SSC, parental selection can yield additional gains in this trait (Table 5). In addition, this trait had high broad-sense heritability ($H^2 = 0.72$ to 0.83) and narrow-sense heritability was moderate to high for SSC ($h^2 = 0.37$ to 0.56), suggesting that clonal selection will allow the capture of non-additive variance in the deployment population. This is consistent with previous estimates of sizable non-additive genetic effects for these traits (Whitaker et al. 2012; Cockerton et al., 2021). These combinations are particularly valuable in commercial breeding programs because high TSS is directly related to fruit sweetness and

consumer preference. Positive heterosis and potence ratio values in all hybrid combinations, except for 'Elsanta × Onebor' (-0.06 and -6.00), reflect hybrid vigour and indicate that these progenies exceed the average of their parents in terms of TSS content. For pH, broad-sense heritability (H^2) was moderate to high, with estimates ranging from 0.66 for 'Elsanta × Onebor' to 0.84 for 'Onebor × Fortuna' (Table 6). Narrow-sense heritability ($h^2 = 0.24$ to 0.60) was low and moderate, suggesting that breeding and selection can result in certain gains, and that genetic gains can be doubled by simply using clonal selection (Table 6). Heterosis values were predominantly positive in all combinations, ranging from 0.02 ('Elsanta × Onebor') to 0.17 ('Onebor × Tecla'), indicating that the performance of hybrid combinations was often greater than the average of the two parents Table 6. In F1 pH trait, the potence ratios were positive in all hybrid combinations; three crosses indicated over-dominance ($> \pm 1$), hybrids performing better than the superior parent, and two indicated partial dominance (0 to $+1$), hybrids outperforming the average but not as good as the superior parent.

Table 6. Statistical analysis of fruit quality colour characteristics in strawberry hybrid combination

Hybrid (Parent♀×♂)	Combination	Brightness (L*)				Chroma Index (C*)				The angle (h °)			
		H ²	h ²	MPH	hp	H ²	h ²	MPH	hp	H ²	h ²	MPH	hp
Onebor × Fortuna		0.77	0.43	0.03	0.49	0.76	0.42	0.01	0.00	0.59	0.13	0.01	-0.40
Onebor × Tecla		0.86	0.65	0.25	3.13	0.91	0.76	12.02	0.24	0.77	0.45	0.04	0.34
Elsanta x Onebor		0.84	0.60	0.05	0.66	0.78	0.47	0.90	0.08	0.78	0.47	0.00	0.33
Benicia × Sugar Lia		0.76	0.43	0.31	0.43	0.73	0.37	2.21	0.04	0.79	0.49	0.05	0.39
Nora × Benicia		0.62	0.17	0.02	0.28	0.59	0.13	-2.13	-0.12	0.64	0.21	0.11	2.65

H² = heritability in broad sense; h² = heritability in narrow-sense; MPH = Mid parent heterosis; hp = potence ratio

Statistical analysis of colour characteristics: L*, C* and h° in F1 strawberry hybrids (Table 6) provides important insights into the genetic potential of different parental combinations to improve fruit colours. Statistical analysis of colour characteristics: L*, C* and h° in F1 strawberry hybrids (Table 6) provides important insights into the genetic potential of different parental combinations to improve fruit colours. Moderately to high heritability of colour values indicated that the colour of

progenies depends on the estimated high heritability of skin colour. The majority of the hybrid combinations had positive heterosis and potence ratio values for colour characteristics, except for the hybrid combination 'Onebor × Fortuna'. Value of the potence ratio for the chroma index in the hybrid combination 'Onebor × Fortuna' was 0, the character is non-dominant. The F1 hybrids have exactly the average value of the two parents, with no dominance effects; the action of the genes is purely additive.

CONCLUSIONS

The genetic parameters of nine commercially significant traits of strawberries for the Romanian strawberry breeding population were calculated by using clonally replicated plant genotypes from controlled crosses in controlled crosses. The presence of high broad-sense heritability for most traits suggests that gains could be achieved for these important characteristics in the strawberry breeding population with regard to clonal deployment. Moderate to high narrow-sense heritability for pH and some colour characteristics indicates a high probability of long-term gains through recurrent selection for these traits. From a yield and biometric parameters perspective, the strawberry hybrid combinations studied showed moderate variability for yield, fruit weight, and shape index. Among these, they are excepted 'Elsanta × Onebor' for fruit weight, whose CV% value was 9.90, which indicates a low variability producing the largest fruits, which may increase market appeal. The hybrid combination that uses 'Onebor' as the male parent was the most successful in terms of productivity and fruit size parameters, with the highest yield and fruit weight among all hybrid combinations studied. Hybrid combinations with 'Onebor' as the female parent, especially when crossed with 'Tecla', showed superior performance in fruit firmness, SSC, and pH. The comparative outcomes demonstrate the strong potential of the tested hybrid combinations to create fruit of quality that is equal to or better than that of the parent varieties and other commercial cultivars. High values for morphometric and biochemical parameters have been observed in hybrid combinations such as 'Elsanta × Onebor' and 'Onebor × Tecla', which indicate great potential as candidates for the development of highly nutritious and commercially valuable crops.

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THE STUDY OF SOME CHOKEBERRY GENOTYPES IN THE CONDITIONS OF SOUTHWESTERN ROMANIA

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Abstract

At the Research-Development Station for Plant Cultivation on Sandy Soil Dabuleni, three genotypes of Aronia melanocarpa were evaluated between 2022-2025. The Viking, Nero, and Melrom genotypes were tested for their suitability for expansion in fruit-tree plantations from the southwestern area of Romania. Following the study, it was found that the evaluated genotypes recorded good yields ranging between 14.25 and 16.00 kg/bush, with an average bush height of 152 to 168 cm in the 7th year after planting. The Melrom variety stood out from the other varieties studied, with an average fruit weight of 0.99 g, the largest number of berries in an inflorescence (22) and an average yield of over 16.00 kg/bush (44 tons/ha).

Key words: *Aronia melanocarpa*, bush vigour, yield, fruit quality.

INTRODUCTION

In the southern part of Romania, the climate with mild winters in the last decade, characterized by maximum temperatures that did not exceed -12°C, has put the fruit growers in the area to the test. So, the mild winters followed by springs that appeared in February, which determined the very early flowering of the stone fruit species, the late frosts that occurred during their flowering period (of peaches and apricots) had negative effects on the production of those species grown on the sandy soils of southern Romania, with negative economic effects felt by the fruit growers of this area. Thus, a real challenge arose for the researchers to find an assortment of species that would escape this spring's late frost from the first of April. One of these species was *Aronia melanocarpa*, a species belonging to the *Rosaceae* family, native to North America, where it was among the most important berries known and first used by the Indians (Bussieres et al. 2008) as a food source. The berries were dried and ground for use in soups, stews, and cakes. The time of year when the chokeberry berries ripened was called the "black cherry month". The Native Americans

used the parts of the entire bush as both medicine and food (Skvortsov, 1982 cited by Dinu et al., 2022). It is used for the vegetative parts as remedies for various ailments. For example, the dried root was chewed and placed on wounds to stop bleeding; the bark was boiled and used together with other additives as a remedy for stomach and intestinal problems; while the roots and branches were used to prepare preparations against coughs, tuberculosis, stomach pains, intestinal worms, and malaria, high fever, colds, and body chills (Strik, 2003).

In Europe, *Aronia melanocarpa* was introduced in the middle of the 18th century from the eastern part of North America. Here, it attracted attention primarily due to its appearance, since its white flowers, blooming in April-May, are considered among the most beautiful in terms of decorative appearance. Also, notable are the leaves of chokeberry in their autumn colours, which seem to glow with a special red colour. For this reason, *Aronia melanocarpa* received the award of the Royal Horticultural Society in 1972.

In Russia, was introduced at the beginning of the 20th century, when the famous Russian biologist

Ivan Michurin experimented with crossing it with other species from the *Rosaceae* family. Since 1946, it has been recognized as a species for fruit production in the Soviet Union, where planting on a larger scale subsequently began. Thus, it became the subject of intense scientific research due to the abundance of active principles, the numerous positive results from research on the preventive and healing effects of ripe fruits, and Russian scientists granting it the status of a medicinal plant (Nicola et al., 2020). Following the Chernobyl nuclear disaster, chokeberry fruits were used to alleviate human health problems caused by radiation. The chokeberry fruits were also called the “cosmonaut fruit” because Russian astronauts regularly consumed them in the 1950s, considering them a source of nutrients and vitamins (Rosca et al., 2019). *Aronia melanocarpa* has proven to be a species with a great capacity for adapting to pedoclimatic conditions (such as humid habitats, but also dunes, rocky slopes, and steep cliffs, as well as on overgrown or bare rocky outcrops) (Dinu et al., 2022). The studies show the spread of chokeberry, as an invasive plant, in the spontaneous flora (Vinogradova et al., 2018; Leostrin et al., 2021), it tolerates arid or excessively wet soils and very low temperatures, and is also resistant to diseases and pests (Cho et al., 2017). These characteristics allow chokeberry cultivation with fewer phytosanitary treatments, as well as rehabilitation by populating abandoned lands with vegetation (Bussieres, 2008), we know that the success of a species grown depends mainly on pedoclimatic conditions (Paltineanu et al., 2020).

MATERIALS AND METHODS

To the Dabuleni Research and Development Station for Plant Culture on Sands, an experimental lot was planted in 2019, with three cultivars: *Viking*, *Nero* and *Melrom*. The planting was carried out at a distance of 3 meters between rows and 1.5 meters between plants within each row, on raised beds, mulched with agrotechtextile, and irrigated by drip. Fertilization in the experimental plot was carried out according to the fertilization scheme

presented in "Technical and Economic Guide - Fruit Trees and Shrubs" (Sumedrea et al., 2014). To identify the growth and fruiting parameters of the studied genotypes we make the following evaluations: the phenological data according to the BBCH scale (Meier, 2001; Cosmulescu, 2022), of the flower buds; the shoot growth dynamics (cm) were evaluated monthly, and the annual average was calculated. The bush volume (V) was calculated according to the formula: $V = \pi (\text{bush diameter in the row direction} + \text{diameter between rows})^2 \times \text{bush height} / 3$. The fruits quality was evaluated by analysing three repetitions for each genotype, annually, for the following characteristics: average fruit weight (g), determined by weighing and measuring berries; fruit diameter (mm); fruit height (mm) were measured with an electronic calliper; the number of berries in clusters. The fruits' biochemistry determinations were carried out, such as: soluble dry matter (% Brix) with the Atago device; vitamin C content (mg %) and fruit pH (Croitoru M., 2021). The results obtained were calculated and statistically interpreted with ANOVA, Duncan's test at $p \leq 0.5$, and the bars in each column of the figures represent the standard deviation, and the letters accompanying the values in the figures represent the significance according to Duncan's test.

RESULTS AND DISCUSSIONS

The development of the fruiting part of the bushes. The Figure 1, show that the swelling of the flower buds in all studied genotypes took place in the third decade of March, and flowering in the third decade of April; thus, according to statistics, this species escapes the incidence of late spring frosts that appear in the first and second decades of April. Monitoring of phenological data did not reveal any differences in earliness or tardiness between the analysed genotypes, so that starting from the end of August the fruits can be harvested. It should be noted that the longer they stay on the bush, the astringent taste of the fruit's decreases, and if the harvest is made after the first autumn frost the astringency of the fruits is much reduced.

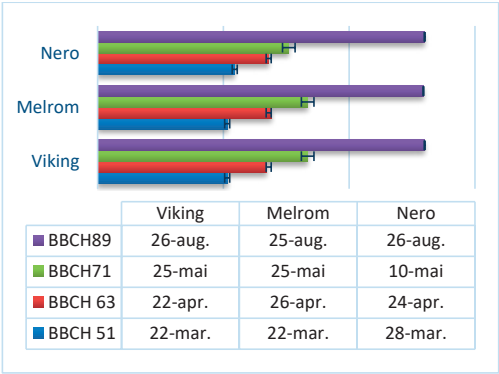


Figure 1. The fruit BBCH at the studied black chokeberry genotypes (BBCH 51 - bud swelling, BBCH 63 - beginning of flowering, BBCH 71 - full flowering, beginning of petal shedding, BBCH 89 - ripen fruit)



Foto 1. The *Melrom* black chokeberry genotype - BBCH71

The bush growth. Analysis of the average data recorded over the entire study period shows that, in terms of bush height growth, they had an annual growth increase of over 30 cm until the 4th year after planting (2022), the differences recorded from one year to another were statistically significant, and starting with the 5th year after planting, the increase in height growth fell into the same statistical class (Figure 2).



Foto 2. The *Melrom* black chokeberry genotype in 2022 year (4th year after planting)

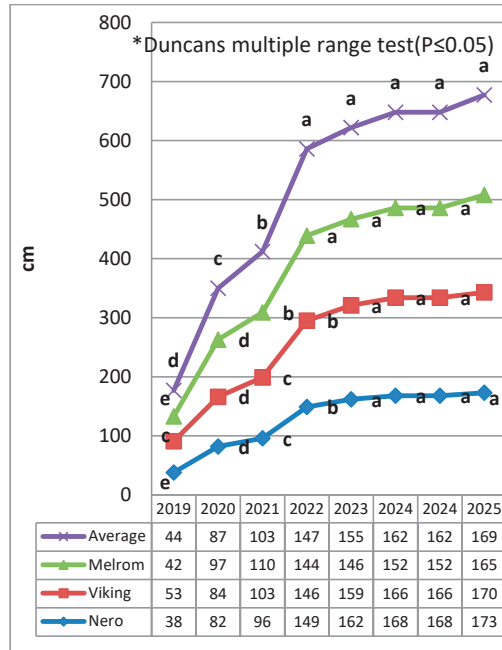


Figure 2. The studied black chokeberry cultivars bushes height (cm)

Regarding the growth of bushes in diameter presented in Figure 3, we note that in the 6th year (2024) from planting the highest average value was recorded for the *Melrom* genotype (140 cm) (Foto 1), and the differences between the study years were statistically assured.

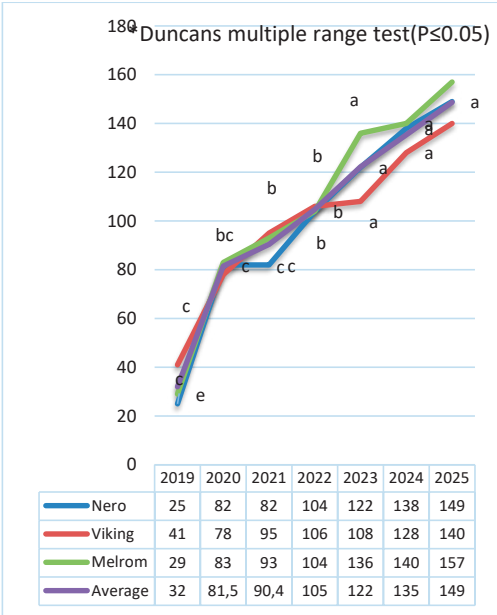


Figure 3. The studied black chokeberry cultivar bushes' thickness (cm)

The evaluation of the bush volume over the entire study period shows that the *Nero* and *Melrom* varieties recorded bush volume values of 1 and 1.1 m³, respectively, although in the first year after planting they recorded lower values than the *Viking* cv. The quality of black chokeberry fruits.

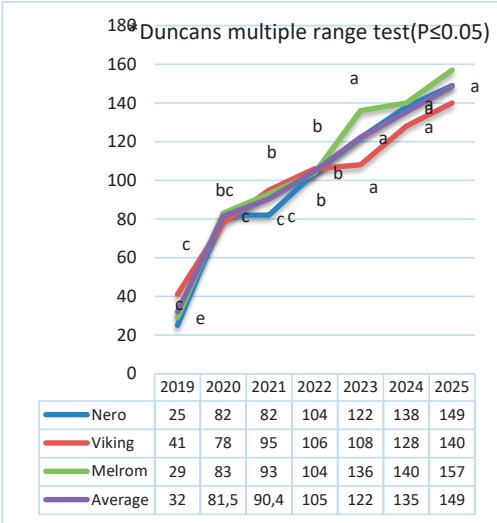


Figure 4. The studied black chokeberry cultivars, bushes volume (m³)

The quality of black chokeberry fruits.

Following the study carried out in Tables 1 and 2, we present the average values recorded over the entire study period for the three cultivars under study. Thus, regarding the average fruit weight, we find that the *Melrom* cv. recorded the highest value of 0.99 g/fruit without a statistically significant difference from the *Nero* cv with an average fruit weight of 0.91 g/fruit, both varieties differed by a difference of 0.30 g from the *Viking* cv. a statistically significant difference assured. In the case of fruit firmness, the annual average values recorded during the study period show that the highest value was recorded for the *Nero* cv. Regarding the biochemical content of the fruits, we find that the *Melrom* cv. recorded the highest vitamin C content, and Nero the highest soluble dry matter content (Tabel 1).

The analysis of the data in Table 2 shows that the annual average values recorded for the biometric characteristics of the fruits to the studied cultivars in each year of the study show that, in the case of the *Nero* cv, there were no statistically significant differences from one year to another in five of the 6 fruit quality characteristics analysed. The analysis of the *Viking* cv in each study year revealed statistically significant differences only in the case of fruit height and diameter, and in the case of the *Melrom* cv, no statistically significant differences were recorded in any of the fruit quality characteristics analysed.



Foto 3. *Melrom* Cv fruit production in 2022 year

The fruit production per bush was recorded annually starting with the third year after planting, so that, on average over the three years after planting, the highest fruit production per plant was recorded for the Melrom variety (16.07 kg/bush) (Figure 5)

Table 1. The black chokeberry fruits quality: average values in the study period

The fruits quality characteristics	Cultivar		
	Nero	Viking	Melrom
Berry weight (g)	0.91 a	0.73 b	0.99 a
Firmness (gF/cm ²)	0.464 a	0.440 b	0.448 ab
pH	4.01	3.68	4.32
Soluble solids (% Brix)	23.57	17.20	19.23
Vitamin C (mg/100g)	22.5	24.5	25.1

Table 2. The black chokeberry fruits biometric characteristics

Cultivar	Year	No berries in cluster	The fruit height (mm)	The fruit thickness (mm)
Nero	2019	22.6 a	12.07 a	11.68 a
	2020	19.6 a	12.26 a	12.07 a
	2021	21.33 a	13.49 a	12.38 a
	2022	22.0 a	12.19 a	12.30 a
	2023	18.54 a	11.55 a	11.85 a
	2024	18.11 a	11.98 a	12.10 a
	2025	21.3 a	12.21a	12.30a
	average	20.6 a	12.31 a	12.06 a
Viking	2019	18.33 a	13.97 a	13.97 a
	2020	19.33 a	13.61 a	12.86 ab
	2021	21.00 a	13.09 ab	12.49 b
	2022	21.33 a	13.95 a	13.56 ab
	2023	16.00 a	11.65 b	12.17 b
	2024	21.00 a	13.07 ab	13.51 ab
	2025	17.31a	13.88 a	13.92 a
	average	19.2 a	13.25 a	13.03 a
Melrom	2019	19.66 a	14.59 a	13.72 a
	2020	24.0 a	13.97 a	13.97 a
	2021	21.33 a	14.61 a	14.20 a
	2022	24.00 a	14.22 a	13.97 a
	2023	23.00 a	14.46 a	14.41 a
	2024	23.11 a	14.14 a	14.22 a
	2025	19.82 a	14.48 a	14.40 a
	average	22.4 a	14.37 a	14.05 a

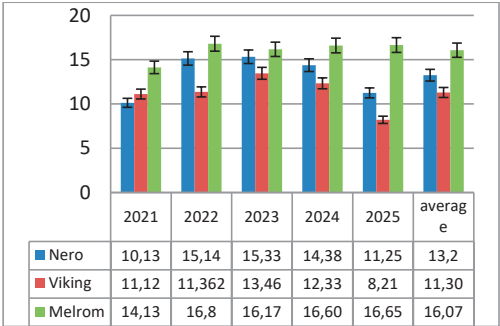


Figure 5. The fruit production to the studied black chokeberry cultivars (kg/bush)

CONCLUSIONS

The black chokeberry studied cultivars bloomed in the last decade of April, so they escaped the frequency of late spring frosts in the last years.

The volume of the bushes throughout the study period had recorded increases in every study year. At the end of the study, the *Nero* and *Melrom* cultivars reached a bush volume of over 1 m³.

The *Melrom* genotype stood out compared to the other studied cultivars, with an average fruit weight of 0.98 g, the highest number of berries in the inflorescence (22) and an average production of over 16.07 kg / bush.

All three black chokeberry cultivars performed according to the consulted literature; it was confirmed to be productive in the sandy soil conditions from Southwestern Romania, and it can be expanded in large commercial plantations.

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MICROBIAL CONTAMINATION IN PLANT TISSUE CULTURE - A REVIEW

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Abstract

Microbial contamination remains one of the most important biological limitations affecting plant tissue cultures used in both horticultural research and commercial micropropagation. Despite continuous improvements in sterilization protocols and aseptic techniques, contamination continues to compromise culture establishment, multiplication efficiency and plant quality. The present review critically synthesizes classic and recent literature addressing the sources of microbial contamination, the types of microorganisms involved, and their biological and physiological impact on in vitro explants. Particular emphasis is placed on latent endophytic microorganisms and secondary contamination arising during repeated subcultures, which challenge the traditional concepts of surface sterilization and absolute axenic culture. During the comparison, the effectiveness, limitations, and applicability of conventional sterilization methods and emerging integrated contamination control strategies are highlighted. In conclusion, the review emphasizes a conceptual shift from complete microbial elimination to case-specific, biologically informed contamination management and discusses future research perspectives aimed at improving the reliability and sustainability of horticultural micropropagation.

Key words: plant tissue culture, in vitro micropropagation, microbial contamination, endophytic bacteria, fungal contamination, aseptic cultures.

INTRODUCTION

Plant tissue culture (micropropagation) has revolutionized modern agriculture, horticulture, and forestry, enabling the rapid large-scale multiplication of disease-free plants, preserving endangered species, and facilitating genetic manipulation (George et al., 2008; Rout et al., 2021; Yapa and Bandalaralage, 2023). The success of this technology is based on the ability to keep explants (cells, tissues or organs) in an artificial, strictly controlled and aseptic environment. However, biological contamination remains a pervasive threat and a major challenge, often compromising months of research and generating substantial financial losses in commercial laboratories (Cassells, 2012; Leifert and Cassells, 2001). The inherent vulnerability of *in vitro* cultures derives from the very nature of the culture media used. The basal medium formulated by Murashige and Skoog (1962), used almost universally in various variants, is extremely rich

in inorganic salts, sources of carbon (sucrose) and vitamins. This high availability of nutrients, combined with optimal incubation temperatures (usually 22-25°C) and high humidity in culture vessels, creates an ideal environment not only for plant cells, but also for the explosive proliferation of heterotrophic microorganisms (Murashige and Skoog, 1962; Permadi et al., 2023).

Historically, efforts have focused on obtaining completely axenic cultures (an *in vitro* culture containing only the intended plant tissue and no detectable contaminating microorganisms). Contaminants have been regarded exclusively as pathogens or "vitro pathogens" (environmental microorganisms that become pathogenic under *in vitro* conditions) (Cassells, 2001). However, research in recent decades has highlighted a much more complex reality. As Herman (2017) points out, the concept of completely sterile plant culture is often an illusion, with many plants hosting dormant endophytic microbial communities that can survive rigorous surface

sterilization procedures. This paradigm shift has led to a clear distinction between harmful contaminants (which cause necrosis, hyperhydricity, or explant death) and neutral or even beneficial endophytes, which can promote plant growth and stress resistance in the *ex vitro* acclimatization phase (Orlikowska et al., 2017; Quambusch et al., 2014).

This review article aims to provide a detailed and up-to-date analysis of the phenomenon of *in vitro* contamination. The types of contaminants, sources of infection, physiological impact on plants, as well as the evolution of detection methods and eradication strategies will be explored, integrating the classic reference literature with the latest discoveries in the field of plant biotechnology.

MATERIALS AND METHODS

In order to carry out this scientific review, a rigorous literature selection methodology was adopted, inspired by the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines. The search was carried out in the main academic databases: Web of Science, Scopus, PubMed, Google Scholar and the MDPI platform. Combinations of keywords such as: "plant tissue culture contamination", "*in vitro* microbial hazards", "endophytic bacteria micropropagation", "virus elimination tissue culture", "mycoplasma detection *in vitro*", and "sterilization protocols explants" were used.

The selection criteria aimed to include the fundamental works, internationally recognized, developed by the pioneers of the field (e.g. Cassells A.C., Leifert C., Herman E.B., Murashige T., Skoog F., Reed B.M., Viss P.R.). To ensure the timeliness of the information, special attention was paid to articles published between 2015 and 2025 in journals with a high impact factor (Q1-Q2), especially from the MDPI portfolio (Plants, Horticulturae, Agronomy, International Journal of Molecular Sciences), as well as from prestigious journals such as Plant Cell, Tissue and Organ Culture, Acta Horticulturae and Scientia Horticulturae. Studies that did not present verifiable data or that did not specifically focus on the plant *in vitro* environment were excluded. In total, more than 35 scientific articles, book chapters and

reviews were selected and analyzed in depth, the data being synthesized to cover all aspects of contamination management: from etiology and diagnosis, to treatment and biotechnological perspectives.

RESULTS AND DISCUSSIONS

Types of contaminants

Biological contamination in plant tissue culture is a heterogeneous phenomenon, involving a diversity of organisms that can be classified according to their biological nature and how they interact with the host plant. Leifert and Cassells (2001) classified microbial hazards into bacteria, fungi (including yeasts), viruses, viroids, mycoplasmas, and microarthropods.

1. Bacteria are the most common and persistent form of contamination *in vitro*. They can be divided into two major categories: epiphytes (which colonize the surface of the explant) and endophytes (which reside in the intercellular spaces or vascular system of the plant) (Orlikowska et al., 2017). While epiphytic bacteria can usually be eliminated by standard surface sterilization protocols, endophytic bacteria are protected inside the tissues and can manifest weeks or months after culture initiation (Reed et al., 1995). Commonly isolated bacterial genera include *Bacillus*, *Pseudomonas*, *Staphylococcus*, *Lactobacillus*, *Agrobacterium*, and *Enterobacter* (Leifert et al., 1989). A particular case is *Agrobacterium tumefaciens*, used as a vector in genetic transformation, whose incomplete post-transformation elimination is a major problem, requiring specific control methods (Viss et al., 1991). Quambusch et al. (2014) identified diverse endophytic bacterial communities in cultures of *Prunus avium* (cherry), demonstrating that difficult-to-propagate genotypes harbored endophytic populations dominated by *Pseudomonas* spp. and *Curtobacterium* spp. - distinctly different from those present in easily propagated genotypes. In *Rubus idaeus* (raspberry) cultures, Trzewik et al. (2025) isolated and identified three persistent bacterial contaminants via DNA sequencing: *Pseudomonas* sp. (Gram-negative, cultivar 'Polka'), *Curtobacterium* sp. (Gram-positive, cultivar 'Norna'), and *Luteibacter* sp. (Gram-negative, cultivar 'Polana'). The researchers

noted that these nitrogen-fixing bacteria did not cause immediate visible symptoms but significantly impaired survival during cold storage (4°C): contaminated cultures became necrotic within 4-6 months, whereas decontaminated cultures survived.

For *Fragaria × ananassa* (strawberry), Reed et al. (1995) demonstrated the presence of latent bacteria in 40-60% of explants tested via indexing on multiple bacteriological media, with *Xanthomonas fragariae* frequently isolated from apparently healthy runners.

2. Fungi and yeasts. Fungal contamination is usually more noticeable and develops faster than bacterial contamination. Fungal spores are ubiquitous in laboratory air and on the surface of donor plants. Genera such as *Aspergillus*, *Penicillium*, *Fusarium*, *Alternaria*, and *Cladosporium* are frequently reported (Permadi et al., 2023). Fungi form mycelial structures (hyphae) that can quickly cover the explant and culture medium, suffocating the plant and depleting nutrients. Yeasts (e.g., *Candida*, *Rhodotorula*) often manifest as mucous colonies, similar to bacterial ones, and can ferment sugars in the environment, drastically altering pH (Leifert and Cassells, 2001).

In *Rosa* sp. cultures, *Botrytis cinerea* and *Fusarium* spp. constitute the dominant fungal contaminants during culture initiation from field buds, particularly during the rainy season (Balo, 2023). Micropropagation studies of *Fragaria × ananassa* report fungal contamination with *Botrytis* spp. and *Alternaria* spp. in 10-25% of explants initiated during warm months; sterilization with 1% sodium hypochlorite for 10 minutes significantly reduces this rate (Anikina et al., 2025).

In *Prunus persica* (peach) and *Prunus avium*, contamination with *Pseudomonas syringae* pv. *syringae* is frequent; Brison et al. (1997) reported that treatment with 250 mg/L cefotaxime reduced bacterial contamination to under 5%.

3. Viruses and Viroids. Unlike bacteria and fungi, viruses and viroids do not grow on the culture medium, but multiply exclusively inside plant cells. Their presence does not cause turbidity of the environment, which makes them invisible to a routine macroscopic inspection. Their passage into the *in vitro* culture is done

systemically, with the explant taken from an infected mother plant. Although meristem culture is the standard technique for eradicating viruses, success is not 100% guaranteed, and viruses such as Plum pox virus (in *Prunus* species) or Yam mosaic virus often require combination therapies (thermo- or cryotherapy) to be completely eliminated (Brison et al., 1997; Elansary et al., 2024).

The Plum pox virus (PPV, "Sharka") severely affects micropropagation cultures of *Prunus* species - peach (*Prunus persica*), apricot (*Prunus armeniaca*), and plum (*Prunus domestica*). Szabó et al. (2024) synthesized literature on virus elimination from *Prunus* spp. and found that meristem culture alone has a highly variable efficacy (20-80%), depending on the species and viral load, whereas the combination of thermotherapy (37°C, 4-6 weeks) + meristem culture increases efficacy to 60-95%. Brison et al. (1997) demonstrated that cryotherapy on a *Prunus* rootstock experimentally infected with PPV eliminated the virus completely; the cryopreserved meristems regenerated healthy plants confirmed by ELISA and RT-PCR.

4. Mycoplasmas (Phytoplasmas). Phytoplasmas are bacteria without a cell wall, necessarily parasites, which colonize the phloem of plants. Due to their extremely small size and membrane flexibility, they can pass through standard microbiological filters (0.22 µm or 0.45 µm) used for cold sterilization of liquid media or thermolabile supplements (Nikfarjam and Farzaneh, 2012). Mycoplasma contamination is insidious, causing abnormalities of growth, yellowing and abnormal proliferation of shoots ("witch broom" symptoms), being extremely difficult to eradicate with conventional antibiotics (Carrillo-Ávila et al., 2023).

5. Microarthropods (mites and thrips). Although they are not microorganisms, microarthropods ("vitro pests") are a major biological vector for cross-contamination in growth chambers. Mites and thrips may enter culture vessels through inadequate ventilation systems or imperfect closures, acting as mechanical vectors of fungal spores and bacteria and facilitating cross-contamination among *in vitro* cultures (Cassells, 2012; Leifert and Cassells, 2001).

Table 1. Summary of the main types of contaminants in plant tissue culture

Contaminant Type	Common Examples	Primary Source	Manifestation / Symptoms	Eradication Difficulty	References
Epiphytic bacteria	<i>Pseudomonas</i> spp., <i>Bacillus</i> spp., occasionally <i>Staphylococcus</i> spp. (handling-related contamination)	Explant surface, water, air, laboratory environment, operator contact	Turbidity in medium; mucilage/biofilm at explant base; growth inhibition; reduced regeneration	Moderate (surface sterilization reduces contamination, efficiency depends on explant type, microbial load and protocol)	Leifert et al. (1989); Reed et al. (1995); Orlikowska et al. (2017)
Endophytic bacteria	<i>Enterobacter</i> spp., <i>Curtobacterium</i> spp., <i>Luteibacter</i> spp., occasionally <i>Agrobacterium</i> spp. <i>Xanthomonas fragariae</i>	Internal tissues of mother plants (<i>Prunus</i> , <i>Rubus</i> , <i>Musa</i>), latent infections in stock plants	Delayed contamination (weeks–months); internal necrosis; growth inhibition; culture decline or failure	Very high (systemic localization makes elimination difficult; antibiotics may suppress but rarely eliminate; phytotoxicity is common)	Quambusch et al. (2014); Viss et al. (1991); Cassells (2012); Trzewik et al. (2025)
Fungi	<i>Aspergillus</i> spp., <i>Fusarium</i> spp., <i>Botrytis cinerea</i> , <i>Penicillium</i> spp. <i>Pseudomonas syringae</i>	Laboratory air, personnel, greenhouse-derived explants, contaminated vessels	Mycelial growth (white, green, black); sporulation; tissue browning and necrosis; incidence varies depending on species and culture conditions	Moderate to high (fungal spores are highly resistant; thermotherapy and aseptic techniques may reduce contamination depending on pathogen and conditions)	Permadi et al. (2023); Anikina et al. (2025); Baló (2023); Cassells (2012)
Viruses	<i>Plum pox virus</i> (PPV), Grapevine leafroll-associated viruses (GLRaVs), <i>Chrysanthemum virus B</i> (CVB)	Systemically infected mother plants	Foliar mosaic, chlorosis, deformation; often asymptomatic in <i>in vitro</i> cultures; detected via RT-PCR or ELISA-based methods	High (meristem culture and thermotherapy may reduce virus presence; success is variable and virus-dependent)	Brison et al. (1997); Szabó et al. (2024); Elansary et al. (2024); Gao et al. (2023)
Phytoplasmas	<i>Candidatus Phytoplasma vitis</i> , <i>Candidatus Phytoplasma solani</i>	Infected mother plants and vegetative propagation material	Abnormal shoot proliferation; stunting; chlorosis; witches' broom;	Very high (phytoplasmas are phloem-limited and difficult to eliminate; antibiotic treatments may temporarily suppress infection but do not ensure eradication)	Nikfarjam and Farzaneh (2012); Carrillo-Ávila et al. (2023); Cassells (2012)
Microarthropods	<i>Rhizoglyphus</i> spp. (mites), <i>Frankliniella</i> spp. (thrips)	Contaminated plant material (explants), greenhouse-derived material, laboratory entry through ventilation or inadequate sealing	Mechanical damage to explants; tissue collapse; facilitation of secondary fungal and bacterial contamination; culture loss	Moderate (prevention through insect-proofing, sanitation, quarantine of plant material, and regular inspection)	Cassells (2012); Leifert and Cassells (2001)

Sources of Contamination

Accurately identifying the source of contamination is the critical first step in implementing an effective quality assurance (HACCP) system in micropropagation laboratories (Cassells, 2012). Sources are often multiple and deeply interconnected.

1. The Initial Explant (Mother Plant)

Plant material collected from natural environments (fields, greenhouses) is heavily

colonized by a multitude of microorganisms. Explants derived from plant parts close to the soil (roots, rhizomes, basal stems) exhibit a significantly higher degree of contamination compared to apical buds. Furthermore, the health status of the mother plant dictates the presence of systemic pathogens (viruses, endophytic bacteria). Irrigating stock plants with unfiltered tap water can introduce resistant

bacteria that will subsequently manifest in culture (Snyman et al., 2011).

2. Culture Medium and Water

Incorrect preparation of the culture medium is a frequent source of failure. Insufficient autoclaving (insufficient sterilization time or temperatures below 121°C) fails to destroy bacterial endospores (e.g., *Bacillus* spp.) or thermoresistant fungal spores. Additionally, the use of contaminated distilled or deionized water, stored improperly, can introduce microorganisms that form highly resistant biofilms on the walls of storage vessels (Karpinski et al., 2021).

Endospores of *Bacillus subtilis*, frequently present in laboratory dust and on work surfaces, easily survive standard autoclaving temperatures if the sterilization cycle falls below 121°C/15 min. Microbiological analyses of distilled water stored under standard laboratory conditions have detected biofilms of *Pseudomonas* spp. and *Sphingomonas* spp. after just 48-72 hours, underscoring the critical importance of using water immediately after preparation or storing it under strictly sterile conditions (Leifert & Cassells, 2001).

3. Laboratory Environment and Air

Laboratory air acts as a major vector for fungal spores and bacteria-carrying dust particles. Poorly maintained ventilation systems, the absence of HEPA filters at the room level, or air currents created by personnel movement can introduce contaminants directly into culture vessels during subculturing. Laminar flow hoods, if not tested and certified periodically, can suffer breaches in the sterile air flow, allowing contaminated outside air to penetrate the workspace (Balo, 2023). Cassells (2012) strongly recommends testing laminar flow hoods every 6-12 months and replacing HEPA filters when filtration efficiency drops below 99.97% for 0.3 µm particles.

4. Personnel and Instruments

Human error and deficient aseptic techniques represent major causes of secondary contamination (during subculturing). The operator's hands, breath, hair, and clothing are rich sources of bacteria (e.g., *Staphylococcus*) and yeasts. Instruments (scalpels, forceps) that are inadequately sterilized between manipulations (via flaming or glass bead sterilizers at 250°C) directly transfer

contaminants from one explant to another (Leifert & Cassells, 2001). Studies cited by Reed et al. (1995) indicate that a single subculture performed with lax aseptic techniques can cross-contaminate 40-60% of the additional cultures processed in the same work session.

Effects of Contamination on Plant Development

The presence of microorganisms in culture vessels has, in the vast majority of cases, detrimental effects on explant development, often resulting in culture loss. These effects can be direct (pathogenesis) or indirect (environmental modification).

1. **Nutrient Competition:** Microorganisms possess a multiplication rate exponentially higher than that of plant cells. They rapidly consume the sucrose, mineral salts, and vitamins from the Murashige & Skoog medium, depriving the explant of the resources necessary for growth. This leads to developmental stagnation and the absence of shoot or root formation (Permadi et al., 2023). A single bacterial colony of 10^6 - 10^8 cells/mL can completely deplete the sucrose from a standard MS vessel within 24-72 hours (Leifert & Cassells, 2001).

2. **pH Modification and Phytotoxicity:** Microbial metabolism generates excretion products that can drastically alter the pH of the culture medium (often lowering it to toxic acidic levels, pH 3-4). Furthermore, many fungi and bacteria secrete phytotoxins, pectolytic enzymes, or phenols that cause severe necrosis of plant tissues, a phenomenon frequently observed as a lethal browning of the explant and surrounding medium (Wijerathna-Yapa et al., 2023). In *Musa* spp., lethal browning - where bacterial contaminants and phenolic oxidation act synergistically - can lead to explant death within 7-14 days of culture initiation (Permadi et al., 2023).

3. **Latent Effects and Somaclonal Variations:** Latent endophytic bacteria may not immediately kill the plant *in vitro*, but they induce chronic stress. This stress can trigger epigenetic or genetic modifications, significantly increasing the rate of somaclonal variations, which is entirely unacceptable in commercial propagation where genetic fidelity (cloning) is paramount (Volk et al., 2022). Moreover, these

latent infections can erupt violently during the *ex vitro* acclimatization phase, when the plant is transferred to soil and humidity drops, leading to massive greenhouse mortality. Trzewik et al. (2025) experimentally demonstrated that raspberry (*Rubus idaeus*) plantlets contaminated with *Luteibacter* sp. and *Curtobacterium* sp. died almost entirely (90-100% necrosis) during dark storage at 4°C for 4-6 months, whereas decontaminated plantlets survived normally.

Opportunities and Beneficial Effects

Although microbial contamination has traditionally been associated with negative effects on *in vitro* cultures, research conducted over the last two decades has demonstrated that certain endophytic microorganisms may exert beneficial effects on plant development. Within the classical paradigm, Leifert and Cassells (2001) advocated the complete elimination of microorganisms from plant cultures, arguing that the risks associated with contamination, including phytotoxicity, reduced regenerative capacity, and the occurrence of somaclonal variation, outweigh any potential benefits. However, an alternative perspective subsequently emerged, suggesting that the effects of contaminants largely depend on the identity and functional role of the microorganisms involved.

An important contribution to this shift in perspective was provided by Cassells and Doyle (2004), who introduced the concept of “controlled biotized culture”. The authors proposed that certain endophytic microorganisms may contribute to maintaining plant physiological status and enhancing survival during *ex vitro* transfer, indicating that complete eradication of the associated microbiota may not always represent the most advantageous strategy. This hypothesis was further supported by the findings of Quambusch et al. (2014), who demonstrated that the success of *in vitro* propagation in *Prunus avium* is influenced by the composition of endophytic microbial communities. Genotypes that were difficult to propagate were associated with the predominance of potentially phytotoxic bacteria belonging to the genus *Pseudomonas*, whereas highly propagable genotypes harbored communities dominated by beneficial bacteria such as *Methylobacterium* and *Sphingomonas*.

These observations suggest that the mere presence of microorganisms is not necessarily detrimental and that their effects on plant development depend on the composition and functionality of the associated microbiota.

This paradigm shift was further reinforced by Herman (2017), who highlighted the role of certain endophytic bacteria as Plant Growth-Promoting Bacteria (PGPB). These microorganisms can enhance plant development through the production of phytohormones such as auxins and cytokinins, biological nitrogen fixation, and the induction of systemic resistance against pathogens. In this context, species belonging to genera such as *Bacillus* and *Pseudomonas* may exert positive effects on plant growth and adaptation, transforming what is conventionally regarded as contamination into a mutualistic interaction with beneficial outcomes.

Collectively, the available evidence indicates that the effects of microbial contaminants on plant development cannot be assessed solely on the basis of their presence or absence. In many cases, microorganisms associated with *in vitro* cultures may contribute to plant growth, acclimatization, and stress tolerance. Therefore, rather than adopting a strategy focused on the indiscriminate eradication of all contaminants, a more appropriate approach involves the selective management of microbial communities based on accurate taxonomic identification and functional assessment of the microorganisms involved in the plant-microbiota system.

Detection and Diagnosis

Early and accurate detection of contaminants is vital to prevent their spread throughout the laboratory. Methods range from simple visual observations to highly precise molecular techniques.

1. Visual Inspection and Indexing on Bacteriological Media

The simplest method is visual monitoring of turbidity in liquid media or the appearance of mucilaginous halos/mycelia on solid media. However, this method is ineffective for slow-growing bacteria or endophytes. For these, “culture plating” is used. Viss et al. (1991) and Reed et al. (1995) developed methods in which the base of the explant is scraped or sectioned

and inoculated onto specific bacteriological media (e.g., Medium 523, Nutrient Agar, Sabouraud Dextrose Agar). If microbial growth is observed on these media after several days of incubation at 28-30°C, the plant line in question is considered contaminated and is discarded.

2. Molecular Methods (PCR and RT-qPCR)

For pathogens that cannot be cultured on artificial media (viruses, viroids, phytoplasmas) or for the precise identification of bacterial species, molecular techniques are the gold standard. Polymerase chain reaction (PCR) using primers specific to the 16S rRNA region allows for the detection and identification of endophytic bacteria even at very low concentrations (Dangariya et al., 2020). For RNA viruses (e.g., Plum pox virus), RT-PCR (Reverse Transcription PCR) or RT-qPCR (quantitative) is used to assess the viral load of explants before and after eradication treatments (Elansary et al., 2024). These methods are rapid and highly sensitive but require expensive equipment and qualified personnel.

3. ELISA (Enzyme-Linked Immunosorbent Assay)

Serological tests such as ELISA are widely used for the initial screening of mother plants for common viruses. Although less sensitive than PCR, ELISA tests are less expensive and allow for the simultaneous processing of a large number of samples, making them ideal for commercial micropropagation laboratories.

Prevention and Control Strategies

Contamination management must be proactive, based on prevention, rather than reactive. Once a culture is visibly contaminated, eradication is extremely difficult, costly, and frequently associated with the phytotoxic effects of antimicrobial treatments.

1. Aseptic Techniques and Laboratory Management

The foundation of prevention is strict adherence to aseptic techniques. This includes the use of protective equipment (lab coats, gloves, masks), regular disinfection of work surfaces with 70% alcohol or chlorine-based solutions, and periodic maintenance of laminar flow hoods. Implementing a unidirectional workflow system in the laboratory (from clean zones to dirty zones) drastically reduces the risk of cross-contamination (Balo, 2023).

2. Explant Sterilization

Surface disinfection of initial explants is achieved using chemical agents such as sodium hypochlorite (NaOCl), calcium hypochlorite, mercuric chloride (HgCl₂), or hydrogen peroxide, often combined with a surfactant (e.g., Tween 20, 0.1%) to ensure penetration into tissue micro-crevices. Exposure time and concentration must be meticulously optimized for each individual species to balance microbial destruction with plant tissue survival (Trzewik et al., 2025).

Reference quantitative data: 1-2% sodium hypochlorite applied for 15-20 minutes - the most widely used protocol for easily sterilized herbaceous species - reduces epiphytic bacterial contamination by 85-95%, but rarely eliminates endophytes (Reed et al., 1995). For woody species, more aggressive protocols are required: 3-5% NaOCl with Tween 20, followed by 70% ethanol for 30 seconds. In *Rosa* sp., researchers reported that immersing explants in 1% NaOCl for 5 minutes plus 70% alcohol for 30 seconds reduced contamination to 10% compared to 100% in the unsterilized control (Balo, 2023). Hydrogen peroxide (H₂O₂) 3-10% for 10-15 minutes represents a less phytotoxic alternative for sensitive tissues, with an efficiency of 75-85% in reducing epiphytic bacteria.

A frequently reported issue in the literature is the complete lack of a universal sterilization protocol. Trzewik et al. (2025) showed that sodium hypochlorite, even at extreme concentrations (0.1-60%) applied via vacuum infiltration, failed to eliminate endogenous bacteria from *Rubus idaeus* cultures; only HgCl₂ 0.05-0.1% (highly toxic) proved completely effective. These results directly conflict with general guidelines promoting NaOCl as a first-line method for all species.

3. Use of Antibiotics and Antifungals

When endophytic contamination persists, researchers resort to incorporating antibiotics (e.g., cefotaxime, kanamycin, rifampicin, streptomycin) or antifungals into the culture medium. Nadha et al. (2012) demonstrated the efficacy of kanamycin (10 µg/mL) in eliminating bacteria from bamboo (*Guadua angustifolia*) cultures. Typical concentrations used in practice are: cefotaxime 250-500 mg/L, rifampicin 50-200 mg/L, streptomycin 10-50 mg/L, and kanamycin 5-20 mg/L (Reed et al.,

1995; Trzewik et al., 2025). Broad-spectrum commercial products, such as PPM™ (Plant Preservative Mixture), are often used prophylactically at 1-2 mL/L, although their efficacy varies considerably between species and bacterial strains. Comparative studies have shown that cefotaxime at 250 mg/L reduces bacterial contamination by 70-85% without significant phytotoxic effects in *Prunus*, *Malus*, and *Pyrus* spp., whereas concentrations >500 mg/L can severely inhibit regeneration in sensitive genotypes (Brison et al., 1997; Viss et al., 1991).

Trzewik et al. (2025) comparatively evaluated the efficacy of rifampicin (50-200 mg/L) versus PPM™ (0.2-4%) in raspberry cultures and found that rifampicin was effective against *Pseudomonas* sp. and *Curtobacterium* sp. without significant phytotoxicity, but failed to completely eliminate *Luteibacter* sp., which reappeared after 2-4 subcultures on antibiotic-free medium. PPM™ failed to produce inhibition zones for any tested contaminant and did not stop bacterial growth, refuting the commercial promises of the product in this specific context.

Leifert and Cassells (2001) explicitly warned that the repeated and prolonged use of antibiotics presents three major risks: (1) Phytotoxicity - broad-spectrum antibiotics, especially aminoglycosides (kanamycin, gentamicin) and rifampicin, inhibit protein synthesis in chloroplasts and mitochondria (organelles of prokaryotic origin), causing chlorosis, reduced photosynthetic rates, and foliar necrosis; (2) Microbial Resistance - suboptimal antibiotic treatment can select for resistant bacterial strains, making them exponentially harder to control later; (3) Infection Masking - bacteria can become latent under antibiotic pressure but are not eliminated, reappearing violently upon antibiotic withdrawal. Reed et al. (1995) confirmed this risk, reporting that 30-40% of mint cultures treated with antibiotics and appearing clean presented detectable bacteria via indexing after 2-3 subcultures without antibiotics.

4. Eradication of viruses: Thermotherapy and Cryotherapy

To obtain virus-free plants, meristem culture (taking the 0.1-0.5 mm apical dome, which is often virus-free due to the lack of vascular

connections) is combined with other therapies. Thermotherapy involves exposing plants *in vitro* to high temperatures (35-38°C) for several weeks to inhibit viral replication. Cryotherapy, a more modern technique, involves immersing meristems in liquid nitrogen (-196°C). Virus-infected cells, having a higher water content (larger vacuoles), are destroyed by ice crystals, while dense meristematic cells survive, regenerating a healthy plant (Brison et al., 1997; Elansary et al., 2024).

The analysis of the scientific literature regarding *in vitro* contamination reveals not only an accumulation of knowledge but also a series of tensions, contradictions, and gaps that demand a highly critical approach. A profound understanding of the field requires evaluating the methodological limitations of existing studies and unresolved debates.

1. Paradigm Evolution: From Total Eradication to Microbiome Management

The literature reveals a constant tension in tissue culture: the desire to obtain completely sterile cultures versus the biological reality of intimate plant-microbe associations. The traditional approach, aiming for total eradication through aggressive biocides and antibiotics, is reaching its limits. As demonstrated by Trzewik et al. (2025) and Leifert and Cassells (2001), bacteria can form biofilms inside culture vessels or hide in deep intercellular spaces, becoming inaccessible to surface sterilants and developing antibiotic resistance.

Examining the literature of the last 30 years, a clear evolution emerges: (1) The Eradication Paradigm (1980s-2000s): Cassells (1991) and Leifert et al. (1989) defined contamination as a one-dimensional threat. The tools - biocides, antibiotics - aimed for "pure axenic culture." (2) The Nuanced Paradigm (2000s-2010s): Herman (2017) reformulated the concept, recognizing that "completely sterile culture is often an illusion" and that some endophytes are inevitable and sometimes beneficial. (3) The Microbiome Paradigm (post-2010): Quambusch et al. (2014) and Orlikowska et al. (2017) applied microbial ecology methods (16S rRNA metagenomics) to characterize complex endophytic communities and correlate them with *in vitro* performance, paving the way for "deliberate biotization" - controlled inoculation with beneficial endophytes.

2. Critical Evaluation of Methodologies

A critical analysis highlights several systematic methodological limitations. First, small sample sizes: many studies use $n < 50$ explants per treatment, limiting statistical power. For example, Leifert et al. (1994) used batches of 20-30 explants, insufficient to detect subtle differences between protocols. Second, lack of biological replication: most studies use a single genotype or cultivar, making it impossible to extrapolate results to the species level. Trzewik et al. (2025) is a notable exception, testing three different *Rubus idaeus* cultivars and demonstrating that antibiotic response varies significantly between genotypes. Third, the gap between laboratory and industrial scale: academic studies are conducted under strictly controlled conditions that do not reflect the variability of a commercial laboratory processing tens of thousands of explants daily. Cassells (2012) estimates that the efficacy of published protocols drops by 20-40% when implemented in real commercial settings.

3. Research Gaps

Several areas remain insufficiently studied. The impact of latent contaminants on the quality of acclimatized plantlets remains poorly documented quantitatively. Furthermore, systematic comparative studies are lacking: few studies compare the efficacy of detection methods (PCR vs. ELISA vs. bacteriological indexing) on the same crop under identical conditions. Molecular mechanisms by which latent contaminants affect somatic embryogenesis and organogenesis are poorly understood; transcriptomic and metabolomic studies elucidating plant-microbe interactions *in vitro* are rare. Finally, there is a glaring lack of international standardization: no ISO standards or harmonized guidelines exist regarding sterilization protocols, antibiotic concentrations, or mandatory detection methods for certifying propagation material.

4. Contradictions and Debates in the Literature

The critical analysis reveals significant contradictions that reflect fundamental differences in approaches and interpretations of the same biological phenomena.

Contradiction 1: Antibiotic Use. Supported by Leifert and Cassells (2001) as essential for controlling endophytic bacteria, but heavily criticized by Reed et al. (1995) and Orlikowska

et al. (2017) due to severe phytotoxicity and resistance selection. Trzewik et al. (2025) showed that rifampicin eliminates bacteria temporarily, but they reappear after subculturing, suggesting a bacteriostatic effect. There is no consensus on optimal treatment duration or safe concentrations.

Contradiction 2: Endophytic Bacteria. Cassells and Doyle (2004) and Quambusch et al. (2014) argue that endophytes can be beneficial (producing phytohormones). In direct contrast, Leifert et al. (1994) advocate for total eradication, reporting 30-50% mortality in contaminated plantlets during *ex vitro* acclimatization compared to $< 10\%$ in sterile plantlets. The impact of endophytes depends critically on the bacterial species, the host plant, and acclimatization conditions.

Contradiction 3: Sterilization Protocols. George et al. (2008) recommend 1-3% NaOCl for woody species, while Anikina et al. (2025) indicate that 0.5-1% is sufficient for herbaceous species and higher concentrations cause necrosis. Trzewik et al. (2025) demonstrated that even 60% NaOCl failed to eliminate endogenous bacteria in raspberry, contradicting general guidelines promoting NaOCl as a universal method.

Future Perspectives

The future of *in vitro* contamination management leans heavily towards advanced technological and ecological solutions. The application of nanotechnology, particularly silver nanoparticles (AgNPs) or zinc oxide, shows enormous potential as antimicrobial agents in culture media, exhibiting low plant toxicity at optimal concentrations and minimal risk of generating microbial resistance. Studies on *Chrysanthemum morifolium* have shown that silver, copper, and gold nanocolloids at 10-50 mg/L can drastically reduce fungal and bacterial contamination without negatively affecting plantlet regeneration (Eisa et al., 2022). However, long-term ecological safety data remain insufficient.

Artificial Intelligence (AI) and Machine Learning are beginning to be applied to model and optimize culture media composition and predict contamination risks based on historical laboratory data (Bhattacharya & Bhattacharya, 2023). Computer vision systems can detect

incipient bacterial or fungal colonies with sensitivity superior to the human eye, allowing early isolation of affected vessels. Furthermore, gene editing (CRISPR/ Cas9) offers the prospect of creating plant lines intrinsically resistant to specific viruses, potentially eliminating the need for laborious thermo- or cryotherapy procedures. Concurrently, research will increasingly focus on *in vitro* biotization - the controlled inoculation of sterile explants with synthetic beneficial microbial consortia (PGPB) to create robust plants capable of resisting pathogenic contaminants independently (Soumare et al., 2021).

CONCLUSIONS

In vitro contamination remains a limitation of plant tissue culture, representing a constant barrier to efficiency and profitability. Bacteria, fungi, viruses, and mycoplasmas can infiltrate cultures through the initial explant, handling errors, or defective equipment, with massive economic consequences: Leifert and Waites (1994) estimated that between 15% and 40% of commercial micropropagation cultures are lost annually due to contamination.

The species-specific examples analyzed in this review - from endophytic bacterial contamination in *Prunus avium* and *Rubus idaeus*, to viral contamination in *Prunus persica* and *Vitis vinifera*, to the synergistic challenges of browning and contamination in *Musa* spp. - demonstrate unequivocally that there is no universal solution. Each species, and often each cultivar, dictates a specific prevention and eradication protocol, adapted to the nature of the explant and the microbiological profile of the mother plant.

Effective management requires a holistic, integrated approach based on HACCP principles: rigorous selection of mother plants, impeccable aseptic techniques, optimized sterilization, and constant monitoring via visual and molecular methods (PCR). While antibiotics and antifungals remain useful tools - with cefotaxime (250-500 mg/L) and rifampicin (50-200 mg/L) demonstrating documented efficacy - their use must be strictly limited due to the severe risks of phytotoxicity and resistance.

The future belongs to ultra-sensitive detection methods (qPCR, next-generation sequencing), the judicious use of nanomaterials, and, fundamentally, a paradigm shift that integrates the beneficial role of the endophytic microbiome into the ecology of *in vitro* systems. The contradictions within the literature analyzed in this review underscore the urgent need for comparative, multi-species studies with standardized methodologies to generate universally applicable, evidence-based practical guidelines for plant biotechnology laboratories.

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USE OF GENERATIVE ARTIFICIAL INTELLIGENCE (GenAI)

The authors declare the use of Generative Artificial Intelligence (GenAI) tools in the preparation of this manuscript. GenAI was used exclusively for language editing and text refinement in the Introduction and Conclusions sections. All scientific content, data analysis, and interpretations were developed and verified by the authors.

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NATURAL PLUM POX VIRUS INFECTION IN PLUM: FIVE-YEAR FIELD ASSESSMENT OF GENOTYPIC RESPONSE

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Abstract

Thirty-four plum genotypes were monitored over a five-year period (2021-2025) to evaluate their response to natural infection with plum pox virus (PPV), the most damaging viral pathogen of stone fruit species. The experimental orchard was established at FRDS Bistrița in spring 2020 using virus-free planting material. Two to six trees per cultivar were planted in the field and visually inspected during each growing season. During the first three vegetative periods, trees showing PPV symptoms were removed, destroyed, and replaced with healthy planting material. In 2024 and 2025, symptomatic trees were no longer removed in order to avoid large age differences within the orchard and to maintain comparable conditions among genotypes. Visual monitoring for PPV symptoms continued during these years, and in 2025 all trees were additionally tested serologically using DAS-ELISA. The elimination of PPV sources during the first three growing seasons limited virus spread, resulting in a low PPV incidence of 2.7% in 2023. After this practice was discontinued in 2024-2025, virus spread increased sharply, with PPV incidence reaching 14.1% by 2025. Nevertheless, thirteen cultivars remained symptomless throughout the five-year period, and PPV infection was not detected by DAS-ELISA.

Key words: cultivars, DAS-ELISA, sharka, survey, visual monitoring.

INTRODUCTION

Plum is one of the most widespread and economically important fruit species in temperate regions, mainly due to the extensive cultivation of *Prunus domestica* L. (Martínez-Gómez et al., 2026). It is used both for fresh consumption and for processed products such as dried plums, jams, juices, and traditional drinks, which increases its economic value in Eastern and Central Europe and supports rural development and agro-biodiversity in traditional growing areas (Wangchu et al., 2021). Despite these advantages, plum growing is often severely affected by viral diseases, among which *Potyvirus plumipoxi* (plum pox virus, PPV), the causal agent of sharka disease, is recognized as the most economically important viral pathogen of stone fruit species (García et al., 2024). Moreover, PPV is considered one of the ten most important plant viruses worldwide (Scholthof et al., 2011), highlighting its global economic impact and the major threat it poses to stone fruit production.

Plum pox virus causes substantial losses in yield and fruit quality (García et al., 2014; Barba et al., 2015). Its spread is facilitated by nearby

infection sources from other *Prunus* species, including *P. spinosa*, *P. cerasifera*, *P. salicina*, and *P. domestica* (Llácer and Cambra, 2006; Barba et al., 2011).

The virus is efficiently transmitted by aphids in a non-persistent manner (Labonne et al., 1995), which allows rapid dissemination within and between orchards. The impact of PPV is particularly significant in countries where plum represents a major fruit crop, such as Romania. Plum ranks first among fruit species in the country in terms of production, exceeding apple production, and Romania currently ranks second worldwide in plum production after China (FAOSTAT, 2026). A survey conducted in Romanian plum orchards showed a PPV incidence of approximately 69%, indicating that the virus has an endemic presence in these growing areas (Zagrai et al., 2010).

A recent survey at the national level conducted in young plum orchards (1 to 5 years old), reported a PPV infection rate of about 23.5%, with values varying widely between 0.5% and 77.5%. (Zagrai I. et al., 2022). These findings confirm that PPV remains a persistent problem in Romania and underline the importance of systematic monitoring, as well as the use of

certified pathogen-free planting material in accordance with EU directives (Zagrai L. et al., 2022). In susceptible cultivars, yield losses can be severe, occasionally reaching total crop failure under high PPV infection pressure (Minoiu & Lefter, 1987; Minoiu, 1997; Zagrai et al., 2001). In addition to quantitative losses, fruit quality deterioration further reduces market value and processing suitability. Cambra et al. (2024) estimated that sharka disease caused losses of about €2.4 billion between 1995 and 2023, while total cumulative losses since its emergence may exceed €13 billion. Therefore, sharka disease is not only a plant health problem but also a serious economic threat to stone fruit production in Europe and in other affected regions, such as Romania.

The response of plum cultivars to PPV represents an essential component for selecting cultivars suitable for a given fruit-growing area (Zagrai et al., 2001; Butac, 2020). When infection sources are present in the surrounding environment, the genotypic response of cultivars becomes a decisive factor influencing orchard productivity and long-term sustainability. The use of PPV-resistant or highly tolerant plum cultivars, combined with virus-free planting material when establishing new orchards, represents one of the most effective preventive strategies to limit virus impact, since removing infected trees and controlling vectors alone do not provide sufficient protection. For this reason, breeding programs increasingly focus on the development of new resistant plum cultivars (Zhang et al., 2024). However, the practical value of genetic improvement must be confirmed through long-term field evaluations under natural infection pressure, which allow a realistic assessment of cultivar behaviour and the identification of genotypes with stable and reliable tolerance or resistance to PPV.

MATERIALS AND METHODS

Experimental trial

A total of 34 plum cultivars, comprising 19 Romanian (Tuleu gras, Diana, Matilda, Elena, Delia, Gras ameliorat, Zamfira, Iulia, Agent, Flora, Andreea, Jubileu 50, Stanley, Ivan, Minerva, Doina, Centenar, Carpatin and Zandra), and 15 foreign genotypes (French improved, Joganta, Jofela, Tophit, Topper,

Topend plus, Top five, Jojo, Anna Spath, President, Blue free, Victoria, Empress, d'Agén, and Black Sun), grafted on Myrobalan 29C, were assessed over five consecutive years (2021-2025) to evaluate their response to natural PPV infection.

The experimental trial (Figure 1) was established at Fruit Research & Development Station Bistrița (FRDS Bistrița) in spring 2020, with two to six trees per cultivar included in the study. Other *Prunus* species scattered in the surrounding area of the plum orchard provided favourable conditions for natural PPV transmission by aphids.



Figure 1. The experimental plum orchard with 34 cultivars (FRDS Bistrița)

Visual monitoring

Each year during the vegetative period, all trees were inspected for typical PPV symptoms (Figure 2) particularly in spring (May - June), when symptom expression on young leaves is most pronounced.

Given that accurate recognition of PPV symptoms in infected plum trees can serve as a practical tool for orchard monitoring (Zagrai et al., 2024), visual assessments of the trees were conducted during the first four years. In the first three growing seasons after planting, symptomatic trees were removed and destroyed to eliminate potential sources of infection within the orchard, thus limiting the spread of the virus. They were subsequently replaced with healthy planting material in order to preserve the integrity of the experimental design. After that, the removal of symptomatic trees was discontinued to prevent excessive age differences within the orchard and to ensure relatively uniform agronomic conditions for comparative evaluation of the studied genotypes.

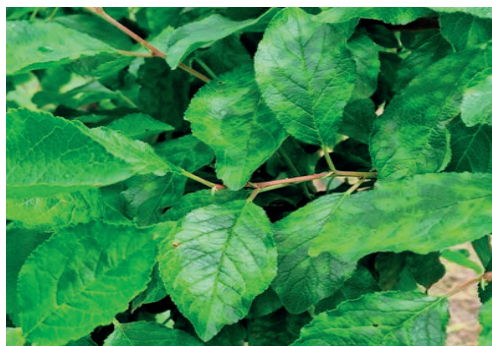


Figure 2. Typical symptoms of PPV infection on *P. domestica* leaves

Serological analyses

In 2025, all trees were additionally subjected to serological testing using the DAS-ELISA method to detect latent or asymptomatic PPV infections, ensuring a more accurate assessment of the overall infection status within the orchard. A minimum of 10 leaves per tree were randomly collected from different parts of the canopy to ensure representative sampling. In the case of symptomatic trees, only leaves showing typical PPV symptoms were sampled. When symptoms were confined to specific branches, samples were collected exclusively from those affected branches. If only a limited number of symptomatic leaves were present, the sample size was adjusted accordingly, consisting exclusively of the available symptomatic leaves. Leaf samples were homogenized at a ratio of 1:20 (w/v) and analyzed using the double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) method described by Clark and Adams (1977). PPV detection was carried out with a PPV-specific commercial kit (Bioreba, Switzerland), following the manufacturer's instructions.

Samples exceeding twice the negative control absorbance were considered positive, with kit controls included in each assay.

RESULTS AND DISCUSSIONS

PPV monitoring by visual observation

During the first three growing seasons after planting, the incidence of natural PPV infections remained low (0.5-2.7%) due to the systematic removal of trees visually identified as infected (Table 1). When the removal of symptomatic

trees was discontinued, the PPV incidence increased to 13.7%. Visual monitoring for PPV symptoms was continued, and in 2025 all trees were additionally tested using DAS-ELISA to detect latent or symptomless PPV infections.

DAS-ELISA assay

Serological testing of all trees (Table 1) showed that PPV incidence had increased to 14.1%, reflecting greater virus spread once infection sources were no longer controlled. Nine Romanian plum cultivars (Matilda, Elena, Delia, Gras ameliorat, Agent, Jubileu 50, Minerva, Carpatin, and Zandra), and four foreign plum cultivars (Joganta, Jofela, Top Five, Jojo) did not display typical PPV symptoms over the five-year period, despite continuous infection pressure. Furthermore, serological analyses did not detect PPV in these genotypes, suggesting a favourable response under natural infection conditions, although longer-term observations are required to confirm this behaviour.

Temporal spread of PPV

The removal of PPV infection sources during the first three growing seasons clearly mitigated virus dynamics (Figure 2). Once this practice was discontinued, PPV incidence increased sharply. The second-degree polynomial model captures this change, reflecting an accelerated rather than linear trend over time. The model's coefficient of determination ($R^2 = 0.868$) indicates that approximately 87% of the observed variability is explained, supporting its adequacy. The data indicate an initial phase of low PPV incidence, followed by a rapid increase in infections after 2023, when infected trees were no longer removed from the orchard. The upward trend can be attributed both to the presence of infection sources within the orchard and to external inoculum pressure from surrounding *Prunus* species.

Infected trees acted as local inoculum sources until removal, while asymptomatic infections likely contributed to viral persistence. During aphid flight periods, the latency between transmission and symptom expression allowed recently infected trees to remain symptomless but infectious, facilitating further spread to neighbouring trees.

Table 1. Visual monitoring followed by serological analyses of plum cultivars to PPV

Cultivar	No. of PPV infected trees/No. of total trees				
	Visual monitoring of PPV/year				DAS-ELISA
	2021	2022	2023	2024	2025
President	0/6	0/6	0/6	1/6	1/6
Tuleu gras	0/6	0/6	0/6	1/2	1/2
Diana	0/6	0/6	0/6	1/6	1/6
French improved	0/6	0/6	0/6	3/6	5/6
Joganta	0/6	0/5	0/6	0/5	0/5
Jofela	0/6	0/6	0/6	0/5	0/5
Tophit	0/6	0/6	1/6	2/5	2/5
Topper	0/6	1*/6	0/5	1/5	0/6
Topend plus	0/6	0/6	0/6	0/6	1/6
Top five	0/6	0/6	0/6	0/6	0/6
Jojo	0/6	0/6	0/6	0/6	0/6
Anna Spath	1*/6	0/6	0/6	1/6	1/6
Matilda	0/6	0/6	0/6	0/5	0/5
Elena	0/6	0/5	0/5	0/6	0/6
Delia	0/6	0/6	0/6	0/6	0/6
Gras ameliorat	0/6	0/6	0/6	0/6	0/6
Zamfira	0/6	0/6	0/6	1/6	1/6
Iulia	0/6	0/6	0/6	1/6	1/6
Agent	0/6	0/6	0/6	0/6	0/6
Flora	0/6	0/6	0/6	1/6	1/6
Andreea	0/6	0/6	0/6	1/6	1/6
Jubileu 50	0/6	0/6	0/6	0/6	0/6
Stanley	0/6	0/6	0/6	1/6	1/6
Ivan	0/6	1*/6	0/5	2/6	1/6
Minerva	0/6	0/6	0/6	0/6	0/6
Doina	0/6	0/6	0/6	1/6	1/6
Centenar	0/6	0/6	1/6	1/6	1/6
Carpatin	0/6	0/6	0/6	0/6	0/6
Blue free	-	0/4	3*/4	0/4	0/4
Victoria	-	0/4	0/4	2/4	2/4
Empress	-	0/4	0/4	1/4	1/4
Zandra	-	0/2	0/2	0/2	0/2
d'Agen	-	0/2	0/1	1/6	1/6
Black Sun	-	0/4	0/4	2/4	2/4
PPV	1/168 (0.5%)	2/186 (1.1%)	5/184 (2.7%)	25/183 (13.7%)	26/184 (14.1%)

*Trees infected with PPV were removed during the first three vegetative seasons and replaced with healthy trees when planting material of the respective cultivars was available. The spaces in the table marked with “-” indicate the absence of the respective cultivars in 2021, as the trees were planted in autumn 2021 or spring 2022.

As no curative treatments are available for viral diseases under field conditions, management depends on preventive strategies, including the use of pathogen-free planting material, the removal of infection sources to limit PPV spread, complemented by physical barriers and resistant cultivars that reduce virus transmission (Cambra & Vidal, 2017).

The small difference between the incidence estimated in 2024 (13.7%, based on visual symptoms) and that determined in 2025 by DAS-ELISA testing (14.1%) can be explained by several biological and epidemiological factors. Most trees infected in 2024 were already

symptomatic and detected through visual inspection.

Under relatively stable infection pressure in the orchard, the proportion of additional latent or asymptomatic infections was likely low. The serological testing performed in 2025 confirmed nearly the same number of infected trees, indicating that visual monitoring was effective in identifying most cases.

Within a single vegetative season, virus spread was probably limited. Plum pox virus is primarily transmitted by aphids, and the infection rate depends on vector abundance, climatic conditions, and cultivar susceptibility.

An additional factor that may have contributed to the limited increase in PPV incidence between 2024 and 2025 was the severe hailstorm on June 4, 2024, which almost completely defoliated the trees. By the time the foliage recovered, the peak aphid flight period had passed, and the population dynamics of aphid vectors capable of transmitting the virus were likely affected, reducing opportunities for new infections. If these factors did not favor intensive transmission during 2024-2025, newly infected trees would remain few. Additionally, the short interval between assessments may have limited detection of recent infections, as newly infected trees might not yet accumulate sufficient viral load or develop visible symptoms. Overall, the close agreement between visual assessment and serological testing suggests that careful visual monitoring provided a reasonable reliable representation of the orchard's

epidemiological status, with DAS-ELISA identifying only a small number of additional latent or weakly symptomatic infections, thereby supporting our previous finding (Zagrai et al., 2024). The differences observed among cultivars under natural PPV infection highlight the importance of cultivar selection for both breeding programs and orchard management. Cultivars that remained symptomless over five-year experiment and tested negative in serological assays may be of interest for further investigation, although longer-term studies are needed to confirm their performance under continued PPV exposure. At the same time, the use of less susceptible cultivars in commercial orchards may be considered in regions where PPV occur, but primarily when these cultivars offer significant advantages in fruit quality, yield, or other agronomic traits.

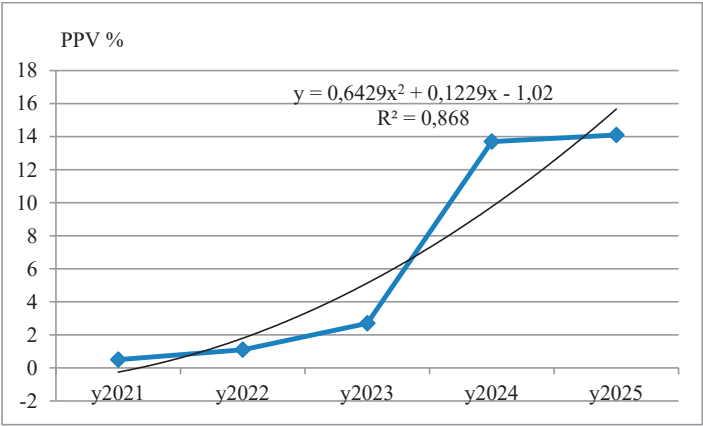


Figure 2. Dynamics of PPV infections in a plum orchard (comparative trial with 34 varieties) where infected trees were removed up to the third year after planting (2023)

CONCLUSIONS

A five-year field evaluation clarified PPV dynamics and the epidemiological factors influencing virus spread within the experimental trial. Several plum cultivars, developed both through Romanian breeding programs, including Zandra, Matilda, Elena, Delia, Gras ameliorat, Agent, Jubileu 50, Minerva and Carpatin, as well as foreign cultivars such as Joganta, Jofela, Top Five and Jojo, remained symptomless and

tested negative for PPV by DAS-ELISA under continuous infection pressure. These findings suggest that these cultivars may be valuable for further studies, although longer-term evaluations are needed to draw definitive conclusions regarding their response to PPV. At the same time, the removal of infected trees during the first three years likely helped limit local inoculum sources, contributing to the low infection levels determined throughout this period.

ACKNOWLEDGEMENTS

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VITICULTURE AND OENOLOGY



PRELIMINARY EVALUATION OF THE PERFORMANCE OF SOME LOCAL GRAPEVINE GENOTYPES UNDER THE PEDOCLIMATIC CONDITIONS OF DĂBULENI RDSPCSS

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Abstract

This study presents preliminary results on the evaluation of local grapevine genotypes cultivated under the pedoclimatic conditions of the Dăbuleni Research and Development Station for Plant Cultivation on Sandy Soils (southern Oltenia, Romania) during the 2025 growing season. The experiment was established in a young vineyard (third year after planting), trained on a trellis system with a 1.2 × 2.5 m spacing, and grown under drip irrigation. The biological material included three traditional cultivars ('Parmac', 'Berbecel', 'Roșioară') and one local genotype ('Gălbioară'). Phenological observations indicated a relatively uniform development of growth stages. Significant differences were recorded in yield components, especially cluster weight and productivity, with higher values in 'Parmac' and 'Roșioară'. Mechanical, morphometric, and physico-chemical analyses revealed genotype-specific patterns in berry structure, sugar accumulation, and acidity, reflecting adaptation to sandy soil conditions. These findings support the evaluation and conservation of regional grapevine genetic resources.

Key words: agrobiological traits, genetic resources, phenology, sandy soils, *Vitis vinifera*, Romania.

INTRODUCTION

The development of viticulture is closely linked to the evolution of human civilization, as the consumption of fresh grapes and their use for wine production have been documented since the Neolithic period, more than 8000 years ago (Estreicher, 2017). Archaeological evidence suggests that early wine production initially relied on collecting fruit from wild grapevines, followed by the cultivation of plants derived from the domestication of the Eurasian grapevine. Grapevine domestication is believed to have occurred at several geographic centers, ranging from ancient regions of Anatolia and the Caucasus to later areas in the western Mediterranean Basin and Central Europe (Muganu & Paolocci, 2013).

Grapevine (*Vitis vinifera* L.) is currently one of the most important cultivated species worldwide

and is grown in most wine-producing regions. Its importance is reflected not only in the economic value of grape and wine production (Fraga et al., 2013; Fraga, 2019) but also in the cultural and nutritional role that this crop plays in many societies.

In recent decades, climate change has become a key factor influencing viticulture (Santos et al., 2020), with many wine-growing regions increasingly exposed to higher temperatures and water scarcity (Mărăcineanu et al., 2025). Under these conditions, identifying grapevine cultivars better adapted to drought represents an important strategy for maintaining production and adapting viticulture to emerging climatic scenarios (Gisbert et al., 2022). Consequently, the evaluation and characterization of existing cultivars are essential for adjusting cultivation strategies and ensuring the stability of grape production.

Climatic conditions strongly influence both grape yield and quality, with these outcomes resulting from complex interactions among temperature, water availability, plant material, and viticultural practices. In established wine-growing regions, growers have historically adapted cultivars and cultivation techniques to local environmental conditions to optimize productivity and fruit quality. However, ongoing climatic changes require further adjustments to both cultivation practices and varietal selection to maintain the balance between yield and quality (van Leeuwen et al., 2019).

The need for adaptation is particularly pronounced in viticulture because grape berry composition, an essential determinant of fruit quality, wine typicity, and market value, is strongly influenced by environmental conditions, annual climatic variability, and their interactions, commonly referred to as *terroir*. Consequently, climatic variability directly affects grape composition and wine characteristics, requiring continuous adaptation of both plant material and cultivation practices (Delrot et al., 2020).

The geographical distribution of grapevine varieties has traditionally been closely related to the climatic characteristics of wine-growing regions, with growers selecting cultivars that perform best under local conditions. Generally, there is strong agreement between current cultivation areas and the optimal climatic conditions for each cultivar, explaining the geographical distribution of grapevine cultivars across different viticultural regions (Fraga et al., 2016).

The traditional grapevine assortment cultivated on the sandy soils of southern Oltenia mainly consisted of the cultivars 'Roşioară', 'Berbecel', and 'Parmac', which are used primarily for the production of white and rosé wines and are well adapted to the pedoclimatic conditions of the region (Băjenaru et al., 2022). Grapevine genetic diversity allows cultivation under a wide range of environmental conditions and provides opportunities for producing grapes intended both for fresh consumption and industrial processing, particularly for winemaking.

Morphological characteristics of clusters and berries, as well as the structure and proportion of their components, directly influence yield and grape quality. The determination of morphometric and physico-chemical parameters of grapes represents an important tool for varietal characterization, assessment of productive potential, and identification of technological suitability. In addition, the analysis of pulp, skin, and seed proportions provides relevant information regarding must yield and the quality of the raw material used in winemaking.

In this context, the present study aimed to analyse several local grapevine genotypes traditionally cultivated on sandy soils in southern Romania by determining cluster and berry morphological characteristics, evaluating the physical composition of grapes, and assessing the relationships among their main components in order to highlight the productive potential and particularities of these local viticultural resources.

MATERIALS AND METHODS

The research was conducted within the ampelographic collection of the Dăbuleni Research and Development Station for Plant Cultivation on Sandy Soils (RDSPCSS), located in Călăraşi, Dolj County, Romania, on the third terrace of the Danube River. The soil in the experimental area is a sandy eutric protosol with lamellar characteristics, with a pedological profile of Ap–Ac–C1–Cla.

The biological material (Figure 1) consisted of local grapevine genotypes 'Parmac' (prime name 'Dimyat' = 'Coarnă Albă' × 'Heunisch Weiss'; VIVC code 5716), 'Berbecel' (VIVC code 1148), 'Gălbioară', as well as the cultivar 'Roşioară' (prime name 'Pamid', VIVC code 8899), cultivated on their own roots. The vineyard was established at a planting distance of 1.2 m between vines and 2.5 m between rows, corresponding to a planting density of 3,333 vines ha⁻¹. The study was conducted in 2025, corresponding to the third year after planting, when training pruning was applied.

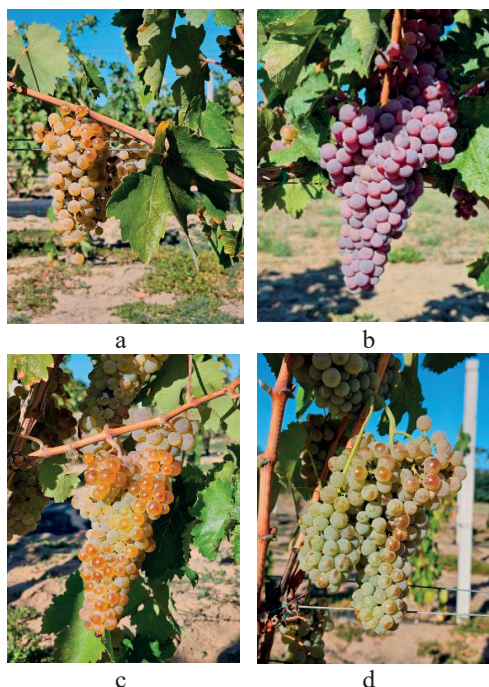


Figure 1. Clusters of the studied grapevine genotypes in the field: 'Gălbioară' (a), 'Roșioară' (b), 'Berbecel' (c), and 'Parmac' (d)

The experiment was arranged in a randomized complete block design (RCBD), with three replicates per genotype, each replicate consisting of three vines. The results represent the mean values of the three experimental replicates. Climatic data for the 2024-2025 agricultural year were recorded by an iMetos meteorological station located in the experimental field (Table 1).

Table 1. Main climate parameters for the agricultural year 2024-2025

Month	Air temperature (°C)			Total rainfall (mm)	No. of rainy days
	Mean	Max.	Min.		
XI	4.65	23.33	-6.99	40.00	13
XII	3.98	17.80	-2.96	128.00	17
I	2.56	17.91	-6.00	4.00	9
II	-0.47	15.27	-13.32	7.20	7
III	9.37	24.10	-5.99	29.20	10
IV	12.55	28.97	-3.97	21.00	9
V	16.07	29.87	1.53	55.40	12
VI	24.32	40.51	9.74	21.40	3
VII	25.75	41.21	10.69	11.60	4
VIII	23.99	40.95	7.53	26.40	5
IX	20.00	35.70	4.87	5.20	4
X	10.77	23.57	-1.96	169.40	11

During the growing season, standard vineyard management practices were applied in accordance with the cultivation technology specific to the region. Preventive phytosanitary treatments were carried out at the main phenological stages, while soil maintenance included mechanical operations, such as shredding plant residues, shallow soil cultivation, and weed control before budburst. The vineyard was managed under drip irrigation conditions.

Experimental observations focused on grapevine phenology, shoot fertility, yield, and grape quality. Phenological stages were monitored according to the BBCH scale, including budburst (BBCH 05-09), flowering (BBCH 61-69), berry growth (BBCH 71-79), veraison (BBCH 81), full ripeness (BBCH 89), and leaf fall (BBCH 93).

Fertility and yield assessments were performed by counting reproductive structures and weighing the harvested grapes for each replicate. For grape quality evaluation, berry samples were analyzed in the laboratory to determine the weight of 100 berries, soluble solids content (°Brix), total sugars, and titratable acidity. For each genotype, samples consisting of 100 berries were collected from each replicate. Berry weight was determined using an analytical balance (Kern PCB 2000-1), while soluble solids were measured using a digital refractometer (Atago PAL-BX|ACID F5; $\pm 0.2\%$). Sugar content (g L^{-1}) was calculated from Brix values according to the STAS 6182/25-73 standard. Titratable acidity was determined by titration with 0.1 N NaOH using phenolphthalein as an indicator, and the results were expressed as $\text{g L}^{-1} \text{H}_2\text{SO}_4$.

Statistical analyses were performed using IBM SPSS Statistics 26. Data were subjected to one-way analysis of variance (ANOVA), and mean comparisons were performed using Duncan's multiple range test at $p \leq 0.05$. Pearson correlation coefficients were calculated to evaluate relationships among the studied parameters, with significance levels set at $p \leq 0.05$ and $p \leq 0.01$. Correlation matrices were graphically represented as lower-triangular heatmaps generated in Python using the Matplotlib library.

RESULTS AND DISCUSSIONS

Grapevine cultivars grown on the sandy soils of the Dăbuleni area have been investigated in several studies, particularly with regard to their adaptation to restrictive pedoclimatic conditions as well as their productive and qualitative potential (Gherman & Vlăduț, 1967; Baniță et al., 1967; Vlădoianu, 1985; Ploae & Baniță, 1994; Rățoi et al., 2010; Rățoi et al., 2021). However, climate changes observed in recent decades, together with the renewal of vineyards, require an update of this information and a reassessment of the performance of local genotypes under current cultivation conditions. In this context, the agrobiological and productive characterization of the local genotypes cultivated in the ampelographic collection of Dăbuleni RDSPCSS provides relevant insights into their adaptability to sandy soil conditions and contributes to the valorization and conservation of local grapevine genetic resources.

Phenological development of local grapevine genotypes

The monitoring of phenological stages revealed minor differences among the studied genotypes (Table 2).

Table 2. Main phenological stages of the studied grapevine genotypes during the 2025 growing season (BBCH scale)

Genotype	Budburst		Flowering		Berry growth (BBCH 71)	Start of veraison (BBCH 81)	Full ripeness (BBCH 89)	Leaf fall (BBCH 93)
	BBCH 05	BBCH 09	BBCH 61	BBCH 69				
‘Gălbioară’	8 Apr	22 Apr	2 Jun	8 Jun	13 Jun	30 Jul	18 Sep	31 Oct
‘Roșioară’	8 Apr	22 Apr	2 Jun	5 Jun	11 Jun	30 Jul	18 Sep	31 Oct
‘Berbecel’	8 Apr	22 Apr	2 Jun	8 Jun	13 Jun	10 Aug	18 Sep	31 Oct
‘Parmac’	10 Apr	22 Apr	2 Jun	9 Jun	13 Jun	30 Jul	18 Sep	31 Oct

The progression of these stages was influenced by the climatic conditions recorded during the 2024-2025 growing season (Table 1), which was characterized by elevated summer temperatures, with maximum values exceeding 40°C during June-August, and relatively low precipitation during the main vegetative period. Budburst

(BBCH 05-09) occurred between 8 and 22 April, with the genotype ‘Parmac’ showing a slight delay compared with the other cultivars. Flowering (BBCH 61-69) took place between 2 and 9 June, with ‘Roșioară’ reaching full bloom slightly earlier than the other genotypes. Berry growth (BBCH 71) was recorded during the first half of June, while the onset of berry coloration (veraison, BBCH 81) occurred between 30 July and 10 August, with ‘Berbecel’ showing a slightly later entry into this stage. Full ripeness (BBCH 89) was reached on the same date (18 September) for all genotypes, whereas leaf fall (BBCH 93) occurred at the end of October. Overall, the relatively uniform phenological behavior suggests good adaptation of the local genotypes to the pedoclimatic conditions of the sandy soils of southern Romania. The elevated summer temperatures recorded during the growing season may also have contributed to the relatively synchronous ripening observed among the studied genotypes.

Shoot fertility and crop load

The productive potential of the studied genotypes was assessed through shoot fertility and crop load parameters (Table 3). The obtained data indicated that all four local genotypes exhibited balanced vegetative development during the first year of production. Crop load per vine ranged from 9.67 buds in ‘Gălbioară’ to 12.00 buds in ‘Roșioară’, indicating moderate differences in vine load intensity.

The number of burst buds and the total number of shoots per vine were similar, reflecting a good budburst capacity across all genotypes, likely favoured by drip irrigation conditions. The number of fertile shoots was relatively high in all cultivars, ranging from 7.67 in ‘Gălbioară’ to 9.67 in ‘Berbecel’, confirming a good fruiting potential even in the first year of production.

The number of inflorescences per vine was relatively uniform, averaging around nine, suggesting a stable reproductive capacity across genotypes. The relative fertility coefficient (Cfr) showed values close to unity, with the highest values recorded for ‘Gălbioară’ and ‘Parmac’, indicating an efficient conversion of fertile shoots into inflorescences.

Table 3. Shoot fertility, inflorescence number, and crop load per vine in the studied genotypes

Genotype	Crop load/vine	Burst buds	Number of shoots/vine	Number of fertile shoots/vine	Number of inflorescences/vine	Fertility coefficients	
						relative	absolute
‘Gălbioară’	9.67±1.15	8.67±1.15	8.67±1.15	7.67±1.15	9.33±0.58	1.08±0.18	1.25±0.29
‘Roşioară’	12.00±1.73	10.00±2.00	10.00±2.00	9.33±1.53	9.00±1.00	0.95±0.27	1.02±0.29
‘Berbecel’	11.33±1.53	10.00±1.00	10.00±1.00	9.67±0.58	9.00±1.00	0.90±0.15	0.91±0.12
‘Parmac’	10.33±1.53	9.00±1.00	9.00±1.00	9.00±1.00	9.33±0.58	1.06±0.12	1.06±0.12

Values are expressed as mean ± standard deviation (SD).

Similarly, the absolute fertility coefficient (Cfa) highlighted a slightly higher reproductive potential in ‘Gălbioară’, suggesting a greater capacity to support fruiting relative to the total number of shoots.

Overall, under the conditions of the first production year, all four local genotypes showed good vegetative and reproductive adaptation, with moderate differences among genotypes that may become more pronounced in subsequent years as the vines' productive potential stabilizes.

Correlation analysis (Figure 2) revealed strong relationships among the parameters describing crop load and vegetative development.

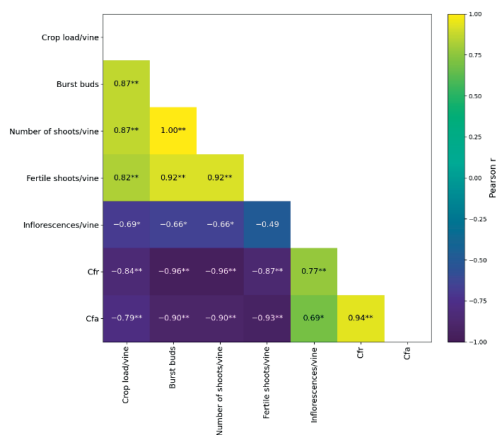


Figure 2. Pearson correlation heatmap of shoot fertility and crop load parameters

Crop load per vine was positively and significantly correlated with the number of burst buds, the total number of shoots, and the number of fertile shoots ($r = 0.82-0.87$; $p \leq 0.01$), indicating that vine load is mainly determined by budburst intensity and shoot development. The number of inflorescences per vine showed negative correlations with most vegetative parameters, suggesting a balance between

vegetative growth and fertility expression. The relative (Cfr) and absolute (Cfa) fertility coefficients were negatively correlated with vigour-related parameters but showed a strong positive correlation between them ($r = 0.94$; $p \leq 0.01$), confirming the consistency of fertility assessment through the two calculation approaches.

Yield components and productivity

Yield performance of the studied genotypes was evaluated through the number of clusters per vine, mean cluster weight, and productivity expressed per vine and per hectare (Table 4). The analysis of grape yield in the first year of production revealed significant differences among the four local genotypes. The number of clusters per vine was relatively uniform, ranging between 8.33 and 9.33, indicating a comparable crop load among cultivars.

Differences in productivity were mainly determined by the mean cluster weight, which varied significantly among genotypes. The cultivars ‘Roşioară’ and ‘Parmac’ recorded the highest average cluster weights, reaching 270 g and 230 g, respectively, values significantly higher than those observed for ‘Berbecel’ and ‘Gălbioară’. This differentiation was reflected directly in productivity per vine and per hectare. ‘Roşioară’ achieved the highest productivity per vine ($2.41 \text{ kg vine}^{-1}$), closely followed by ‘Parmac’ ($2.13 \text{ kg vine}^{-1}$), both were statistically superior to the other two genotypes. When expressed per hectare, productivity ranged from $\sim 3 \text{ t ha}^{-1}$ in ‘Gălbioară’ to $\sim 8 \text{ t ha}^{-1}$ in ‘Roşioară’, while ‘Parmac’ reached $\sim 7 \text{ t ha}^{-1}$. ‘Berbecel’ showed an intermediate productivity level ($\sim 3.8 \text{ t ha}^{-1}$), but was significantly lower than ‘Roşioară’ and ‘Parmac’.

Overall, the results indicate that under the conditions of the first production year and drip

irrigation, ‘Roşioară’ and ‘Parmac’ exhibit a superior productive potential, mainly determined by cluster size, whereas ‘Berbecel’ and ‘Gălbioară’ show a lower fruiting capacity typical of the juvenile phase of the plantation. Băjenaru et al. (2025) reported that, for the period 1969-2000, yields ranged between 11.5 and 23.95 t ha⁻¹ for ‘Roşioară’, between 16.07 and 22.35 t ha⁻¹ for ‘Parmac’, and between 11.88

and 15.65 t ha⁻¹ for ‘Berbecel’ under the pedoclimatic conditions of Dăbuleni RDSPCSS. The yields obtained in the present study were lower, which can be mainly explained by the young age of the plantation (third year after planting), when the vines’ productive potential is not yet fully expressed, as well as by the climatic conditions specific to the studied year.

Table 4. Yield components and productivity of the studied genotypes

Genotype	Number of clusters/vine	Mean cluster weight (g)	Productivity (kg vine ⁻¹)	Productivity (kg ha ⁻¹)
‘Gălbioară’	9.33±0.58	100±0.03 ^b	0.91±0.35 ^b	3036.67±1141.82 ^b
‘Roşioară’	9.00±1.00	270±0.04 ^a	2.41±0.22 ^a	8036.33±739.50 ^a
‘Berbecel’	8.33±0.58	140±0.01 ^b	1.13±0.15 ^b	3851.67±539.32 ^b
‘Parmac’	9.33±0.58	230±0.01 ^a	2.13±0.21 ^a	7092.33±325.48 ^a

Values are expressed as mean ± standard deviation (SD). Different letters indicate statistically significant differences among genotypes ($p \leq 0.05$) according to ANOVA followed by Duncan’s multiple range test.

Correlation analysis among yield components (Figure 3) revealed a very strong positive correlation between mean cluster weight and productivity expressed in kg ha⁻¹ ($r = 0.97$; $p \leq 0.01$), indicating that yield variation was mainly determined by cluster mass rather than cluster number.

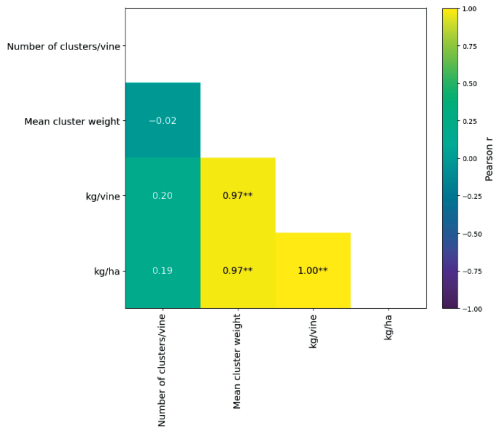


Figure 3. Pearson correlation heatmap of yield components in the studied genotypes

The number of clusters per vine did not show significant correlations with mean cluster weight ($r = -0.02$) or productivity ($r = 0.19$), suggesting that, under the conditions of this study, yield was influenced primarily by cluster size rather than by numerical crop load. The

almost perfect correlation between productivity expressed in kg vine⁻¹ and kg ha⁻¹ ($r = 0.999$; $p \leq 0.01$) reflects the direct mathematical relationship between these two variables, determined by the constant planting density.

Physico-chemical characteristics of grapes at harvest

The technological maturity of the grapes was assessed by soluble solids content, total sugars, and titratable acidity (Table 5). The analysis of physico-chemical characteristics at harvest revealed differences among the studied genotypes in terms of sugar content, soluble solids, and total titratable acidity.

‘Parmac’ genotype recorded the highest values of total sugars (174 g L⁻¹) and soluble solids (15.77%), indicating a more advanced level of technological maturity compared with the other genotypes. The genotypes ‘Gălbioară’, ‘Roşioară’, and ‘Berbecel’ showed similar values of total sugars (153-156 g L⁻¹) and soluble solids (14.27-14.45%), suggesting a relatively uniform ripening process.

Regarding titratable acidity, ‘Roşioară’ presented the highest value (3.40 g L⁻¹ H₂SO₄), while the other cultivars showed lower values (2.19-2.63 g L⁻¹ H₂SO₄), indicating differences in the balance between sugar accumulation and acid degradation during the ripening period.

Table 5. Physico-chemical characteristics of grape must at technological maturity

Genotype	Total sugar (g L ⁻¹)	Soluble solids (%)	Total titratable acidity g L ⁻¹ H ₂ SO ₄
‘Gălbioară’	153.00±10.15 ^b	14.45±0.89 ^b	2.19±0.25 ^b
‘Roşioară’	156.00±5.57 ^b	14.33±0.43 ^b	3.40±0.17 ^a
‘Berbecel’	155.33±5.86 ^b	14.27±0.46 ^b	2.38±0.23 ^b
‘Parmac’	174.00±5.57 ^a	15.77±0.43 ^a	2.63±0.38 ^b

Values are expressed as mean ± standard deviation (SD). Different letters indicate statistically significant differences among genotypes ($p \leq 0.05$) according to ANOVA followed by Duncan’s multiple range test.

Compared with the data reported by Băjenaru et al. (2025) for the same viticultural area (1969-2000), the sugar content values determined in the present study fall within the ranges reported for ‘Roşioară’ (148-180 g L⁻¹) and ‘Berbecel’ (152-154 g L⁻¹), but exceed those previously reported for ‘Parmac’ (138-143 g L⁻¹). In contrast, titratable acidity values recorded in this study were lower than the ranges reported in the literature (2.6-4.5 g L⁻¹ for ‘Roşioară’, 4.3-5.44 g L⁻¹ for ‘Parmac’, and 3.9-4.98 g L⁻¹ for ‘Berbecel’). These differences may be associated with the climatic conditions of the study year and the ripening stage at harvest.

Mechanical composition of grape clusters (1 kg basis)

The mechanical composition of grapes expressed on a 1 kg basis (Table 6) revealed notable differences among the studied genotypes.

Table 6. Mechanical components of grape clusters (1 kg basis) in the studied genotypes

Genotype	Rachis weight (g)	Berry weight (g)	No. of berries	Must (cm ³)	Skins and structural solids (g)	Seeds (g)	No. of clusters
‘Gălbioară’	108.53±1.65 ^a	906.64±5.48 ^c	927.33±7.51 ^b	560.00±5.00 ^c	280.75±3.77 ^b	63.93±1.20 ^c	6.67±0.58 ^a
‘Roşioară’	63.69±1.35 ^c	936.41±4.40 ^b	708.67±6.51 ^c	590.00±5.00 ^b	291.53±2.65 ^a	49.33±0.75 ^d	4.33±0.58 ^b
‘Berbecel’	78.40±1.55 ^b	946.87±4.35 ^a	1120.33±10.02 ^a	600.00±5.00 ^a	263.42±2.87 ^c	82.93±1.30 ^b	5.33±0.58 ^b
‘Parmac’	55.87±1.25 ^d	948.13±3.90 ^a	685.33±5.03 ^d	550.00±5.00 ^d	251.78±2.95 ^d	93.06±1.35 ^a	4.33±0.58 ^b

Values are expressed as mean ± standard deviation (SD). Different letters indicate statistically significant differences among genotypes ($p \leq 0.05$) according to ANOVA followed by Duncan’s multiple range test.

Correlation analysis among the mechanical components of grape clusters (expressed per 1 kg of grapes) revealed several statistically significant relationships (Figure 4). Rachis

weight was highest in ‘Gălbioară’ (108.53 g kg⁻¹) and lowest in ‘Parmac’ (55.87 g kg⁻¹), indicating differences in the proportion of the structural woody part of the clusters.

Berry weight was similar in ‘Berbecel’ and ‘Parmac’ (~947-948 g kg⁻¹), although the number of berries differed considerably. ‘Berbecel’ showed the highest number of berries (1120 berries kg⁻¹), whereas ‘Parmac’ recorded the lowest value (685 berries kg⁻¹). These results suggest that ‘Berbecel’ produces smaller but more numerous berries, while ‘Parmac’ produces larger but fewer berries.

Must volume was higher in ‘Berbecel’ (600 cm³ kg⁻¹) and ‘Roşioară’ (590 cm³ kg⁻¹), while lower values were recorded in ‘Parmac’ (550 cm³ kg⁻¹), indicating differences in winemaking potential among the genotypes. The proportion of skins and structural solids reached the highest values in ‘Roşioară’ (291.53 g kg⁻¹) and the lowest in ‘Parmac’ (251.78 g kg⁻¹), whereas seed weight was highest in ‘Parmac’ (93.06 g kg⁻¹) and lowest in ‘Roşioară’ (49.33 g kg⁻¹). The number of clusters per kilogram was higher in ‘Gălbioară’ (6–7 clusters kg⁻¹) and lower in ‘Parmac’ and ‘Roşioară’ (4–5 clusters kg⁻¹).

Overall, the significant differences among cultivars reflect varietal particularities in both morphological traits and the distribution of yield within 1 kg of grapes. These findings are relevant for cultivar selection depending on the intended use, either for fresh consumption or for winemaking, and for estimating must yield and the proportion of non-usable solid components.

weight showed a strong positive correlation with the number of clusters ($r = 0.88$; $p \leq 0.01$), suggesting that a greater structural mass is associated with a higher cluster load.

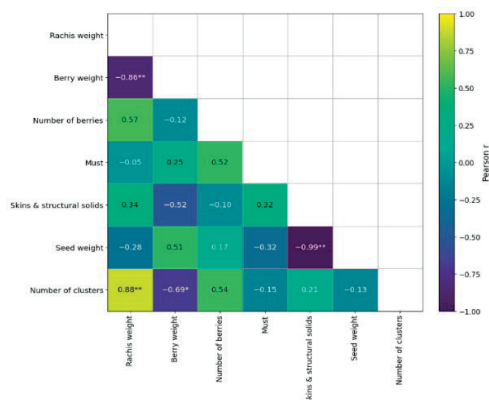


Figure 4. Pearson correlation heatmap of mechanical components of grape clusters (1 kg basis)

Conversely, rachis weight showed a strong negative correlation with berry weight ($r = -0.86$; $p \leq 0.01$), which may indicate a trade-off between cluster structural development and berry mass accumulation. Berry weight was also negatively correlated with the number of clusters ($r = -0.69$; $p \leq 0.05$), suggesting that a higher crop load per vine may reduce individual berry mass.

A very strong negative correlation was observed between skins and solid parts and seed weight ($r = -0.99$; $p \leq 0.01$), highlighting an inverse

relationship between these structural components of the grape berry. The remaining correlations were low or not statistically significant.

Morphometric traits of clusters, berries, and seeds

The analysis of morphometric traits of clusters, berries, and seeds (Table 7) in the four studied genotypes ('Gălbioară', 'Roșioară', 'Berbecel', and 'Parmac') revealed significant differences among them. Regarding cluster dimensions, cluster length ranged from 14.10 cm in 'Gălbioară' to 17.57 cm in 'Roșioară', while cluster width varied between 6.93 cm in 'Berbecel' and 9.80 cm in 'Parmac'. The peduncles were longest in 'Roșioară' (2.60 mm) and shortest in 'Gălbioară' (1.20 mm). The number of cluster wings was relatively constant in most cultivars, with the exception of 'Parmac', which presented a slightly higher number (three wings).

Berry size also differed among genotypes. The greatest berry length and width were recorded in 'Parmac' (14.6 x 13.5 mm), while the longest seeds were observed in 'Berbecel' (6.1 mm). The weight of a single seed ranged from 0.05 g in 'Gălbioară' and 'Parmac' to 0.06 g in 'Roșioară' and 'Berbecel'.

Table 7. Morphometric descriptors of clusters, berries, and seeds in the studied genotypes

Genotype	Cluster length (cm)	Cluster width (cm)	Peduncle length (mm)	No. of cluster wings	Berry length (mm)	Berry width (mm)	Seed length (mm)	Seed weight (g)
'Gălbioară'	14.10±0.20 ^c	9.53±0.15 ^a	1.20±0.10 ^c	2.00±0.00	11.90±0.20 ^c	12.27±0.15 ^c	5.90±0.10 ^b	0.05±0.01
'Roșioară'	17.57±0.25 ^a	8.77±0.15 ^b	2.60±0.10 ^a	3.00±0.00	13.20±0.20 ^b	12.90±0.20 ^b	5.40±0.10 ^a	0.06±0.01
'Berbecel'	14.80±0.20 ^b	6.93±0.15 ^c	1.50±0.10 ^b	2.00±0.00	11.87±0.15 ^c	11.60±0.20 ^d	6.10±0.10 ^a	0.06±0.01
'Parmac'	14.90±0.20 ^b	9.80±0.20 ^a	1.40±0.10 ^b	3.00±0.00	14.60±0.20 ^a	13.50±0.20 ^a	5.80±0.10 ^b	0.05±0.01

Values are expressed as mean ± standard deviation (SD). Different letters indicate statistically significant differences among genotypes ($p \leq 0.05$) according to ANOVA followed by Duncan's multiple range test.

Correlation analysis among morphological descriptors (Figure 5) revealed strong structural relationships between cluster and berry parameters. Cluster length showed an almost perfect positive correlation with peduncle length ($r = 0.99$; $p \leq 0.01$), indicating a clear structural dependency between these two components. The number of cluster branches ("wings") showed strong positive correlations with berry length ($r = 0.89$; $p \leq 0.01$) and berry width ($r = 0.87$; $p \leq 0.01$), suggesting that a more complex cluster architecture is associated with larger berry size. Additional correlations highlighted

significant relationships among berry dimensions. Berry length was strongly correlated with berry width ($r = 0.91$; $p \leq 0.01$), indicating a consistent proportional development of berry size across genotypes. In contrast, seed length showed negative correlations with several cluster and berry parameters, including cluster length ($r = -0.79$; $p \leq 0.01$) and peduncle length ($r = -0.75$; $p \leq 0.01$), suggesting an inverse relationship between seed size and certain structural characteristics of the cluster.

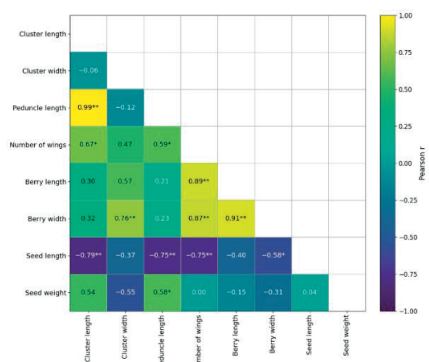


Figure 5. Pearson correlation heatmap of morphometric descriptors of clusters, berries, and seeds

Overall, these relationships highlight the interdependence between cluster architecture and berry morphology, reflecting varietal particularities that may influence both grape yield structure and technological potential.

Mechanical composition of 100 berries

The physical composition of berries, assessed on a sample of 100 berries for each genotype (Table

8), revealed significant differences among the studied cultivars in terms of total berry weight, distribution of berry components, and seed characteristics.

The total weight of 100 berries varied within a wide range, with the lowest values recorded for ‘Gălbioară’ (114.79 g) and ‘Berbecel’ (112.03 g), and significantly higher values for ‘Roşioară’ (154.72 g) and especially for ‘Parmac’ (179.69 g). These differences reflect substantial variation in berry size and mass among genotypes. Băjenaru et al. (2025) reported, for the period 1969–2000, values of 100-berry weight ranging between 184 and 255 g for ‘Roşioară’, approximately 302 g for ‘Parmac’, and 213 g for ‘Berbecel’, values higher than those obtained in the present study (154.72 g for ‘Roşioară’, 179.69 g for ‘Parmac’, and 112.03 g for ‘Berbecel’). The observed differences may be mainly attributed to the young age of the plantation analysed and to the climatic conditions specific to the study year; these factors can limit dry matter accumulation and final berry size.

Table 8. Mechanical components of berries based on 100-berries

Genotype	Total weight (g)	Skin (g)	Pulp (g)	Seed weight (g)	No. of seeds	Weight of 100 seeds (g)
‘Gălbioară’	114.79±2.94 ^c	25.54±1.35 ^c	78.12±1.38 ^c	7.85±0.27 ^b	192.33±3.51 ^a	3.84±0.11 ^b
‘Roşioară’	154.72±6.39 ^b	39.73±1.41 ^a	89.27±0.97 ^{ab}	7.09±0.13 ^b	185.33±3.21 ^{ab}	3.87±0.03 ^b
‘Berbecel’	112.03±3.67 ^c	23.21±0.99 ^c	85.93±1.55 ^b	8.65±0.41 ^b	162.67±4.51 ^c	3.91±0.06 ^b
‘Parmac’	179.69±22.60 ^a	33.94±3.67 ^b	92.88±6.53 ^a	13.71±1.68 ^a	177.67±7.64 ^b	6.89±0.15 ^a

Values are expressed as mean ± standard deviation (SD). Different letters indicate statistically significant differences among genotypes ($p \leq 0.05$) according to ANOVA followed by Duncan’s multiple range test.

Skin weight was highest in ‘Roşioară’ (39.73 g), followed by ‘Parmac’ (33.94 g), while ‘Gălbioară’ and ‘Berbecel’ showed significantly lower values (approximately 23–26 g). Pulp represented the largest fraction of the total berry mass, with values ranging from 78.12 g in ‘Gălbioară’ to 92.88 g in ‘Parmac’, the latter genotype standing out for its higher pulp content, which is favorable for must production. The total seed weight of the 100-berry sample also showed significant differences among genotypes. ‘Parmac’ recorded the highest seed weight (13.71 g), while ‘Gălbioară’, ‘Roşioară’, and ‘Berbecel’ showed lower values ranging from 7.09 to 8.65 g. The number of seeds was highest in ‘Gălbioară’ (192.33 seeds per 100 berries) and lowest in ‘Berbecel’ (162.67 seeds

per 100 berries), highlighting varietal differences in seed formation.

The weight of 100 seeds varied considerably among genotypes, with the lowest values recorded in ‘Gălbioară’, ‘Roşioară’, and ‘Berbecel’ (approximately 3.8–3.9 g), while the highest value was observed in ‘Parmac’ (6.89 g). This indicates that in ‘Parmac’, seeds are larger and heavier, contributing significantly to the increase in total berry mass.

Overall, the results demonstrate significant differences among genotypes in berry structure at the 100-berry level. The genotype ‘Parmac’ is characterized by larger berries with a higher proportion of pulp, but also heavier seeds, whereas ‘Gălbioară’ and ‘Berbecel’ show smaller berries with lower proportions of skins and

seeds. These characteristics are important for assessing grape quality and determining the technological suitability of each genotype. Correlation analysis of the mechanical composition of 100 berries (Figure 6) revealed several significant relationships among the parameters.

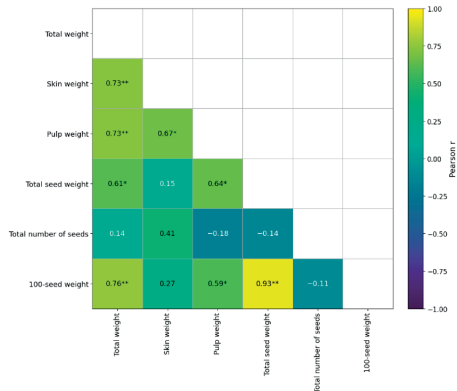


Figure 6. Pearson correlation heatmap of mechanical components based on 100-berry analysis

Total berry weight showed significant positive correlations with skin weight ($r = 0.73$; $p \leq 0.01$), pulp weight ($r = 0.73$; $p \leq 0.01$), and the weight of 100 seeds ($r = 0.76$; $p \leq 0.01$), indicating a proportional distribution of mass among the main berry components.

Pulp weight was positively correlated with total seed weight ($r = 0.64$; $p \leq 0.05$) and with the weight of 100 seeds ($r = 0.59$; $p \leq 0.05$), suggesting a coordinated development between fleshy tissues and reproductive structures. The strongest association was observed between total seed weight and 100-seed weight ($r = 0.93$; $p \leq 0.01$), confirming the internal consistency of the seed-related parameters.

The total number of seeds did not correlate significantly with the other analyzed variables, indicating that berry mass is more closely related to seed weight than to the number of seeds.

The results can also be interpreted in the context of the classical information reported by Alexei et al. (1970) for the cultivars ‘Parmac’, ‘Berbecel’, and ‘Roşioară’, grown in other viticultural regions of Romania.

For ‘Parmac’, the literature reports relatively high fertility indices (absolute fertility coefficient Cfa 1.8-2.6; relative fertility coefficient Cfr ≈ 2.1) and sugar contents ranging between 156 and 187 g L⁻¹, with total acidity between 3.4 and 7.7 g L⁻¹. In the present study, sugar accumulation (174 g L⁻¹) falls within the previously reported range, whereas the measured acidity (2.63 g L⁻¹ H₂SO₄) was lower. In addition, both yield level and mean cluster weight were below the upper limits reported in the classical literature (108-411 g per cluster), which can be explained by the young age of the plantation and by the specific conditions of sandy soils.

For the cultivar ‘Berbecel’, Alexei et al. (1970) describe a highly fertile variety (Cfa 1.8; Cfr 1.7; approximately 97% fertile shoots), with sugar contents between 149 and 210 g L⁻¹ and very high productivity (32-53 t ha⁻¹). The values obtained in the present study indicate a lower productive potential than in the literature, while the mean berry and cluster weights were within the lower limits of previously reported ranges (1.1-2.2 g per berry; 100-290 g per cluster). The sugar content recorded in this study (155 g L⁻¹) falls within the lower end of the reported interval, suggesting adequate technological maturity, although it does not reach the maximum potential described for other viticultural areas. Similarly, Gorjan and Botu (2013) mention ‘Berbecel’ as a cultivar characterized by moderate sugar accumulation and relatively low acidity under the conditions of the Drăgăşani vineyard.

Regarding the cultivar ‘Roşioară’, the literature reports fertility indices of approximately Cfa 1.8 and Cfr 1.1, sugar contents ranging between 143 and 202 g L⁻¹, and high yields (32-41 t ha⁻¹), with cluster weights between 100 and 450 g. Under the conditions of the present study, sugar content (156 g L⁻¹) and acidity (3.40 g L⁻¹) fall within the previously reported ranges; however, the productivity recorded was considerably lower. These differences can be attributed to the plantation's young age, the climatic conditions of the 2025 growing season, and the specific pedological characteristics of the sandy soils of southern Oltenia.

Overall, the comparison with previous literature indicates that although the productivity values obtained in the present study are lower than the historical potential reported for these cultivars, the ripening parameters and morphological traits remain within the specific ranges characteristic of each genotype, confirming their adaptability to the ecological conditions of the studied area. The obtained results also highlight the adaptation of the studied local genotypes to the pedoclimatic conditions specific to the sandy soils of southern Oltenia, as well as their particular morphological, productive, and technological characteristics. The characterization of these genotypes contributes not only to evaluating their agronomic and oenological potential but also to the valorization and conservation of the local viticultural heritage. In this context, the present study is part of the ongoing efforts to identify, preserve, and enrich the ampelographic collection of the Dăbuleni Research and Development Station for Plant Cultivation on Sandy Soils, with local genotypes considered valuable genetic resources for the adaptation of viticulture to current and future climatic conditions.

CONCLUSIONS

The present study enabled a comparative evaluation of local grapevine genotypes cultivated on the sandy soils of southern Romania, highlighting differences among them in phenology, fertility, yield level, morphological traits of clusters and berries, and the physico-chemical composition of grapes.

Fertility parameters were closely correlated with vegetative development, confirming the interdependence between crop load and shoot dynamics, while productivity was mainly influenced by mean cluster weight.

Mechanical and morphometric analyses revealed structural relationships among cluster, berry, and seed components, suggesting possible associations between structural elements and the accumulation of usable berry mass.

From a technological perspective, the genotypes reached maturity parameters consistent with the climatic conditions of the study year, with

variations observed in sugar accumulation and must acidity.

Overall, the results indicate that these local genotypes are adapted to the ecological conditions of the study area, highlighting their potential to maintain viticultural diversity and to be suitable for cultivation under restrictive pedoclimatic conditions.

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SUITABILITY OF SOME *V. VINIFERA* VARIETIES AND CLONES IN THE CURRENT CLIMATIC CONTEXT OF SOUTHWESTERN ROMANIA

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Abstract

The selection and promotion of valuable grapevine clones and varieties in vineyards can be a way in the strategy for adaptation and resilience to climate change, but also a possibility to offer consumers with a diverse range of wines characterized by complexity and typicity. The aim of this study was to evaluate the adaptation potential of some grapevine varieties and clones in the context of seasonal climate variability in South-West Romania. Seven V. vinifera varieties and clones were studied: 'Chardonnay' clones R8, VCR4 and 95, 'Tămâioasă românească', 'Fetească neagră', 'Merlot'; and 'Cabernet Sauvignon' clone ISV 117, grafted on V. Berlandieri x V. Riparia SO4 rootstock. Aspects regarding phenological stages, bioproductive parameters (bud fertility, grape yield, grape weight) and qualitative parameters (soluble sugars, total acidity) were evaluated. Rising temperatures and water scarcity are constant challenges in the wine-growing areas of southwestern Romania. The effects of warming and drying from flowering to full ripening generated a decrease in production by 38.56%, production losses ranging from 25.41% ('Chardonnay' clone 95) to 44.59% in Merlot.

Key words: climatic change, grapevine, phenological stages, high temperatures, bud fertility.

INTRODUCTION

The viticulture and winemaking sector plays an important role in Romania's agriculture and economy.

Climatic resources, along with other terroir factors, play a primary role in the growth and development of grapevine, influencing metabolism and physiological processes, with implications for the production and quality of grapes and, implicitly, the quality of wines (Costea et al., 2023).

Medium- and long-term climate scenarios show important variations in atmospheric patterns (Rességuier et al., 2020; Schultz, 2016), which can affect viticultural geography (Jones et al., 2005) grapevine phenology, grape production, and wine quality (Muñoz et al., 2021; Neethling et al., 2019). Modeling the effects of increased temperatures, according to different scenarios, on the evolution of climate change at the geoviticultural level is an important concern and

provides useful information on the choice of suitable varieties for vineyards (Duchêne et al., 2010; Zapata et al., 2017), establishing the optimal harvest time according to the desired sugar content for a certain type of wine (Molitor et al., 2020).

The climate change noticed in recent decades generates significant challenges for the vine and wine sector at the global and European levels (Cardell et al., 2019; Santos et al., 2020). Vineyards in Eastern Europe are experiencing increased water stress amid rising temperatures, erratic rainfall, and prolonged periods of drought (Filimon et al., 2024; Florea et al., 2025). In recent decades, in all wine-growing regions in Romania, there has been a significant increase in the average annual temperature (Iliescu et al., 2019) and in the frequency and intensity of heat waves (Bucur & Babeș, 2016; Croitoru et al., 2016), as well as a reduction in the frequency of freezing periods (Cichi et al., 2021).

The production and quality of the viticultural raw material and, implicitly, of the wine are directly influenced by climatic conditions (Van Leeuwen et al., 2019). The consequences of climate warming in the veraison-ripening interval on the quality of grapes at harvest must be taken into account in future grapevine breeding programs (De Cortazar Atauri et al., 2017) or cultivation practices (Bucur et al., 2024).

The stress caused by water scarcity can have the effects of limiting photosynthesis, slowing down the growth of shoots, and reducing the size of the berries, but also stimulating the synthesis of some phenolic compounds (van Leeuwen et al., 2008) and some volatile organic compounds (Savoi et al., 2016).

Recent studies focusing on wine-growing areas in the southwestern Romania region have shown that the changes and trends detected (1961-2021 period) for a series of climate indices used in viticultural climatology may generate pressure to obtain quality wines from certain grape varieties, amid the increase in temperature and reduced amounts of rainfall during the growing season (Irimia et al., 2018; Mărăcineanu et al., 2021; Vlăduț et al., 2023).

At national and regional levels, there is ongoing interest from the scientific community, but also from operators in the viticulture and wine sector, in identifying solutions to counteract the negative effects of the challenges generated by climate change (Nistor et al., 2022).

The use of genetic variability in grapevines (varieties, clones, rootstocks) is a possibility intensely explored in research aimed at adapting to climate change (Cichi et al., 2022; Zaldea et al., 2021), along with the identification of new cultivation zones (Irimia et al., 2018; Licurici et al., 2025) or various training systems (Băducă et al., 2020; Gatti et al., 2016; Trought et al. 2015). The selection and promotion of valuable grapevine clones and varieties in vineyards can be a way in the strategy of adaptation and resilience to climate change, but also a possibility to offer consumers a diverse range of wines, characterized by complexity and typicity. The aim of this study was to evaluate the adaptation potential of some traditional grapevine varieties and new grapevine clones introduced in the assortment, in the context of seasonal climate variability in southwestern Romania.

MATERIALS AND METHODS

Location and climatic characteristics. The study was conducted for the 2023 and 2024 vintages in a wine grape vineyard located at the Didactic Research Station of the University of Craiova (Dealurile Craiovei vineyard, *Hills of Muntenia and Oltenia winegrowing Region*), in southwestern Romania. The vineyard area is located between the parallels of 44°29' north latitude and 23°87' east longitude (190 m elevation). Hills of Muntenia and Oltenia winegrowing ranks second in terms of winegrowing area at the national level, the Oltenia winegrowing sub-region being representative for the quality and diversity of its wines.

Climate characteristics. The climatic data were obtained from the following source: Klein Tank et al, 2002 (data and metadata available at <http://www.ecad.eu> for the Craiova meteorological station- 44°13' latitude and 23°52' longitude, 192 m altitude, Dolj County). Climate suitability was assessed by bioclimatic indices such as: average annual temperature, sum of average daily temperature > 10°C, April 1st to September 30th (SAT), the growing season average temperature (April 1st to September 30th), the Huglin Index -IH), the annual and growing season rainfall (mm), the Winkler Index (Winkler et al., 1974), the number of days with maximum temperatures >30 °C (April 1st to September 30th), the De Martonne Aridity Index, and the Nights Cold Index -CI (Tonietto & Carbonneau, 2004).

Plant material. Seven *V. vinifera* varieties and clones were studied: 'Chardonnay' clones 95, R8 and VCR4, 'Tămâioasă românească', 'Fetească neagră', 'Merlot'; and 'Cabernet Sauvignon' clone ISV 117, grafted onto *V. Berlandieri* x *V. Riparia* SO4 rootstock. The study was conducted on 9 year-old vines. The vines were cultivated under the same growing conditions, on reddish-brown soil, with the 2.2 x 1.2 m spacing, a semi-tall shape of the stem (with a trunk of 0.8 m), Cordon spur pruning: 32 buds/vine, without irrigation. The viticultural management was applied for all varieties and clones in the same way. The randomized experimental design was used. Five vines per variety/clone were selected for the study in three replications.

Phenology data. Budburst (BBCH 07), flowering (BBCH 65), veraison (BBCH 81) and

fully ripening (BBCH 89) were considered (Lorenz et al., 1995). The Day of the Year (DOY) and the cumulated Growing Degree Day (GDD) since DOY to reach the main phenological stages were determined.

Agrobiological, quantitative, and qualitative characteristics. The absolute and relative fertility index (Afi and Rfi) were established and calculated according to Cichi et al., 2022.

Sampling measurement, and analyses. The measurements and analyses of yield and quality of the production were conducted at harvest. The average yield (kg/vine), the average weight of grapes (in g) were analysed. Sugar content (g/L) was measured and determined using a Kruss Optronic Hand Refractometer Hrot 32. Titratable acidity (g/L H₂SO₄) was determined by the titration method using NaOH 0,1N until pH 7.0. Sugar content and titratable acidity measurements were performed in three replicates.

Statistical analysis. The analysis of variance (one-way ANOVA) was applied for each variable evaluated. All variables significant in the F test were analysed using Tukey's HSD test for $p < 0.05$. The data are presented as mean $\pm$ standard deviation of each variable.

RESULTS AND DISCUSSIONS

Climatic characteristics. The main climatic characteristics during the study period are shown in Table 1. Compared with the multiannual average values (1961-2021), evident warming is observed during the study period. The average annual temperature was higher by 2.43° (2023) and 3.43° (2024). The values of growing degree days from 1 April to 31 October (Winkler index) total 2000 to

2312 units, which places the investigated area and the study years in Winkler climate class IV (temperate, 2023) and V (warm, 2024), indicating a favorable climate for grape varieties suitable for producing high-quality wines (Winker et al., 1974).

Compared to the 2023 growing season, there was significant warming in the 2024 growing season, the number of days with maximum temperatures $>30^{\circ}\text{C}$ being 92 days, of which 60 days occurred during July-August, during the veraison-fully ripening period. The increase in the number of days with maximum temperatures $>30^{\circ}\text{C}$ before harvest has also been reported in other vineyards worldwide (Van Leeuwen et al., 2019; Vršič et al., 2024), with effects on the rate and timing of ripening and the quality of grapes and wines. The year 2024 was also marked by a significant deficit of precipitation, being suboptimal both in terms of annual rainfall and during the growing season. The De Martonne Aridity Index growing season clearly demonstrates the arid character of the climate in the 2024 growing season (Table 1).

Phenological characteristics. In the context of climate change, knowing the projection of phenological stages in grapevines will play an important role in the decisions that grapevine growers and wine producers will make (Piña-Rey et al., 2021).

The climate specificity of the harvest year had a significant effect, in terms of the time of reaching the main phenological stages, as well as the amount of GDD for budburst and full ripening (Table 2).

The variety had a significant influence through GDD in achieving veraison (Table 3).

Table 1. Main climatic indexes of the experimental site

Climatic Index	Average 1961-2021	2023 Year	2024 Year
Average annual temperature	11.2*	13.63	14.33
Growing average season temperature (April 1 st to September 30 th)	-	19.78	22.00
SAT (Sum of average daily temperature $> 10^{\circ}\text{C}$, April 1 st to September 30 th)	-	3585	3988
Winkler Index (April 1 st to October 31 th)	1706*	2000	2312
Huglin's heliothermal index (IH)	2261*	2484	2923
Number of days with maximum temperatures $>30^{\circ}\text{C}$ (April 1 st to September 30 th)	-	46	92
Annual Rainfall (mm)	606.6**	575.4	357.3
Rainfall in the growing season (mm, April 1 st to September 30 th)	349.7*	318.2	175.2
De Martonne Aridity Index (I _{DM} , year)	-	24.35	14.69
De Martonne Aridity Index growing season (I _{DM} , April 1 st to September 30 th)	-	10.69	5.47
Nights Cold Index CI ($^{\circ}\text{C}$)	12.1*	15.02	13.72

*Source: Vlăduț et al., 2023 ; **calculated

Table 2. Seasonal evolution (DOY) and cumulated Growing degree days (GDD) in grapevine at main phenological stages by vintage

Vintage year	Budburst (BBCH 08)		Flowering (BBCH 65)		Veraison (BBCH 81)		Fully Ripening (BBCH 89)	
	DOY	GDD	DOY	GDD	DOY	GDD	DOY	GDD
2023	114±4.26 ^a	78.57±10.64 ^b	162±8.29 ^a	346.86±25.82	215.14±4.06 ^a	1108±48.56	252 ±3.37 ^a	1,622±43.34 ^b
2024	104±2.34 ^b	132.86±19.13 ^a	145±4.90 ^b	370.86±20.46	197.86±2.91 ^b	1144±53.24	242±5.50 ^b	1,977±93.76 ^a
Significance	***	***	**	NS	NS	NS	**	**
Minimum date	10 –April		22- May		12- Jul.		24-Aug.	
Maximum date	3 -May		16- Jun.		11-Aug.		14-Sept.	

Note: *** = significant at $P \leq 0.001$, ** = significant at $P \leq 0.01$, * = significant at $P \leq 0.05$, NS = non-significant.

Table 3. Cumulated growing degree days (GDD) and DOY at main phenological stages by varieties and clone (mean 2023-2024 vintage)

Variety/Clone	Budburst (BBCH 08)		Flowering (BBCH 65)		Veraison (BBCH 81)		Fully Ripening (BBCH 89)	
	DOY	GDD	DOY	GDD	DOY	GDD	DOY	GDD
Chardonnay clone 95	106	87	150	335	204	1,087 ^b	243±8	1,749±218
Chardonnay clone R8	108	101	151	339	205	1,103 ^{ab}	244±6	1,754±257
Chardonnay clone VCR4	108	101	152	344	205	1,107 ^{ab}	244±7	1,752±239
Tămăioasă românească	109	108	154	364	204	1,076 ^b	247±11	1,802±190
Fetească neagră	108	100	153	354	207	1,128 ^{ab}	245±8	1,773±231
Merlot	111	114	156	385	209	1,169 ^{ab}	250±8	1,844±283
Cabernet Sauvignon	116	132	157	394	213	1,215 ^a	255±3	1,924±339
Mean	109±6 ^{NS}	106±32 ^{NS}	153±9 ^{NS}	359±26 ^{NS}	207±10 ^{NS}	1,126±52 [*]	247±7 ^{NS}	1,800±197 ^{NS}

Note: *** = significant at $P \leq 0.001$, ** = significant at $P \leq 0.01$, * = significant at $P \leq 0.05$, NS = Non-significant.

There were no significant differences between the three clones of 'Chardonnay' concerning the attainment of the phenological stages or GDD. Budburst occurred earliest on 10 April (in 'Chardonnay' clone 95) and at the latest on 3 May (in 'Cabernet Sauvignon' ISV 117 clone). Budburst ranged from 87 GDD ('Chardonnay' clone 95) to 132 GDD ('Cabernet Sauvignon' ISV 117 clone). Flowering (BBCH 65) ranged from 335 GDD to 394 GDD, occurring from 22 May to 16 June. Early flowering is noticeable in the 'Chardonnay' clones and, among the varieties for red wines, in the 'Fetească neagră' variety. The veraison range was from 1076 GDD to 1215 GDD, with earliest occurring on 12 July 2024 and the latest on 11 August. There are statistically significant differences regarding the early veraison only for 'Chardonnay' clone 95 and 'Tămăioasă românească' compared to 'Cabernet Sauvignon' ISV 117 clone (Table 3). In order to establish fully ripened fruit, the minimum level of 190 g/L of sugar was established for the clones of 'Chardonnay' and 'Tămăioasă românească' and the minimum level of 204 g/L for the red wine varieties, in accordance with the regulations in force provided in the *Banu Mărăcine DOC Wine Specifications* submitted in 2011 to the

European Commission – according to Art. 118s, of Regulation (EC) 1234/2007.

Fully ripening ranged from 1749 GDD in the 'Chardonnay' clone 95 to 1924 GDD in the 'Cabernet Sauvignon' ISV 117 clone. The average duration of the veraison-fully ripening interval was 41 DOY in the two years of study, decreasing compared to previous years. Against the background of high average daily temperatures, a high number of maximum daily temperatures above 30°C (NDT 30) throughout the 2024 growing season (of which 56 DOY in the veraison-ripening interval) and a significant deficit of rainfall (Table 1), the full ripening of grapes had an advance (precocity) between 4-15 days compared to 2023. An exception, however, was the 'Cabernet Sauvignon' ISV 117 clone, which retains its late character in full grape ripening (Table 3).

Fertility and productivity. In terms of fertility, the 'Chardonnay' 95 clone in 2023 and the 'Cabernet Sauvignon' ISV 117 clone in 2024 were noted by a higher number of inflorescences reported to the total number of shoots per vine (R.f.i). Regarding the number of inflorescences on fertile shoots (A.f.i), significant differences between genotypes were highlighted only in 2024, showing the the 'Cabernet Sauvignon' ISV

117 clones showing an average of 1.50 inflorescences/fertile shoot (Table 4). In relation to the interannual variability of fertility, significant differences were observed in the variety 'Tămâioasă românească' for R.f.i, respectively in the the 'Chardonnay' R8 and

VCR4 clones and the the the 'Cabernet Sauvignon' ISV 117 clone for A.f.i. In vintage 2024, with the exception of the 'Cabernet Sauvignon' ISV 117 clone, all genotypes showed a lower number of inflorescences on the fertile shoots (A.f.i), Table 4.

Table 4. Fertility and productivity characteristics in varieties and clones*

Variety/Clone	Vintage	R.f.i	A.f.i	Bunch weight (g)	Yield (Kg/vine)	± % Yield Vintage 2024/2023
Chardonnay clone 95	2023	0.78 ±0.14 ^{abA}	1.24 ±0.07 ^{aA}	166.86 ±14.03 ^{cA}	1.43 ±0.24 ^{cdA}	- 25,41%
	2024	0.82 ±0.06 ^{bcA}	1.17 ±0.09 ^{bcA}	134.62 ±9.71 ^{bcB}	1.07±0.16 ^{aA}	
Chardonnay clone R8	2023	0.70 ±0.12 ^{abA}	1.25 ±0.07 ^{aA}	161.04 ±15.60 ^{cdA}	1.98±0.19 ^{bcA}	- 33,05%
	2024	0.71 ±0.06 ^{cA}	1.01 ±0.07 ^{cB}	110.33 ±18.88 ^{bcB}	1.33±0.13 ^{abB}	
Chardonnay clone VCR4	2023	1.06 ±0.19 ^{aA}	1.42 ±0.09 ^{aA}	191.63 ±17.56 ^{bcA}	2.25 ±0.48 ^{bcA}	-43,11%
	2024	0.83 ±0.07 ^{bcA}	1.13 ±0.04 ^{bcB}	150.97 ±19.20 ^{bcA}	1.28±0.08 ^{abB}	
Tămâioasă românească	2023	0.65 ±0.07 ^{bB}	1.40 ±0.20 ^{aA}	250.13 ±39.68 ^{abA}	2.58 ±0.33 ^{bA}	-36,60%
	2024	0.88 ±0.04 ^{bcA}	1.27 ±0.06 ^{bA}	184.07 ±14.19 ^{aA}	1.64 ±0.18 ^{abB}	
Fetească neagră	2023	0.76 ±0.08 ^{abA}	1.23 ±0.07 ^{aA}	261.35 ±33.09 ^{aA}	2.16 ±0.33 ^{bcA}	-43,12%
	2024	0.78 ±0.07 ^{bcA}	1.11 ±0.07 ^{bcA}	159.38 ±15.54 ^{abB}	1.23 ±0.10 ^{abB}	
Merlot	2023	0.96 ±0.15 ^{abA}	1.40 ±0.11 ^{aA}	164.07 ±18.03 ^{cA}	3.11 ±0.28 ^{abA}	-44,59%
	2024	0.90 ±0.04 ^{bA}	1.26 ±0.03 ^{bA}	125.34 ±15.83 ^{bcB}	1.72 ±0.32 ^{abB}	
Cabernet Sauvignon ISV 117	2023	0.89 ±0.10 ^{abA}	1.14 ±0.10 ^{abB}	143.03 ±16.11 ^{cdA}	2.12 ±0.30 ^{bcA}	-38,30%
	2024	1.06 ±0.06 ^{aA}	1.50 ±0.08 ^{aA}	101.17 ±16.52 ^B	1.31 ±0.26 ^{abB}	
Mean 2023		0.85±0.20 ^A	1.32 ±0.14 ^A	191.17 ±48.49 ^A	2.23±0.33 ^A	-38.56%
Mean 2024		0.85±0.12 ^A	1.21 ±0.16 ^B	137.98 ±30.57 ^B	1.37±0.07 ^B	

*Note: Means ± S.D.; Means separation by HSD Tukey's test at p≤ 0.05; Means with the same superscript are not statistically significant. In the column: the small letters indicate the significance of the differences between the varieties and clones for the same variable and vintage; capital letters represent the significance of the differences between the vintages

Table 5. Main qualitative characteristics in grapevine varieties and clones at harvest*

Variety/Clone	Vintage	Sugar content (g/L)	Titrate acidity (g/L H ₂ SO ₄)
Chardonnay clone 95	2023	209.93±3.05 ^{bcB}	4.26±0.07 ^{abA}
	2024	218.20±3.55 ^{bcA}	3.85±0.08 ^{cB}
Chardonnay clone R8	2023	204.73±6.00 ^{cA}	4.25±0.10 ^{abA}
	2024	214.94±4.51 ^{cA}	3.96±0.09 ^{bcB}
Chardonnay clone VCR4	2023	208.34±5.92 ^{bcA}	4.14±0.06 ^{bA}
	2024	218.87±2.96 ^{bcA}	3.82±0.15 ^{cB}
Tămâioasă românească	2023	218.23±2.25 ^{bB}	4.46±0.06 ^{aA}
	2024	224.80±2.92 ^{bA}	4.15±0.07 ^{abB}
Fetească neagră	2023	224.60±2.85 ^{abB}	4.43±0.09 ^{aA}
	2024	236.87±2.30 ^{aA}	4.24±0.04 ^{abB}
Merlot	2023	227.07±2.38 ^{abB}	4.20±0.13 ^{bA}
	2024	234.67±2.61 ^{aA}	4.24±0.05 ^{aA}
Cabernet Sauvignon ISV 117	2023	231.03±4.25 ^{aA}	4.42±0.09 ^{aA}
	2024	236.00±2.72 ^{aA}	4.21±0.10 ^{abB}
Mean 2023 vintage		217.70±10.28 ^B	4.31±0.14 ^A
Mean 2024 vintage		226.33±9.19 ^A	4.07±0.19 ^B

*Note: Means ± S.D.; Means separation by HSD Tukey's test at p≤ 0.05; Means with the same superscript are not statistically significant. In the column: the small letters indicate the significance of the differences between the varieties and clones for the same variable and vintage; capital letters represent the significance of the differences between the vintages.

Significantly greater differences in the average weight of the grape in vintage 2023 were recorded for the 'Fetească neagră' variety (261.35 g). With the exception of the clone 'Chardonnay' VCR 4 and the 'Tămâioasă

românească' variety, the average weight of grapes in 2024 was significantly reduced compared to 2023, ranging between 101.17 g ('Cabernet Sauvignon' ISV 117) and 184. 07 g ('Tămâioasă românească'). Production was

decreased by an average of 38.56% in vintage 2024 compared to vintage 2023, with production losses ranging from 25.41% ('Chardonnay' clone 95) to 44.59% in 'Merlot'. There were no statistically significant differences between the three 'Chardonnay' clones in terms of grape weight and yield/vine.

Quality characteristics. Knowing the ripening time and the time needed to reach the sugar concentrations in the traditional vine varieties for the wine region ((Fraga et al., 2014), depending on the temperature dynamics in the growing season or veraison-ripening and the desired style of wine, are very important for obtaining balanced wines, with variety typicity and specificity for the viticultural terroir.

The climatic conditions of the vintages and the genetic potential of the grape varieties significantly influenced the levels of the main chemical constituents of the grapes (Table 5). Under heat stress and water deficit conditions in the 2024 season, all varieties recorded higher sugar content and lower acidity at harvest, although the ripening phenophase occurred 4-15 days earlier compared to vintage 2023. The clones 'Chardonnay' VCR 4 and 95 and 'Cabernet Sauvignon' ISV 117 did not experience significant sugar increases between the two vintages. The dehydration of berries explains, to a certain extent, the increase in sugar concentrations at harvest, which was evident in 2024, under conditions of high temperatures in the veraison-fully ripening interval and water deficit. From the point of view of acidity, it is found that the varieties for red wines, under the same restrictive climate conditions, registered a fairly good content compared to some varieties/clones for white wines in which the acidity decreased significantly.

Interestingly, the Merlot variety, although not known as a drought-tolerant variety, did not register significant differences in acidity under conditions of water scarcity in the 2024 growing season; however, the production losses recorded were significant.

CONCLUSIONS

Rising temperatures and water scarcity are constant challenges in the wine-growing areas of southwestern Romania. The effect of warming and drying during flowering-fully ripening

generated a decrease in production of 38.56 %, production losses were between 25.41 % ('Chardonnay' clone 95) and 44.59 % in Merlot. Complex approaches are needed regarding the specific response reactions of grapevine varieties (traditional/recently introduced in the assortment) in the current climatic context and agro-management practices in order to better understand the genotype-terroir interaction, to have a predictable picture of the evolution,, and an adequate response for the viticulture and winemaking sector in the context of the prefigured climate scenarios in the medium and long term. The development and implementation of adequate strategies for adaptation to climate change in the wine-growing areas of the southwestern region of Romania are a pressing necessity to ensure sustainable viticulture, the preservation of the typicality of the wines, and the specificity of the terroirs of this region.

The overall analysis of the results highlights, in the current context, the necessary conditions for obtaining high-quality red wines from the Cabernet Sauvignon, Merlot, and Fetească neagră varieties, provided that the evolution of grape ripening is rigorously monitored every year and the selection of the most appropriate harvesting times is done when the ratios between sugars and acidity are fully in line with the type of wine to be produced.

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IMPACT OF UNFAVORABLE CLIMATIC CONDITIONS IN 2025 ON CERTAIN TABLE GRAPEVINE GENOTYPES FROM THE MURFATLAR VITICULTURAL CENTER

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Abstract

Grapevine is particularly vulnerable to late spring frosts, especially during the early stages of vegetative growth. Between 26 and 30 April, a severe frost event was recorded in the Murfatlar viticultural center, with minimum temperatures reaching -1°C , which caused complete necrosis of the primary shoots at BBCH phenological stages 11-13. The present study aimed to evaluate the effects of this climatic accident on the vegetative and productive behavior of four table grapevine genotypes: Cardinal, Aurana, Muscat Hamburg, and Afuz Ali 93 Mf. The results indicated a significant reduction in the number of fertile shoots and inflorescences per vine across all analyzed genotypes, with the most pronounced losses observed in Aurana and Cardinal. The real fertility coefficient showed values below the multiannual average (2015-2024), highlighting the low fertility of secondary buds. Productivity indices were also reduced; however, the average cluster weight was maintained or slightly increased in Muscat Hamburg and Afuz Ali 93 Mf. Among the genotypes, Afuz Ali 93 Mf demonstrated the highest biological and productive stability under unfavorable climatic conditions.

Key words: grapevine, bud fertility, productivity indices, genotypes, climatic stress.

INTRODUCTION

In the context of ongoing climate change, the frequency and intensity of extreme weather events have increased significantly, generating considerable challenges for agricultural systems worldwide. Viticulture is particularly sensitive to climatic variability because the growth, development, and productivity of grapevines are strongly influenced by environmental conditions during the vegetative cycle. Temperature fluctuations, especially during the transition from winter dormancy to active growth, can cause significant disturbances in grapevine physiology and yield formation (Jones et al., 2005; Hannah et al., 2013).

Among the climatic hazards affecting vineyards, late spring frosts represent one of the most damaging events, particularly in temperate wine-growing regions. These frosts occur after budburst, when young shoots and developing tissues have low tolerance to subzero temperatures. Even short exposures to temperatures between -1°C and -3°C can lead to severe damage, including necrosis of primary shoots and destruction of inflorescences (Keller, 2015). Consequently, grapevine recovery largely

depends on the development of secondary or tertiary buds, which usually have a lower fertility potential and a reduced capacity to ensure normal yield levels (Friend and Trought, 2007). The impact of spring frost on vineyard productivity depends on several factors, including the phenological stage at the time of the event, the intensity and duration of low temperatures, vineyard topography, and the genetic characteristics of the cultivated varieties (Molitor et al., 2014). In recent years, earlier budburst induced by warmer winters and springs has increased the vulnerability of grapevines to late frost episodes, particularly in regions characterized by high interannual climatic variability (Leolini et al., 2018). Consequently, identifying varieties or genotypes with greater resistance to climatic stress has become an important objective in modern viticulture.

The damage caused by spring frost can significantly alter the vegetative balance of grapevines, reducing the number of fertile shoots and inflorescences and ultimately affecting yield components such as the number of clusters, cluster weight, and berry composition. However, certain grapevine geno-

types may exhibit different levels of tolerance and recovery capacity, which may partially mitigate yield losses (Keller, 2015). Therefore, evaluating the response of different varieties to frost events is essential for improving vineyard management strategies and selecting plant material better adapted to changing climatic conditions. The damage caused by spring frost can significantly alter the vegetative balance of grapevines, reducing the number of fertile shoots and inflorescences and ultimately affecting yield components such as the number of clusters, cluster weight, and berry composition. However, certain grapevine genotypes may exhibit different levels of tolerance and recovery capacity, which may partially mitigate yield losses (Keller, 2015). Therefore, evaluating the response of different varieties to frost events is essential for improving vineyard management strategies and selecting plant material better adapted to changing climatic conditions. Under these conditions, the present study investigates the effects of a late spring frost

event recorded between April 26 and April 30 in the Murfatlar viticultural center on the vegetative and productive behavior of several table grape genotypes. The research focuses on assessing the impact of this climatic event on fertility parameters, productivity indices, and vegetative behavior characteristics of four grapevine genotypes: 'Cardinal', 'Aurana', 'Muscat Hamburg', and 'Afuz Ali 93 Mf'. The results provide useful information regarding the biological stability and adaptive capacity of these genotypes under unfavorable climatic conditions.

MATERIALS AND METHODS

In 2025, during the period April 26-30, a severe frost event was recorded in the Murfatlar viticultural center, with minimum temperatures reaching -1°C , which caused complete necrosis of the main shoots in the BBCH 11-13 phenophases (Figure 1).



'Cardinal'



'Aurana'

Figure 1. - Frost damage observed on young grapevine shoots

The present study aimed to evaluate the effects of this climatic event on the vegetative and productive behavior of four table grape genotypes: 'Cardinal', 'Aurana', 'Muscat Hamburg', and 'Afuz Ali 93 Mf', cultivated in the vineyards of the Murfatlar Research and Development Station for Viticulture and Oenology.

The studied varieties were grafted onto the same rootstock, Oppenheim Selection 4, clone 4, and the training system adopted was the classic Guyot, with a load of 34-38 buds per vine and a planting distance of 2.2 m between rows and 1.1 m between vines. The plots cultivated with these

varieties are located on land with an east-west exposure, a 2% slope, and a calcareous chernozem soil with a clayey texture and granular-glomerular porous structure, containing 2.3% humus. In order to describe the response of grapevine varieties to the climatic factors specific to the viticultural year and the local area, observations on the succession of vegetative phenophases were carried out between November 1, 2024 and October 30, 2025, recording the date when 50% of the plants physiologically reached each stage.

To monitor climatic elements, the automatic meteorological station iMetos 3.3 was used, located in the center of the vineyard plantation at the Murfatlar Research and Development Station for Viticulture and Winemaking. Daily observations were performed and the main climatic parameters were recorded: maximum and minimum temperatures, sunshine duration, relative air humidity, and precipitation. Based on these records, the evolution of abiotic stress during the viticultural year November 1, 2024–October 30, 2025 was determined and compared with the multiannual average (2015–2024).

To characterize the heliothermal and water resources during this interval, several synthetic ecological indicators were used: the real heliothermal index (Branas et al., 1946), the hydrothermal coefficient (Selezninov, 1936), the grapevine bioclimatic index (Constantinescu et al., 1936), the oenoclimatic aptitude index (Teodorescu, 1987), the heliothermal index (IH) (Huglin, 1978), and the cool night index (IF) (Tonietto et al., 2000). Grape yield was determined at harvest by establishing the average yield per hectare. The relationships between agrobiological and productivity indicators were evaluated by calculating the Pearson correlation coefficient (r), used to determine the intensity and direction of the linear relationship between two numerical variables. The correlation analysis was performed based on the average values determined for each variety in 2025, using Microsoft Excel (Sedgwick, 2012).

RESULTS AND DISCUSSIONS

In 2025, the main specific indicators were monitored and evaluated in relation to the ecological conditions characteristic of the reference year.

For each variety, the values of the relevant agrobiological and technological indicators were recorded and interpreted, following the evolution of the vegetative cycle and phenological stages, growth dynamics, fertility and productive potential, as well as the adaptation relationships to the dominant ecological factors. Monitoring these parameters allowed the identification of the behavioral particularities of each variety in relation to the climatic and edaphic characteristics of the area.

The comparative analysis of the main climatic elements presented in Table 1 for the average period 2015–2024 and for the year 2025 reveals several important changes in the thermal, pluviometric, radiative, and bioclimatic regime of the cultivation area of the varieties ‘Cardinal’, ‘Aurana’, ‘Muscat Hamburg’, and ‘Afuz Ali 93 Mf’.

The thermal balances (global, active, and useful) recorded lower values in 2025 compared to the average of the period 2015–2024: the global thermal balance ($\Sigma t^{\circ}g$) decreased from 5336.8°C to 4934.4°C; the active thermal balance ($\Sigma t^{\circ}a$) from 4723.1°C to 4379.7°C; and the useful thermal balance ($\Sigma t^{\circ}u$) from 2677.1°C to 2253.3°C.

These decreases, ranging between 7.3% and 15.9%, together with the late frost episodes (the first episode: April 7–11, 2025 with minimum temperatures between –1.9°C and –0.9°C – BBCH 03–07, and the second episode: April 26–30, 2025 with temperatures between –1.0°C and 0.3°C in the air and –2.0°C to –4.7°C at ground level – BBCH 11–13), suggest a reduction in the thermal energy available for biogeophysical processes, reflecting a shorter period of accumulation of useful temperatures ($>10^{\circ}C$).

Although the annual average temperature remained constant (14.8°C), the seasonal redistribution of heat becomes evident. The average temperature in July increased slightly (from 27.0°C to 27.5°C), while August and September recorded decreases of –1.2°C and –1.5°C, respectively. This phenomenon indicates a shift of the thermal maximum toward mid-summer, with an earlier cooling of autumn. Moreover, the absolute minimum temperature in the air (–13.2°C) and at the soil surface (–16.1°C) indicates a moderation of winter thermal extremes, characteristic of a climate with milder winters.

Therefore, the thermal regime in 2025 can be characterized as moderately warm, with reduced seasonal amplitudes but with a total thermal energy lower than the multiannual average. Annual precipitation decreased from 476.3 mm (average 2015–2024) to 342.0 mm in 2025, representing a reduction of approximately 28%. During the vegetative period, the decrease was even more pronounced, from 259.5 mm to 165.2 mm, indicating a deficit of more than 40%. Such a level of precipitation, correlated with the high

temperatures during the summer period, resulted in a hydrothermal coefficient (HC) of 0.5, well below the threshold of 1 that, according to Seleaninov's methodology (apud Teodoreanu, 1980), delineates areas with a semiarid regime. Regarding the heliothermal regime, the total annual duration of actual sunshine increased from 2178.3 hours (2015-2024) to 2357.4 hours in 2025, corresponding to an increase of approximately 10% in actual radiation. In

contrast, during the vegetative period, the duration of sunshine decreased slightly (from 1560.6 to 1463.8 hours), suggesting a contraction of the bioactive season as a result of the early autumn cooling. The real heliothermal index (IHr) recorded a higher value (4.4 compared with the multiannual average of 4.0), confirming an increased intensity of radiation and energy available for photosynthesis, particularly during the summer months.

Table 1. Analysis of the climatic conditions during the period 2024-2025 compared with the multiannual average for the interval 2015-2024

Analyzed climatic elements	Multiannual mean 2015-2024	Viticultural year 2024-2025
Global thermal balance ($\Sigma t^{\circ}g$)	5336.8	4934.4
Active thermal balance ($\Sigma t^{\circ}a$)	4723.1	4379.1
Useful thermal balance ($\Sigma t^{\circ}u$)	2677.1	2253.3
Average temperature in July ($^{\circ}C$)	27.0	27.5
Average temperature in August ($^{\circ}C$)	26.3	25.1
Average temperature in September ($^{\circ}C$)	21.1	19.6
Absolute minimum air temperature ($^{\circ}C$)	-15.0	-13.2
Absolute minimum temperature at soil surface ($^{\circ}C$)	-18.8	-16.1
Mean annual temperature ($^{\circ}C$)	14.8	14.8
Maximum air temperature ($^{\circ}C$)	37.8	39.9
Total annual precipitation (mm)	475.6	342.0
Total precipitation during the vegetative period (mm)	259.5	165.2
Total annual sunshine duration (hours)	2178.3	2357.4
Sunshine duration during the vegetative period (hours)	1560.6	1463.8
Average maximum temperature in August ($^{\circ}C$)	36.0	30.7
Average temperature in the first and second decades of June	23.7	23.3
Number of days with maximum temperatures $> 30^{\circ}C$	74.3	61.0
Duration of the bioactive period (days)	194.0	195.0
Real heliothermal index (IHr)	4.0	4.4
Hydrothermal coefficient (CH)	0.7	0.5
Bioclimatic index (Ibcv)	13.5	15.5
Oenoclimatic aptitude index (IAOe)	5410.0	5197.2
Huglin heliothermal index (IH)	3209.3	2434.0
Cool night index (IF)	14.2	12.9

This combination of high insolation and reduced precipitation accentuates the continental and arid character of the climate, favoring an increased risk of atmospheric drought (Marinică, 2006).

The bioclimatic index (Ibcv) increased from 13.5 (multiannual average) to 15.5 in 2025, indicating an improvement in the thermal comfort of grapevines, although this aspect is counterbalanced by generalized water stress. The cool night index (IF) decreased from 14.1 (average for 2015-2024) to 12.9 in 2025, suggesting cool nights, a value very close to the threshold of temperate nights, a phenomenon

favorable for sugar accumulation, acidity preservation, and aroma intensification in grapes.

Regarding the oenoclimatic aptitude index (IAOe), the values decreased from 5410 to 5197.2, while the Huglin index (IH) decreased significantly (from 3209.3 to 2434.0).

This reduction of 24.1% reflects less favorable conditions for the ripening of grape varieties with very high thermal requirements. The overall climatic values analyzed for 2025 compared with the 2015-2024 average indicate a clear trend toward the aridization and continentalization of the regional climatic

regime. Although the annual average temperature remained constant, the decrease in active and useful thermal balance, together with the significant reduction in precipitation and the increase in insolation, outlines a warm and dry regime characteristic of periods of climatic imbalance. Thus, the year 2025 can be considered a year with unfavorable agroclimatic potential, especially for grapevine cultivation, due to the late spring frosts followed by a pronounced water deficit and a reduction in useful thermal resources. The observed trends are consistent with the context of regional climatic changes recorded in recent decades in southeastern and eastern Romania, characterized by the alternation of warm and dry years with short periods of pluviometric recovery (Bogdan & Marinică, 2007; Cheval et al., 2017; Dumitrașcu et al., 2017). The data presented in Table 2 reflect the dynamics of the phenological stages (budburst,

flowering, veraison, and ripening) for the four grapevine genotypes – ‘Cardinal’, ‘Aurana’, ‘Muscat Hamburg’, and ‘Afuz Ali 93 Mf’ (Figure 2) – reported in relation to the average values for the period 2015-2024 and the specific values recorded in 2025. The analysis highlights significant differences in the rate of development and the duration of the vegetative period, correlated with the climatic conditions specific to the year 2025. This delay (1-4 days) in budburst, associated with a colder and more unstable spring, was reflected in the decrease in the active thermal balance at the beginning of the vegetative season. Flowering also occurred later than the multiannual average, by 8-11 days. This delay was caused by the deficit of accumulated heat during the early summer period (May-June), also confirmed by the reduction in the active and useful thermal balances in 2025.

Table 2. Evolution of the vegetative phenophases of the table grape varieties ‘Cardinal’, ‘Aurana’, ‘Muscat Hamburg’, and ‘Afuz Ali 93 Mf’ in 2025 compared with the period 2015-2024 at the Murfatlar Research and Development Station for Viticulture and Winemaking (S.C.D.V.V. Murfatlar)

Genotype Variety/clone	Year	Budburst	Flowering	Veraison	Ripening	No. Days from budburst to full ripening
‘Cardinal’	Average*	9.04-26.04 17.04	24.05-10.06 1.06	18.07-2.08 25.07	24.08-30.08 27.08	132
	2025	20.04	12.06	24.07	29.08	131
	Difference	+3 (Delayed) **	+11 (Delayed)	+1 (Delayed)	+2 (Delayed)	-1 (Early) ***
‘Aurana’	Average	12.04-22.04 17.04	25.05-9.06 1.06	22.07-8.08 29.07	25.08-30.8 27.08	132
	2025	21.04	10.06	01.08	30.08	131
	Difference	+4	+9	+3	+3	-1
‘Muscat Hamburg’	Average	9.04-28.04 19.04	30.05-9.06 4.06	24.07-9.08 1.08	2.09-9.09 5.09	140
	2025	20.04	13.06	13.08	17.09	150
	Difference	+1	+9	+12	+12	+10
‘Afuz Ali 93 Mf’	Average	14.04-29.04 21.04	29.05-10.06 4.06	5.08-19.08 11.08	2.09-18.09 10.09	141
	2025	22.04	12.06	19.08	23.09	154
	Difference	+1	+8	+8	+13	+13

*Multiannual mean 2015-2024; **Delayed; ***Early.

From a physiological perspective, this stage depends on the accumulation of a minimum thermal sum of approximately 200-300°C above 10°C, which in 2025 was achieved more slowly. The veraison stage (the onset of sugar accumulation in the berries) shifted significantly toward the end of summer, occurring one day earlier for the ‘Cardinal’ variety, while delays of approximately 3 days were recorded for

‘Aurana’, 12 days for ‘Muscat Hamburg’, and 8 days for ‘Afuz Ali 93 Mf’. The delay in veraison was a consequence of the moderate temperatures recorded in July and the pronounced water deficit, which limited active growth and photosynthetic metabolism. Full ripening was the most affected stage. Compared to the multiannual average for the period 2015-2024, it was reached 2-3 days later for the ‘Cardinal’ and

'Aurana' varieties. For the mid-season and late varieties, 'Muscat Hamburg' and 'Afuz Ali 93 Mf', ripening was significantly delayed (by 12-13 days). This delay was caused by the lack of useful thermal resources during the August-September period, when average temperatures were lower than the multiannual normal (-1.2°C in August and -1.5°C in September). The total duration of the vegetative period (budburst – full ripening) showed slight variation among the varieties. Early varieties ('Cardinal', 'Aurana') maintained a relatively constant duration of the vegetative period, adapting better to the climatic conditions of 2025. In contrast, the mid-season and late varieties ('Muscat Hamburg', 'Afuz Ali') experienced an extension of the vegetative period by 10-13 days, indicating a slowdown of physiological processes due to the decrease in useful thermal balance and the water deficit during the ripening period.

The ability of grapevine varieties to differentiate fruit buds and form inflorescences on shoots represents fertility. Biological fertility and productivity are essential indicators for evaluating the performance of grapevine varieties, especially in the current context of climate change, which significantly affects growth and yield parameters. Determining the fertility of varieties each year aims to identify variations in their performance under potentially unfavorable conditions, as well as to highlight genotypes that exhibit high biological stability. The comparative analysis of the data obtained in 2025, in relation to the multiannual average (2015-2024), highlights significant changes in the vegetative and productive behavior of the four studied genotypes. The indicators related to bud fertility, fruiting coefficients, and productivity indices demonstrate a general imbalance in reproductive potential, with variations specific to each variety.

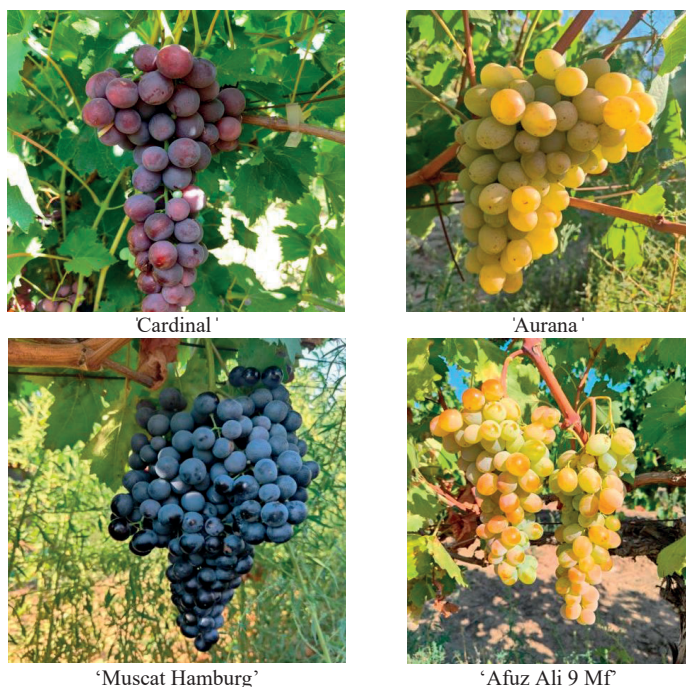


Figure 2. Appearance of the table grape varieties studied in 2025 at full ripeness in the vineyards of the Murfatlar Research and Development Station for Viticulture and Winemaking (S.C.D.V.V. Murfatlar)

The comparative analysis of the data obtained in 2025, in relation to the multiannual average (2015-2024), highlights significant changes in the vegetative and productive behavior of the

four studied genotypes. The indicators related to bud fertility, fruiting coefficients, and productivity indices demonstrate a general imbalance in reproductive potential, with variations

specific to each variety. Referring to the dynamics of vegetative growth and shoot fertility, it can be observed in Table 3 that the total number of shoots per vine recorded lower values in 2025, particularly in the varieties ‘Cardinal’ (–42%) and ‘Muscat Hamburg’ (–54%), indicating reduced vegetative vigor. This decrease is attributed to unfavorable climatic conditions during the early stages of vegetative growth, specifically the late frosts recorded in April.

The number of fertile shoots decreased drastically in all genotypes, especially in ‘Aurana’ (–77%) and ‘Cardinal’ (–64%). Consequently, the coefficient of fertile shoots (LF%) was considerably reduced (from 72-62% to 45-16%), indicating low bud fertility. The number of inflorescences per vine also decreased significantly in all varieties; the reduction was more pronounced in ‘Aurana’ (from 15 to 3) and ‘Cardinal’ (from 18 to 5). As a result, the real fertility coefficient (Cfr) recorded lower values than the multiannual average (0.2-0.5 compared to 0.7-1.0), confirming the weak fertility of secondary buds that developed after the late April frost, which affected the primary shoots in the BBCH 11-13 development stage.

The values of the absolute productivity index (Ipa) and the real productivity index (Ipr) showed significant decreases in most varieties, reflecting the reduction in production potential.

However, in 2025 the average cluster weight remained relatively constant compared with the multiannual average or increased slightly in ‘Muscat Hamburg’ and ‘Afuz Ali 93 Mf’, indicating a favorable redistribution of assimilates toward the formed clusters.

Compared with the multiannual average for the period 2015-2024, the clone ‘Afuz Ali 93 Mf’ showed the highest physiological stability, maintaining moderate values of fertility and productivity indices even under unfavorable conditions. ‘Muscat Hamburg’ compensated for shoot losses through an increase in cluster weight, while ‘Aurana’ showed the lowest bud fertility (LF = 16%), highlighting a high sensitivity to climatic stresses, particularly late frosts and water deficit. Due to the late frosts recorded in April, grape yield was drastically affected, remaining well below the multiannual average (reference period 2015-2024) for all the studied varieties. The lowest yield was recorded for the ‘Aurana’ variety. Although the berries remained relatively large, the reduced number of clusters led to a significant decrease in total yield, from a multiannual average of 13.8 t/ha (2015-2024) to 4.36 t/ha. The ‘Cardinal’ variety was the most severely affected by the late frosts in 2025; the number of clusters was very low, and the total yield reached only 5.70 t/ha compared with the multiannual average of 12.9 t/ha (2015-2024).

Table 3. Fertility and productivity parameters determined for the table grape varieties ‘Cardinal’, ‘Aurana’, ‘Muscat Hamburg’, and ‘Afuz Ali 93 Mf’ in 2025 compared with the average for the period 2015-2024 at the Murfatlar Research and Development Station for Viticulture and Winemaking (S.C.D.V.V. Murfatlar)

Genotype Variety/Clone	Year	Total shoots per vine	Fertile shoots per vine	Fertile shoots coefficient (LF) %	Inflorescences	Fertility coefficients		Productivity indices		Cluster weight
						Cfr	Cfa	Ipa	Ipr	
‘Cardinal’	Average*	19	14	72	18	0.9	1.3	379.6	262.8	292
	2025	11	5	45	5	0.4	1.0	276.0	110.4	276
‘Aurana’	Average*	21	13	62	15	0.7	1.1	463.1	294.7	421
	2025	19	3	16	3	0.2	1.0	252.0	70.4	352
‘Muscat Hamburg’	Average*	24	17	72	24	1.0	1.4	238.0	333.2	238
	2025	11	5	45	6	0.5	1.2	346.8	144.5	289
‘Afuz Ali 93 Mf’	Average*	19	11	54	13	0.7	1.2	518.4	302.4	432
	2025	15	5	33	7	0.5	1.4	222	201.6	444

*Multiannual average 2015-2024

For the ‘Muscat Hamburg’ variety, although the average cluster weight increased as a result of the reduced crop load per vine, the total yield remained significantly lower. The variety

partially compensated for the reduced number of clusters through their higher weight. Compared with the multiannual average, the yield was 6.04

t/ha lower (7.16 t/ha compared with the average yield of 13.2 t/ha for the period 2015-2024). The clone ‘Afuz Ali 93 Mf’ proved to be more resistant and better adapted to the variable climatic conditions of this year, showing the best

performance by achieving a yield close to the multiannual average (12.78 t/ha compared with 13.7 t/ha, the average yield for the period 2015-2024) (Table 4).

Table 4. Potențialul de producție ale soiurilor de masă de la S.C.D.V.V. Murfatlar în anul 2025 comparativ cu intervalul 2015-2024

Genotype Variety/clone	Year	Clusters/vine	Mean cluster weight g	Yield kg/but	Yield t/ha
‘Cardinal’	Average*	11	292	3.30	12.90
	2025	5	276	1.38	5.70
‘Aurana’	Average *	8	421	3.30	13.80
	2025	3	352	1.06	4.36
‘Muscat Hamburg’	Average *	14	238	3.30	13.20
	2025	6	289	1.73	7.16
‘Afuz Ali 93 Mf’	Average *	8	432	3.50	13.70
	2025	7	444	3.09	12.78

*Multiannual average 2015-2024.

The analysis of the relationships between agrobiological and productivity indicators, performed using the Pearson correlation coefficient (r) (Table 5), highlighted the existence of strong correlations between shoot fertility, the number of inflorescences, and yield components. The relative fertility coefficient (Cfr) shows a very strong positive correlation with the number of inflorescences (r = 0.966), while the absolute fertility coefficient (Cfa) is

strongly correlated with the relative productivity index (Ipr) (r = 0.956), indicating a direct relationship between shoot fertility and the productive potential of the vines. At the same time, the relative productivity index shows a very strong positive correlation with yield per vine (r = 0.965), while the absolute fertility coefficient is also strongly correlated with yield (r = 0.961).

Table 5. Pearson correlation matrix among agrobiological and productivity indicators of the studied grapevine cultivars (2025)

	Total shoots	Fertile shoots	LF %	Inflorescences	Cfr	Cfa	Ipa	Ipr	Cluster weight	Clusters/vine	Yield kg/but	Yield t/ha
Total shoots	1.000											
Fertile shoots	-0.870	1.000										
LF %	-0.996	0.911	1.000									
Inflorescences	-0.561	0.878	0.629	1.000								
Cfr	-0.739	0.943	0.790	0.966	1.000							
Cfa	-0.091	0.522	0.171	0.866	0.739	1.000						
Ipa	-0.632	0.278	0.580	0.046	0.287	-0.208	1.000					
Ipr	-0.331	0.736	0.411	0.967	0.871	0.956	-0.158	1.000				
Cluster weight	0.577	-0.102	-0.502	0.338	0.089	0.709	-0.777	0.567	1.000			
Clusters/vine	-0.561	0.878	0.629	1.000	0.966	0.866	0.046	0.967	0.338	1.000		
Yield kg/but	-0.092	0.564	0.179	0.869	0.713	0.961	-0.398	0.965	0.758	0.869	1.000	
Yield t/ha	-0.094	0.566	0.182	0.870	0.715	0.961	-0.396	0.965	0.756	0.870	1.000	1.000

The number of inflorescences and the number of clusters per vine show strong positive correlations with yield (r = 0.869), suggesting the important role of these indicators in determining production levels. Cluster weight shows a moderate positive correlation with yield per vine (r = 0.758),

indicating a smaller contribution of this indicator compared with the number of clusters. A very strong negative correlation is also observed between the total number of shoots and the percentage of fertile shoots (r = -0.996), which confirms the low fertility of secondary buds that developed after the late April frost,

which affected the primary shoots in the BBCH 11–13 developmental stage.

CONCLUSIONS

From a pedological perspective, the Murfatlar area provides excellent conditions for the cultivation of table grape varieties, with an optimal level of soil fertility, drainage, and water retention capacity. However, the analysis of the climatic parameters indicates a tendency toward aridization, with direct effects on the biological performance and productivity of the varieties.

The varieties ‘Cardinal’ and ‘Aurana’ showed increased sensitivity to thermo-hydric stress, requiring protective management practices such as irrigation and balanced pruning. ‘Muscat Hamburg’ shows moderate adaptation, maintaining stable vigor but with reduced fertility. The clone ‘Afuz Ali 93 Mf’ demonstrates the highest phenological and productive stability under the conditions of 2025 and is therefore recommended as a reference variety for semiarid viticultural areas in Dobrogea.

The correlation coefficients indicate that the variation in yield among the analyzed varieties is mainly associated with shoot fertility and the number of inflorescences formed per vine, indicators that show the strongest relationships with yield. In contrast, cluster weight contributes to a lesser extent to the differences in productivity among genotypes.

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CLIMATE INFLUENCE ON QUALITY PARAMETERS OF GRAPES, WINE, AND ECONOMIC PERFORMANCE IN FETEASCĂ NEAGRĂ

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Abstract

The consistent production of high-quality wines, characterized by typicality and authenticity, is fundamental to market competitiveness. Achieving this trait requires the ongoing adaptation of agronomic techniques to harmonize the biological needs of the cultivar with the prevailing climatic variables of each growing season. The research was conducted between 2022 and 2024 in the Miniș-Măderat vineyard, focusing on Fetească neagră, widely regarded by specialists and consumers as Romania's most valuable grape cultivar. The climatic conditions over the four-year period were analyzed in detail, with particular attention to their influence on both grape and wine quality parameters, as well as key economic indicators. The quality parameters assessed for grapes and wine included sugar content, protein content, macro- and microelement concentrations, vitamins, and tannins. Economic indicators considered were production costs, unit costs, production value, and profit. Correlations and regression analyses were subsequently performed to evaluate the relationships between selected climatic variables and the measured quality and economic parameters. Significant variations were observed in several indicators across the four years, highlighting the impact of climatic fluctuations on grapevine performance and wine quality.

Key words: grapes, economic performance, quality, wine.

INTRODUCTION

Viticulture is one of the agricultural sectors highly sensitive to climatic variability, as vine development, productivity, and the quality of the raw material intended for winemaking are strongly influenced by environmental factors (Dobrei et al., 2023). Air temperature, precipitation regime, solar radiation, and thermal amplitudes during the vegetation period determine the dynamics of the physiological processes of the vine, the accumulation of biochemical compounds in grape berries, and consequently the organoleptic and technological characteristics of the resulting wines (Bucur & Dejeu, 2022). In recent decades, increasing climatic variability has intensified the need to analyze the relationships between meteorological conditions during the growing season and the quality parameters of grapes and wines, making this topic particularly relevant

from both a viticultural and economic perspective (Filimon et al., 2024).

The quality of grapes intended for winemaking is determined by a series of physicochemical and technological indicators, including sugar content, total acidity, pH, phenolic composition, and the balance of aromatic compounds (Dobrei et al., 2021). These parameters result from the interaction between the genetic potential of the variety, the pedoclimatic conditions of the vineyard area, and the applied cultivation technology (Frîncu et al., 2024). Among these factors, annual climatic conditions play a decisive role, influencing vine phenology and the processes of accumulation and transformation of compounds in grape berries during ripening (Dominguez et al., 2024).

The Fetească Neagră cultivar, one of the most valuable native grape varieties for red wine production in Romania, is distinguished by its high oenological potential and its capacity to

reflect the climatic particularities and terroir of the cultivation area (Constantinescu et al., 2024). Wines obtained from this variety are appreciated for their intense color, complex phenolic structure, and distinctive aromatic profile (Cichi et al. 2024). However, the expression of these qualitative characteristics may vary significantly from one vintage to another, depending on the thermal, hydric, and radiation regimes during the vegetation period (Bocăneală et al., 2025).

In addition to its impact on product quality, climatic variability also affects the economic performance of wine farms through its influence on production levels, production costs, market value of wines, and overall production efficiency (Costea et al., 2025). Therefore, correlating agrobiological and oenological indicators with economic indicators allows for an integrated analysis of the viticultural system, providing valuable information to support technological and managerial decision-making (Stroe et al., 2025).

The objective of this study was to evaluate the relationships between climatic variability and the main quality indicators of grapes and wines obtained from the Fetească Neagră variety in the Miniș wine-growing center over the period 2021-2024. The study aims to identify the influence of bioclimatic factors on the physicochemical characteristics of grapes and to highlight the correlations between climatic conditions and oenological parameters using multivariate statistical approaches.

MATERIALS AND METHODS

Study area and experimental design. The research was conducted during the 2022-2024 growing seasons in the Miniș-Măderat vineyard, located in western Romania within the Crișana wine region, one of the traditional viticultural areas recognized for the production of high-quality red wines. The study focused on the Romanian autochthonous grape cultivar Fetească Neagră, which is well adapted to the pedoclimatic conditions of the region and is known for its high oenological potential.

The experimental vineyard was located in the Miniș viticultural center (Arad County, Romania), at approximately 46°05' N latitude and 21°37' E longitude, at altitudes ranging

between 120 and 300 m above sea level. The area is characterized by a moderate continental climate with Mediterranean influences, which provides favorable thermal conditions for grapevine development and the accumulation of sugars and phenolic compounds in grape berries. The soils in the experimental plot are predominantly well-drained brown soils and cambisols with moderate fertility, typical of the Miniș-Măderat vineyard. The vineyard is situated on gently sloping terrain (5-12%) with predominantly southern and south-western exposure, ensuring good solar radiation during the growing season and creating favorable microclimatic conditions for grape ripening. The Fetească Neagră cultivar vines were trained using a single Guyot training system, with a planting density of approximately 4,000 vines per hectare. Vineyard management practices followed the principles of integrated viticultural management, including canopy management, soil maintenance, and phytosanitary treatments. The experimental design covered three consecutive wine-growing years (2022-2024), each year representing a distinct climatic scenario. The experiment was organized using a randomized sampling design with three replicates. Each replicate consisted of 10-15 vines of the Fetească Neagră cultivar, selected to ensure uniformity in vigor and productivity.

Climatic data and bioclimatic indices. To characterize the climatic conditions specific to each growing year (2022-2024), the main meteorological parameters relevant for grapevine development were analyzed during the vegetation period (April-October). The climatic data included (AT) average air temperature (°C), average (ST) soil temperature (°C), total (PP) precipitation (mm), air hygroscopicity (%) (AH), and solar radiation expressed as sunshine duration (hours) (SR).

The average air temperature (AT) represents one of the most important factors influencing vine phenology, berry development, and the accumulation of sugars and phenolic compounds in grapes. Soil temperature (ST) plays a significant role in root activity, nutrient uptake, and overall plant metabolism. The precipitation (PP) regime (mm) determines the water availability during the vegetation period and strongly influences vegetative growth, berry size, and grape composition. The air

hygroscopicity (%) (AH), reflecting atmospheric humidity conditions, contributes to the regulation of plant transpiration processes and may also influence the incidence of fungal diseases during the growing season. Additionally, sunshine duration (hours) (SR) represents a key parameter controlling photosynthetic activity, which directly affects carbohydrate accumulation and grape maturation. Based on these meteorological parameters, several bioclimatic indices commonly used in viticultural research were calculated, including the global thermal balance (Σ_{tog}), active thermal balance (Σ_{toa}), useful thermal balance (Σ_{tou}), hydrothermal coefficient (CH), drought index (I-ar-DM), heliothermal index (Ihr), viticultural bioclimatic index (Ibcv), oenoclimatic aptitude index (IAOe), and Huglin heliothermal index (IH). These indices allowed the characterization of the climatic potential of each growing season and facilitated the evaluation of the relationships between climatic variability and grape quality indicators.

Biological material. Grape sampling was performed at technological maturity, determined based on the stabilization of sugar accumulation and the balance between sugars and acidity in the must. For each replicate, approximately 3-5 kg of grape clusters were randomly collected from different parts of the canopy, ensuring representative sampling. Immediately after harvest, the grape samples were transported to the laboratory, where they were subjected to physicochemical and biochemical analyses to determine the main quality parameters. In parallel, part of the harvested grapes was used for micro-vinification, carried out under controlled conditions, in order to obtain wines representative of each study year.

To evaluate the quality of the raw material, several chemical composition parameters were determined in the grape samples. The following indicators were analyzed: sugar content (g/L must), protein content (g/100 g), phosphorus (mg/100 g), copper (mg/100 g), potassium (mg/100 g), magnesium (mg/100 g), iron (mg/100 g), calcium (mg/100 g), sodium (mg/100 g), vitamin C (total ascorbic acid, mg/100 g), vitamin B6 (mg/100 g), vitamin E (α -tocopherol, mg/100 g) and tannin content (g/100 g).

Physicochemical and biochemical analyses.

The sugar content of the grape must was determined using a refractometric method, expressed as grams of sugar per liter of must (g/L). The analyses were performed on freshly pressed grape juice obtained from representative berry samples. Measurements were carried out using a digital refractometer (Atago PAL-1, Atago Co., Ltd., Tokyo, Japan), calibrated with distilled water before each series of determinations.

The total protein content was determined using the Kjeldahl method. The digestion and distillation processes were carried out using a Kjeldahl digestion and distillation system (Kjeltec™ 8400 Analyzer, Foss Analytical, Denmark). The mineral composition of the samples, including phosphorus (P), copper (Cu), potassium (K), magnesium (Mg), iron (Fe), calcium (Ca), and sodium (Na), was determined after wet mineralization of the samples using a mixture of nitric and perchloric acids. The concentrations of the elements were measured using atomic absorption spectrometry (AAS) with a PerkinElmer AAnalyst 400 spectrometer (PerkinElmer Inc., USA). Phosphorus was determined spectrophotometrically using a UV-Vis spectrophotometer (Shimadzu UV-1800, Kyoto, Japan). The vitamin C (ascorbic acid) content was determined by spectrophotometric titration based on its reducing capacity using 2,6-dichlorophenolindophenol, with measurements performed using a UV-Vis spectrophotometer (Shimadzu UV-1800). The concentrations of vitamin B6 (pyridoxine) and vitamin E (α -tocopherol) were determined using high-performance liquid chromatography (HPLC). The analyses were performed using an HPLC system (Agilent 1260 Infinity II, Agilent Technologies, USA) equipped with a UV detector and appropriate chromatographic columns for vitamin separation. The tannin content was determined using the Folin-Ciocalteu spectrophotometric method, which allows the quantification of total phenolic compounds present in grape berries. Absorbance measurements were performed using a UV-Vis spectrophotometer (Shimadzu UV-1800), and the results were expressed as grams per 100 g of fresh sample (g/100 g). All analyses were performed in triplicate to ensure the accuracy and reproducibility of the experimental data.

Statistical analysis. The experimental data obtained for the climatic indices and grape quality indicators were statistically processed to evaluate the relationships between climatic conditions, grape, and wine composition. Descriptive statistics (mean and standard deviation) were calculated for all variables. Differences between the studied years were assessed using analysis of variance (ANOVA) at a significance level of $p \leq 0.05$. To explore the relationships between bioclimatic parameters and grape quality indicators, Principal Component Analysis (PCA) was applied. This multivariate method allowed the reduction of data dimensionality and the identification of the main factors explaining the variability between the studied years. All statistical analyses and graphical representations were performed using IBM SPSS Statistics (Version 26.0, IBM Corp., USA) and XLSTAT 2023 (Addinsoft, France).

RESULTS AND DISCUSSIONS

The comparative analysis of the climatic indicators (Table 1) shows that the 2022-2024 growing seasons were characterized by warmer and sunnier conditions than the multi-year average, while the precipitation remained within limits close to the vine’s ecological optimum. These climatic conditions favored vegetative growth, the physiological ripening of grapes, and the accumulation of quality compounds, indicating high viticultural potential in the studied area and favorable conditions for producing high-quality wines. The hydrothermal coefficient (CH) averaged 1.13, suggesting a relative balance between thermal conditions and total rainfall, while the real heliothermal index (Ihr) indicates a high potential for solar radiation in combination with available thermal resources.

Table 1. Values of the main climatic indicators in the Miniş-Măderat vineyard (2022-2024)

Parameter	2022	2023	2024	Mean 2022-2024	Mean 1971-2020
Average air temperature (°C)	13.40	14.37	14.57	14.11	11.70
Average soil temperature (°C)	13.90	14.48	14.70	14.36	12.90
Precipitation (mm)	560.30	693.85	631.10	628.41	646.40
Hygrosopicity (%)	73.00	68.23	69.23	70.15	78.80
Sunshine duration (hours)	2130.0	2258.6	2905.0	2431.2	1982.8
Σ toa* (total active temperatures, °C)	4423.4	3896.8	4605.6	4308.6	3949.3
Σ tou* (useful active temperatures, °C)	2152.4	1994.9	2435.6	2194.3	1750.0
Hydrothermal coefficient (CH)*	1.30	1.10	1.00	1.13	1.10
Real heliothermal index (Ihr)*	2.60	2.80	3.70	3.03	2.20
Heliothermal index (IH)*	2507.4	2523.5	2937.2	2656.03	5753.1

*Σ toa = sum of active temperatures (all temperatures above 10 °C); Σ tou = sum of useful temperatures (temperatures for vine development, typically above 10 °C but adjusted for phenology); CH, Ihr, IH are standard viticultural climatic indices.

The data summarized in Table 2 illustrate the dynamics of the main biochemical and nutritional parameters of the analyzed grapes during the 2022-2024 period, offering a comprehensive view of how composition responds to year-to-year climatic variation. The primary quality indicators, including sugars, acids, phenolic content, and antioxidant activity, showed a high degree of stability, with only moderate fluctuations between vintages. This relative consistency aligns with findings from other multi-year investigations, which also report vintage-dependent variability in grape composition driven by climatic factors such as temperature, precipitation, and growing degree days. For example, long-term studies have

demonstrated that climatic conditions during key phenological stages can significantly influence sugar accumulation, acidity decline, and phenolic profiles, with warmer and drier years often associated with increased sugar content and altered secondary metabolite levels in grapes and wine (van Leeuwen et al., 2016; Bai et al., 2025). Other multi-year field experiments have similarly highlighted the effects of annual climate variability on phenolic composition and technological maturity across different grape cultivars, reinforcing the notion that environmental conditions - though variable - often exert a predictable influence on biochemical traits (Romero et al., 2016).

Table 2. Grape quality parameters (Mini's vineyard during 2022–2024 growing seasons)

Parameter	Year			Mean
	2022 / ($\pm$ SD)	2023 / ($\pm$ SD)	2024 / ($\pm$ SD)	
Sugar content (g/L)	206.00 $\pm$ 9.50	225 $\pm$ 9.50	218 $\pm$ 9.50	216.33
Protein content (g/100 g)	5.621 $\pm$ 0.039	5.698 $\pm$ 0.039	5.675 $\pm$ 0.039	5.66
Phosphorus (mg/100 g)	91.175 $\pm$ 0.076	91.326 $\pm$ 0.076	91.238 $\pm$ 0.076	91.246
Copper (mg/100 g)	0.604 $\pm$ 0.021	0.645 $\pm$ 0.021	0.629 $\pm$ 0.021	0.626
Potassium (mg/100 g)	270.87 $\pm$ 0.44	271.72 $\pm$ 0.44	271.53 $\pm$ 0.44	271.37
Magnesium (mg/100 g)	29.87 $\pm$ 0.22	30.31 $\pm$ 0.22	30.26 $\pm$ 0.22	30.14
Iron (mg/100 g)	2.59 $\pm$ 0.08	2.74 $\pm$ 0.08	2.68 $\pm$ 0.08	2.67
Calcium (mg/100 g)	362.17 $\pm$ 1.66	365.43 $\pm$ 1.66	364.11 $\pm$ 1.66	363.90
Sodium (mg/100 g)	9.31 $\pm$ 0.015	9.34 $\pm$ 0.015	9.33 $\pm$ 0.015	9.32
Vitamin C, total ascorbic acid (mg/100 g)	11.511 $\pm$ 0.005	11.521 $\pm$ 0.005	11.519 $\pm$ 0.005	11.517
Vitamin B6 (mg/100 g)	0.439 $\pm$ 0.018	0.473 $\pm$ 0.018	0.467 $\pm$ 0.018	0.459
Vitamin E (alpha-tocopherol) (mg/100 g)	2.218 $\pm$ 0.18	2.265 $\pm$ 0.18	2.549 $\pm$ 0.18	2.344
Tannins (g/100 g)	0.48 $\pm$ 0.22	0.91 $\pm$ 0.22	0.79 $\pm$ 0.22	0.72

Regarding sugar content, an increase was observed in 2023, likely reflecting favorable climatic conditions for photosynthesis and carbohydrate accumulation in the berries. Such vintage effects on sugar concentration have been reported previously, linking warmer, sunnier seasons to accelerated technological maturation (van Leeuwen et al., 2016; Rácz et al., 2026).

Protein content showed limited variability, suggesting a stable nitrogen status in the grapes, consistent with studies indicating that vine nitrogen uptake often buffers protein synthesis against inter-annual climatic variation (Camps & Ramos, 2012). Mineral composition remained largely constant. Magnesium ranged from 29.87–30.31 mg/100 g, while phosphorus showed high stability. Iron and copper exhibited moderate fluctuations, likely influenced by soil composition and element bioavailability (Pradubsuk & Davenport, 2011). Bioactive compounds showed mostly stable levels: vitamin C remained constant, supporting antioxidant potential, while vitamin B6 varied slightly. Notably, α -tocopherol (vitamin E) increased in 2024, reflecting ecological influences on antioxidant metabolism (Şeker et al., 2012). Tannin content varied among years, reflecting the effect of climate on phenolic biosynthesis and maturation, in agreement with previous observations on vintage effects in grapes (Echave et al., 2025).

The Principal Component Analysis (PCA) biplot illustrates the relationships between the analyzed biochemical parameters and climatic variables during 2022–2024 vintages. The first principal component (F1) explains 74.79% of the total variance, while the second component

(F2) explains 25.21%, indicating that the two axes together capture the overall variability of the dataset and provide a reliable representation of the multivariate relationships among variables. The F1 axis is mainly associated with variables related to grape biochemical composition, including sugars, tannins, vitamin C, vitamin B6, and protein content, which show positive loadings. The grouping of variables on the positive side of the axis indicates a strong positive correlation among them. In contrast, CH located on the negative side of F1, suggesting a relationship with the biochemical variables associated with grape maturation.

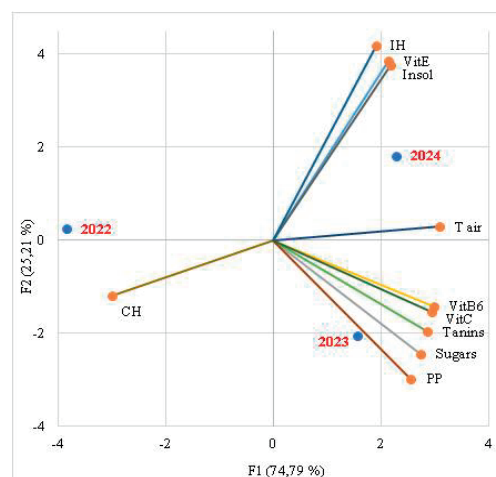


Figure 1. Principal Component Analysis (PCA) biplot showing the relationships between grape berry quality parameters and climate variables during the 2022–2024 vintages

Similar correlations between sugar accumulation and phenolic compounds during grape ripening have been reported in other grape quality studies (Ramos et al., 2024; Rouxinol et al., 2023). The F2 axis is mainly influenced by variables such as vitamin E, insoluble components, and the heliothermal index (IH). Their proximity indicates a positive association, suggesting that increased thermal and solar conditions may favor the synthesis of antioxidant compounds in grapes. Previous research has shown that environmental factors such as temperature, sunlight, and water availability strongly influence the biosynthesis of antioxidants and phenolic compounds in grape berries (van Leeuwen & Darriet, 2016; Kok et al., 2025; Crupi et al., 2023). Samples from the 2023 growing season are closely associated with higher levels of sugars, tannins, vitamin C, vitamin B6, and proteins, suggesting favorable climatic conditions for grape maturation and the accumulation of primary and secondary metabolites. Similar relationships between climatic conditions and the accumulation of sugars and phenolic compounds in grapes have been reported in previous studies (Rouxinol et al., 2023; Cataldo et al., 2023). In contrast, samples from the 2024 growing season show stronger associations with vitamin E, insoluble components, and the heliothermal index, indicating that environmental conditions during that year may have favored the biosynthesis and accumulation of antioxidant compounds. Temperature and solar radiation are known to influence the production of antioxidant molecules in grape berries (van Leeuwen et al., 2024; Ramos et al., 2024). The 2022 growing season, however, appears less strongly associated with the main biochemical variables, suggesting comparatively lower concentrations of these compounds. Such inter-annual variability is commonly attributed to differences in climatic conditions during berry development and ripening, which significantly influence grape composition and metabolic processes (Shahab et al., 2023).

The evolution of the main biochemical and nutritional parameters during the 2022-2024 vintages (Table 3) indicates a generally stable composition of the wine, with moderate variations associated with the specific

conditions of each growing season. The low variability of protein content suggests a relatively stable nitrogen fraction, which is important for the colloidal and physicochemical stability of wine. The slight increase observed in 2023 may be related to fermentation conditions and the composition of the initial must. Nitrogen compounds are known to influence fermentation dynamics and wine stability (Christofi et al., 2022).

Macroelements showed a relatively uniform distribution during the analyzed period. Potassium remained stable, confirming its key role in the ionic balance of wine and in tartaric stabilization processes (Han et al., 2023). Calcium and phosphorus also presented relatively constant values, contributing significantly to the mineral composition of the wine, while sodium remained at low levels typical of quality wines. Moderate variations in iron and copper may be associated with both the characteristics of the raw material and the influence of technological equipment during the winemaking process (Wang et al., 2023).

The analysis indicates relatively stable levels of vitamin C, suggesting a consistent antioxidant potential of the wine across years. Phenolic compounds, particularly tannins, play a key role in determining the antioxidant capacity and sensory characteristics of wine. The observed variation in tannin content reflects the influence of grape ripening conditions and phenolic extraction during fermentation (Baris et al., 2026).

Vitamin E (α -tocopherol) showed relatively similar values (4.478-4.543 mg/100 g), with an average of 4.533 mg/100 g. This antioxidant contributes to oxidative protection and may support wine stability during maturation and storage (Akbaba et al., 2023). Overall, the results indicate a high degree of compositional stability during the 2022-2024 vintages, with only minor inter-annual variations. These findings suggest consistent quality of both the grapes and the winemaking process, resulting in a wine with a balanced biochemical profile and stable quality parameters. The comparison between grapes and wine also reflects the biochemical transformations occurring during fermentation and maturation, which contribute to the development of the mineral, antioxidant, and phenolic profile of the wine.

Table 3. Wine quality parameters (Minis vineyard during 2022-2024 growing seasons)

Parameter	2022/ ($\pm$ SD)	2023/ ($\pm$ SD)	2024 /($\pm$ SD)	Mean 2022-2024 /($\pm$ SD)
Protein (g/100 g)	4.707 $\pm$ 0.036	4.775 $\pm$ 0.036	4.762 $\pm$ 0.036	4.748 $\pm$ 0.036
Phosphorus (mg/100 g)	97.813 $\pm$ 0.025	97.863 $\pm$ 0.025	97.852 $\pm$ 0.025	97.842 $\pm$ 0.025
Copper (mg/100 g)	0.938 $\pm$ 0.025	0.987 $\pm$ 0.025	0.976 $\pm$ 0.025	0.967 $\pm$ 0.025
Potassium (mg/100 g)	381.56 $\pm$ 0.13	381.87 $\pm$ 0.13	381.75 $\pm$ 0.13	381.72 $\pm$ 0.13
Magnesium (mg/100 g)	47.76 $\pm$ 0.60	48.92 $\pm$ 0.60	48.81 $\pm$ 0.60	48.49 $\pm$ 0.60
Iron (mg/100 g)	3.89 $\pm$ 0.035	3.96 $\pm$ 0.035	3.93 $\pm$ 0.035	3.92 $\pm$ 0.035
Calcium (mg/100 g)	396.78 $\pm$ 0.88	398.51 $\pm$ 0.88	398.11 $\pm$ 0.88	397.80 $\pm$ 0.88
Sodium (mg/100 g)	11.69 $\pm$ 0.03	11.75 $\pm$ 0.03	11.73 $\pm$ 0.03	11.72 $\pm$ 0.03
Vitamin C (mg/100 g)	14.475 $\pm$ 0.10	14.683 $\pm$ 0.10	14.612 $\pm$ 0.10	14.59 $\pm$ 0.10
Vitamin B6 (mg/100 g)	0.649 $\pm$ 0.011	0.671 $\pm$ 0.011	0.665 $\pm$ 0.011	0.661 $\pm$ 0.011
Vitamin E (α -tocopherol, mg/100 g)	4.478 $\pm$ 0.033	4.543 $\pm$ 0.033	4.508 $\pm$ 0.033	4.533 $\pm$ 0.033
Tannins (g/100 g)	3.59 $\pm$ 0.18	3.95 $\pm$ 0.18	3.81 $\pm$ 0.18	3.78 $\pm$ 0.18

The principal component analysis (PCA) diagram illustrates the relationships between wine quality components and climatic bioindicators for the 2022-2024 vintages. The first two principal components explain 99.94% of the total variance, with PC1 accounting for 74.83% and PC2 for 25.11%. Positive PC1 values are associated with vitamin B6, vitamin C, tannins, proteins, and vitamin E, indicating that these variables strongly contribute to the compositional profile of wines produced under favorable ripening conditions. Similar relationships between phenolic compounds, vitamins, and grape maturity have been reported in previous studies, highlighting the influence of climatic conditions on the accumulation of antioxidant metabolites in wines (Varo et al., 2022; Cojocaru & Antoce, 2017).

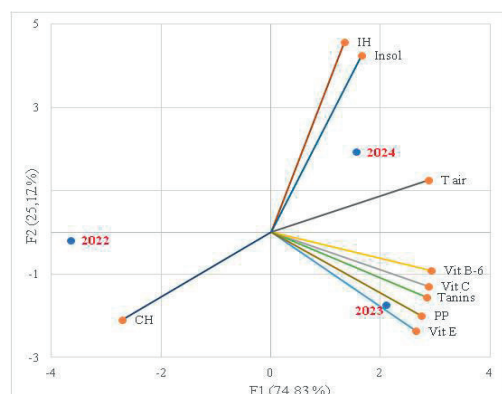


Figure 2. Principal Component Analysis (PCA) biplot showing the relationships between wine quality parameters and climate variables during the 2022-2024 vintages

The 2023 vintage shows strong correlations with higher concentrations of sugars, tannins, vitamin

C, vitamin B6, proteins, and vitamin E, suggesting that the climatic conditions favored the biosynthesis and accumulation of phenolic and antioxidant compounds that contribute to wine quality and oxidative stability (Tudorache et al., 2014). The 2024 vintage is associated with vitamin E, insoluble components, air temperature (T_{air}), and the heliothermal index (IH), indicating that higher temperatures and heliothermal conditions may have stimulated antioxidant compound production, particularly tocopherols. Environmental factors such as temperature and solar radiation are known to influence grape metabolism and antioxidant synthesis (Cataldo et al., 2023; Eder et al., 2023). In contrast, the 2022 vintage shows weaker correlations with most biochemical variables and is positioned closer to the CH climatic indicator, suggesting less favorable conditions for the accumulation of bioactive compounds. Inter-annual climatic variability is widely recognized as a major factor influencing grape composition and wine quality (Fotescu et al., 2024).

Table 4. Main economic indicators for the Fetească Neagră cultivar (EUR: 2022-2024)

Indicator	2022	2023	2024	Average
Production costs (€/ha)	1933	1987	2071	1997
Unit cost (€/kg grapes)	0.28	0.23	0.22	0.24
Winemaking cost (€/L wine)	0.39	0.39	0.42	0.40
Production value (€/ha)	2719	3432	3741	3297
Gross profit (€/ha)	787	1445	1671	1301

The analysis of production costs (Table 4) indicates a gradual increase, ranging from €1,933 ha⁻¹ in 2022 to €2,071 ha⁻¹ in 2024, with a multiannual average of €1,997 ha⁻¹. This trend can be attributed to the rising costs of

agricultural inputs (materials, treatments, energy, and tillage), as well as adjustments in vineyard management practices required to adapt to the climatic conditions of each growing season (Calugar et al., 2021). A relevant indicator for assessing economic efficiency is the unit cost of grapes, which decreased from €0.28 kg⁻¹ in 2022 to €0.22 kg⁻¹ in 2024, with an average of €0.24 kg⁻¹. This reduction suggests improved production efficiency, likely due to higher yields and more efficient use of technological resources. Favorable climatic conditions during the 2023 and 2024 growing seasons may have contributed to better grape development and ripening, thus improving the economic return of the vineyard (van Leeuwen et al., 2024). The winemaking cost remained relatively stable, with values between €0.39 L⁻¹ and €0.42 L⁻¹. The slight increase observed in 2024 may be related to higher energy and processing costs associated with the transformation of grapes into wine. Nevertheless, the limited variation of this parameter indicates a relatively stable winemaking technology (Marchuk et al., 2025). A key indicator of economic performance is the production value per hectare, which increased from €2,719 ha⁻¹ in 2022 to €3,741 ha⁻¹ in 2024, with an average of €3,297 ha⁻¹. This trend reflects both higher grape yields and improvements in raw material quality, which can increase the market value of the resulting wine. Consequently, gross profit increased from €787 ha⁻¹ in 2022 to €1,671 ha⁻¹ in 2024, confirming the improved economic efficiency of the vineyard. These results indicate that the Fetească Neagră variety has significant economic potential under the analyzed pedoclimatic conditions.

CONCLUSIONS

This study provides an integrated evaluation of grape composition, wine quality, and economic performance of the Fetească Neagră variety cultivated in the Miniș Vineyard over the 2022-2024 vintages. The results demonstrated a generally stable biochemical profile of grapes, with moderate inter-annual variations mainly associated with climatic conditions during the growing season. Higher concentrations of sugars, tannins, and antioxidant compounds

observed in the 2023 vintage indicate favorable ripening conditions, while the 2024 vintage was more strongly associated with the accumulation of vitamin E and climatic bioindicators related to temperature and the heliothermal index. The wine quality parameters showed limited variability across the studied vintages, suggesting consistent technological processing and stable raw material characteristics. The presence of phenolic compounds and antioxidant vitamins contributed to oxidative stability and the overall compositional balance of the wines. Multivariate statistical analysis (PCA) highlighted clear relationships between climatic bioindicators and wine biochemical composition, confirming the influence of annual climatic variability on metabolite accumulation and wine quality. From an economic perspective, although production costs increased slightly, the unit cost of grapes decreased, while production value and gross profit per hectare increased, indicating production efficiency and favorable economic performance. The results confirm that the Miniș vineyard provides suitable pedoclimatic conditions for the cultivation of Fetească Neagră, enabling the production of wines with stable compositional characteristics and good economic profitability. These findings highlight the importance of adapting vineyard management and winemaking practices to climatic variability in order to sustain both product quality and economic efficiency in viticultural systems.

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PRODUCTIVITY ELEMENTS AND QUALITY INDICES IN SOME RED WINES GRAPE VARIETIES - COMPARATIVE STUDY

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Abstract

The study aimed to evaluate the productivity parameters and quality indices of three grape varieties for red wines. The study was conducted in the 2023-2024 wine year, in the area of the Recea Wine Center. Three grape varieties for red wines were considered in the study: "Fetească Neagră" (Gv1), "Pinot Noir" (Gv2) and "Cabernet Sauvignon" (Gv3). The vegetation diagram recorded 125 days for Gv1, 146 days for Gv2, and 145 days for Gv3 from the start of vegetation to the ripening of the grapes. Grapevine varieties showed differentiated values for productivity parameters and productivity indices of productivity, yield, and quality. Close values were recorded for total vine shoots (Tvs), but variant Gv2 was noted for fertile vine shoots (Fvs), and trunk inflorescence number (Tin). The Gv1 variant stood out for average grape weight (Agw), and the Gv3 variant stood out for yield (Y/plt). Regarding quality indices, the Gv3 variant presented higher values for total soluble solids (TSS), and anthocyanin content (Ant). The results are important for the management of vineyard plantations in relation to the yield and quality indices considered.

Key words: correlation, productivity elements, quality indices, red wine cultivars, vineyard plantation management.

INTRODUCTION

The long history of grapevine cultivation is supported by consistent databases, with textual, archaeobotanical, archaeological, iconographic, and morphometric information, traditions and traditional practices, etc. (Limier et al., 2018). The grapevine is considered a plant species, with an essential role in the long history of humankind (Vignani & Scali, 2024).

The high plasticity of the grapevine was evaluated and quantified in relation to eco-anatomical elements specific to wood (e.g. age, maturity), specific to leaves, fruits, seeds, environmental conditions, etc. (Limier et al., 2018). Morphological and genetic traits of the vine have undergone certain changes as a result of the "domestication" process, genotype selection and improvement programs, and technological practices in vine cultivation (Wen et al., 2018; Grassi & Arroyo-Garcia, 2020).

Associated with the effects of climate warming, changes in conditions for vines have been recorded in areas dedicated to the crop; the expansion of cultivated areas has been recorded; and new areas suitable for vines have been presented (Bănuță et al., 2024; van Leeuwen et al., 2024; Corbeels et al., 2025; Lisek &

Popińska, 2025). In the context of climate change, the selection of varieties adapted to the specifics of the growing area was considered important (Baltazar et al., 2025). To improve adaptive mechanisms of the grapevine, different future scenarios based on genetic modifications were considered (Vignani & Scali, 2024).

Although the grapevine has high natural biodiversity, some studies have drawn attention to "cultivated biodiversity", the "concentration" of the number of varieties, and the possibility of "diversity decline" in the grapevine (Boursiquot et al., 2025). For the purpose of objectively studying biodiversity and trends of change identified in grape germplasm, certain "adapted indices" have been introduced to ensure neutrality and objectivity in specific studies and investigations (Boursiquot et al., 2025).

Interactions of the vine with the soil and climate, as a "terroir" ecosystem, have been studied in terms of vegetative growth, the formation of berries in grape clusters, and the qualitative compositional suitability of red grapes for quality wines (van Leeuwen et al., 2018). Climate change and the "soil - climate" interaction have been quantified in vegetative growth and in viticultural performance in

quantitative and qualitative terms (Vanden Heuvel & Centinari, 2021).

High variability was recorded in grapevines in shoots and small berries in relation to ripening, compared with developed shoots and larger berries (Wisdom & Considine, 2022). Although it has high adaptability, the grapevine responds to environmental factors, stressors, and technological factors through variations in yield and production quality (Tănase et al., 2022; Gayretli et al., 2024; Catania et al., 2025).

The influence of some abiotic and biotic stress factors was analyzed in relation to the negative effects they presented on yield values and some quality indices in the vine under specific technological conditions (Lu et al., 2023).

Factors, but especially combinations of soil and climatic factors, have influenced the yield of white wine varieties differently than red or rosé wine varieties, and the authors concluded the need to develop research to quantify the interactions of environmental factors with an impact on the formation of grapevine yield (Fernandez-Mena et al., 2023).

The morphological specificity of the vine and the response to influencing factors are important for optimizing the balance between vegetative growth, yield, and quality of grape production (Karn et al., 2024). Different adaptation and response mechanisms have been reported in the grapevine, in relation to climate change, the succession of vegetation stages, yield, and quality indices of wine production (Costea et al., 2023; Bonfante et al., 2025). The development of floral organs, inflorescence formation, vegetative initiation, and yield aspects in grapevines has been studied in relation to the vegetative dormancy period (Li et al., 2025).

Through cultivation technology, the expression of "terroir" can be corrected in relation to the purpose of the final wine production (van Leeuwen et al., 2018). Through comparative analysis, the impact of different types of organic and mineral fertilizers on the soil (physical and chemical parameters) and on vine yield of the vine was evaluated (Wu et al., 2024). Treatments with organic and mineral fertilizers, biofertilizers, and biostimulators generated differentiated effects in the soil and plants in two grapevine varieties cultivated in acidic soil conditions with low humus content (Lisek &

Popińska, 2025). Technological practices (e.g. post-harvest practices), such as stimulating pruning and foliar treatments, were promoted in order to favor the differentiation of secondary floral buds (Li et al., 2025). Soil maintenance systems through mechanical work and covering with a vegetal carpet (vegetable carpet by sowing) have been studied in relation to the root system of the vine and the sustainability of vineyards (Lenari et al., 2025).

The impact of climate change has been recorded in the yield and quality of wine production, and recent studies have communicated "adaptive measures", which target biological material and elements of technology for the adoption of medium and long-term strategies to compensate for climatic constraints (Villalobos-Soublett et al., 2022).

The yield potential of some red wine grape varieties, in different soil conditions and semi-arid areas, was evaluated in relation to water regime and irrigation methods (Ma et al., 2023). To ensure the sustainability of vineyards, "yield gaps" in vines have been studied and analyzed in relation to soil and climatic factors of influence (Fernandez-Mena et al., 2023). Under conditions of moderate, experimentally induced water stress, an increase in the content of accumulated soluble sugar and the amount of nitrogen (metabolized by yeasts) in grape berries on the vine was recorded (Ma et al., 2023).

Appropriate models and selection methods were used to objectively identify specific zonal indicators with effects on grapevine yield gaps (Fernandez-Mena et al., 2023). Relief elements and specific soil indicators (e.g. fertility indicators) were identified through multiple regression analysis as "significant predictors" for morphological parameters, productivity, and quality elements in the vineyard (Karn et al., 2024).

This study aimed to compare three red grape varieties, "Fetească Neagră", "Pinot Noir" and "Cabernet Sauvignon", cultivated in the Recaș wine-growing area, based on elements of productivity, yield and grape quality indices.

MATERIALS AND METHODS

The study was conducted in the area of the Recaș Wine Center, Timis County, Romania, during

the 2023-2024 wine years. The geographical position, through the specific relief (altitudes between 100-300 m, predominantly southern exposure, SE-SW), the soil conditions (zonal and azonals soils - luvisol class) and the climate (hydrothermal coefficient 1.15, heliothermal index 2.37, bioclimatic index 7.18, and oenoclimatic aptitude index 4.665), gives the area a viticultural favorability recognized nationally and internationally. In the wine year 2023- 2024, the average precipitation was 69.5 mm in 2023, and 54.2 mm in 2024. Active precipitation totaled 835 mm in 2023 and 650

mm in 2024. Mechanical works, applied periodically, ensured the maintenance of the vineyard. The bud load on the vine was consistent with each variety and the cultivation technology in the area. Three grape varieties were considered in the study: “Fetească Neagră”, “Pinot Noir” and “Cabernet Sauvignon” (Figure 1). The study was conducted according to the flow chart (Figure 1). For reasons of graphic representation, the presented abbreviations were considered in the article.

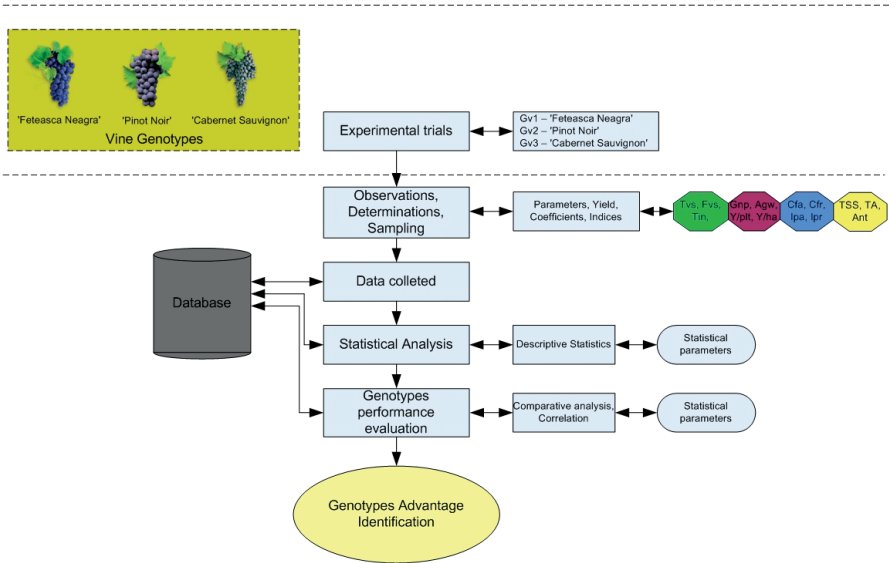


Figure 1. Flow chart

The three genotypes were evaluated in terms of: productivity elements (Tvs – total vine shoots; Fvs – fertile vine shoots; Tin – trunk inflorescence number; Agw – average grape weight), yield (Y/plt – yield per plant; Y/ha – yield per unit area); performance coefficients and indices (Cfa – Coefficient of absolute fertility, Cfr – Coefficient of relative fertility, Ipa – Index of productivity absolute, and index of productivity relative – Ipr), quality indices (TSS – total soluble solids, TA – total acidity, Ant – anthocyanins). The determination of some parameters was done by numerical counting (e.g. Tvs, Fvs, Tin), by weighing with a laboratory balance (e.g. Agw),

by calculation (e.g. Y/ha; Cfa, Cfr, Ipa, Ipr). The quality indices were determined by laboratory methods, respectively the refractometric method (TSS, °Brix), the titrimetric method (TA, g tartaric acid/L), UV-VIS spectrophotometry (Anthocyanins, mg Ant/L, calculated as malvidin-3-glucoside). The experimental data were analyzed to comparatively evaluate the three grape varieties for each parameter considered. Correlation analysis was used to evaluate the interdependence between the parameters considered. The calculation module in EXCEL, the PAST software v.4.17. (Hammer et al., 2001), and the JASP software v 0.16.2 (2022)

were used for data analysis and graphical representations.

RESULTS AND DISCUSSIONS

Under the study conditions, the three grapevine genotypes presented differentiated values for the evaluated parameters.

In the case of elements associated with grapevine shoots, Tvs recorded values between 12.40 ± 0.31 (Gv3) and 13.40 ± 0.31 (Gv1), Fvs recorded values between 10.20 ± 0.48 (Gv3) and 11.80 ± 0.48 (Gv2), and Tin recorded values between 12.20 ± 1.92 (Gv2) and 18.80 ± 1.92 (Gv3) (Table 1).

Table 1. The productivity elements of the grapevine varieties analyzed

Grape variety	Tvs	Fvs		Tin
	(No)	(No)	(%)	(No)
Gv1	13.40	10.60	79.10	12.20
Gv2	12.60	11.80	93.65	18.80
Gv3	12.40	10.20	82.26	16.20
SE	± 0.31	± 0.48	± 4.42	± 1.92

Comparative analysis of Tvs values showed a positive difference of the Gv1 genotype compared to the experiment average ($\overline{Tvs} = 12.80$) in conditions of statistical safety ($p < 0.01$, **), and differences without statistical safety ($p > 0.05$) in the case of Gv2 and Gv3 (Table 2).

Table 2. Comparative analysis results for the Tvs parameter

Statistical parameters	Gv1	Gv2	Gv3
Given mean	13.40	12.60	12.40
Sample mean	12.80		
95% conf. interval	(12.392 13.208)	(12.392 13.208)	(12.392 13.208)
Difference	0.60	0.20	0.40
95% conf. interval	(0.19176 1.0082)	(-0.20824 0.60824)	(-0.0082409 0.80824)
t	-3.3891	1.1297	2.2594
p (same mean)	0.0095	0.2913	0.0538
Significance	**	Ns	ns

In the case of the Fvs parameter, the average of the experiment was at the level = 10.87. In relation to the calculated average value, the Gv2 genotype presented a positive difference ($p < 0.01$, **), and the Gv3 genotype presented a

negative difference ($p < 0.05$, o). In the case of the Gv1 genotype, the difference did not present statistical safety (Table 3). The graphic representation illustrates the level of values achieved by genotypes and the averages calculated at the experiment level (Figure 2).

Table 3. Results of the comparative analysis for the Fvs parameter

Statistical parameters	Gv1	Gv2	Gv3
Given mean	10.60	11.80	10.20
Sample mean	10.87		
95% conf. interval	(10.227 11.506)	(10.227 11.506)	(10.227 11.506)
Difference	0.27	0.93	0.67
95% conf. interval	(-0.37312 0.90646)	(0.29354 1.5731)	(0.026876 1.3065)
t	0.9611	-3.3640	2.4028
p (same mean)	0.3646	0.0099	0.0430
Significance	ns	**	o

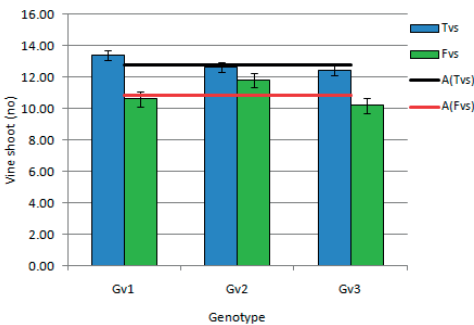


Figure 2. Graphical representation of Tvs and Fvs parameters by genotypes and experimental average
Note: Tvs – Total vine shoot; Fvs – fertile vine shoot; A(Tvs) – average of Tvs; A(Fvs) – average of Fvs

In the case of the parameter Tin (total inflorescence number), the average value calculated at the experiment level was $\overline{Tin} = 15.73$ (Table 4, Figure 3). The Gv2 genotype presented a value higher than the average ($p < 0.05$, *), and the Gv1 genotype presented a value lower than the average ($p < 0.05$, o). In the case of the Gv3 genotype, the difference did not present statistical certainty ($p > 0.05$).

The increase in the number of shoots was associated with an increase in grapevine yield but negatively influenced the diameter of the berries, and for the quality of the bunches, control of the bunches on the plant was recommended (Somkuwar et al., 2012). The

association of shoot characteristics (e.g. development, size) and grape berries with ripening suggested differentiated management of the vine through technological work, in relation to plant vigour (Wisdom & Considine, 2022).

Table 4. Comparative analysis results for the Tin parameter

Statistical parameters	Gv1	Gv2	Gv3
Given mean	12.20	18.80	16.20
Sample mean	15.73		
95% conf. interval	(13.178 18.289)	(13.178 18.289)	(13.178 18.289)
Difference	3.53	3.07	0.47
95% conf. interval	(0.97763 6.089)	(0.51097 5.6224)	(-2.089 3.0224)
t	3.1881	-2.7670	-0.4211
p (same mean)	0.0128	0.0244	0.6848
Significance	o	*	ns

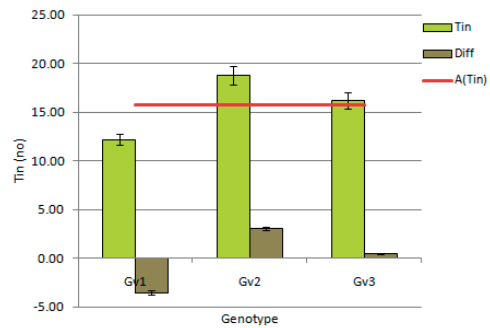


Figure 3. Graphical representation of the Tin parameter by genotypes and experimental average

The number of nodes, the number of shoots,, and other productivity elements in the vine showed variations in relation to the pruning system and influenced the ripening of the berries, the yield,, and the quality indices of the grapes (Epee et al., 2024).

Productivity elements (Gnp; Agw), and yield (Y/plt; Y/ha) were determined for the three grapevine genotypes (Table 5). Grape number on plant (Gnp) recorded values ranging between 14.56±1.34 (Gv1) and 15.44±1.34 (Gv2). Average grape weight (Awg) presented values ranging between 0.124±0.004 kg (Gv2) and 0.139±0.004 kg (Gv1). Yield per plant (Y/plt) recorded values ranging between 1.91±0.17 kg/plt (Gv2) and 2.46±0.17 kg/plt (Gv3).

Similarly, Y/ha recorded a low value in the case of Gv2, 8.70±0.76 t/ha, and a high value in the case of Gv3, 11.20±0.76 t/ha (Table 5).

Table 5. Elements of productivity and yield in grapevine varieties

Grape variety	Gnp	Agw	Y/plt	Y/ha
	(No)	(kg)	(kg/plt)	(t/ha)
Gv1	14.56	0.139	2.02	9.20
Gv2	15.44	0.124	1.91	8.70
Gv3	18.96	0.130	2.46	11.20
SE	±1.34	±0.004	±0.17	±0.76

The comparative analysis showed the level of differences and statistical significance for the productivity elements.

In the case of Gnp (grape number per plant) the average value calculated at the experiment level was $\bar{Gnp} = 16.32$ (Table 6, Figure 4). The Gv3 genotype recorded a value higher than the average, with a statistically significant difference ($p < 0.01^{**}$), while the other two genotypes presented differences without statistical significance in relation to the average value (Table 6).

Table 6. Results of the comparative analysis for the Gnp parameter

Statistical parameters	Gv1	Gv2	Gv3
Given mean	14.56	15.44	18.96
Sample mean	16.32		
95% conf. interval	(14.532 18.108)	(14.532 18.108)	(14.532 18.108)
Difference	1.76	0.88	2.64
95% conf. interval	(-0.028237 3.5482)	(-0.90824 2.6682)	(0.85176 4.4282)
t	2.2696	1.1348	-3.4044
p (same mean)	0.0529	0.2893	0.0093
Significance	ns	ns	**

In the case of the Agw parameter, the average value calculated at the experiment level was $\bar{Agw} = 0.131$ kg (Table 7, Figure 4). Compared to the average value, the Gv1 genotype recorded a higher value, with a statistically significant difference ($p < 0.05$, *), and the Gv2 genotype presented a lower value ($p < 0.05$, o).

In the case of yield per plant (Y/plt) the average calculated value was = 2.13 kg/plt (Table 8), and in the case of yield per unit area (Y/ha) the

average calculated value was $\overline{Y/ha} = 9.70 \text{ t/ha}$ (Table 9).

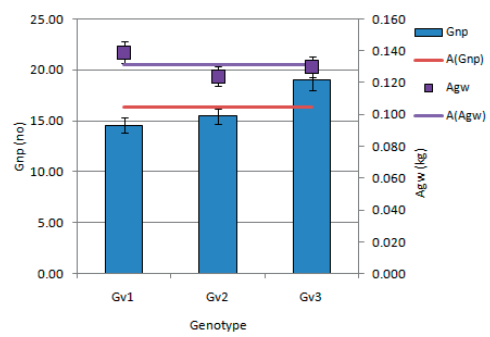


Figure 4. Representation of Gnp and Agw parameters by genotypes and experimental average

Table 7. Comparative analysis results for the Agw parameter

Statistical parameters	Gv1	Gv2	Gv3
Given mean	0.139	0.124	0.130
Sample mean	0.131		
95% conf. interval	(0.12531 0.13669)	(0.12531 0.13669)	(0.12531 0.13669)
Difference	0.008	0.007	0.001
95% conf. interval	(0.0023124 0.013688)	(0.0013124 0.012688)	(-0.0046876 0.0066876)
t	-3.2435	2.8381	0.4054
p (same mean)	0.0118	0.0219	0.6958
Significance	*	o	ns

Table 8. Comparative analysis results for the Y/plt parameter

Statistical parameters	Gv1	Gv2	Gv3
Given mean	2.02	1.91	2.46
Sample mean	2.13		
95% conf. interval	(1.9056 2.3544)	(1.9056 2.3544)	(1.9056 2.3544)
Difference	0.11	0.22	0.33
95% conf. interval	(-0.11436 0.33436)	(-0.0043644 0.44436)	(0.10564 0.55436)
t	1.1306	2.2611	-3.3917
p (same mean)	0.2910	0.0536	0.0095
Significance	ns	ns	**

Compared to the average calculated values, the Gv3 genotype presented values above the average (Figure 5), under conditions of statistical safety ($p < 0.01$, **), and in the case of the other two genotypes the differences did not present statistical safety ($p > 0.05$).

Table 9. Results of the comparative analysis for the parameter Y/ha

Statistical parameters	Gv1	Gv2	Gv3
Given mean	9.20	8.70	11.20
Sample mean	9.70		
95% conf. interval	(8.6844 10.716)	(8.6844 10.716)	(8.6844 10.716)
Difference	0.50	1.00	1.50
95% conf. interval	(-0.51559 1.5156)	(-0.015591 2.0156)	(0.48441 2.5156)
t	1.1353	2.2706	-3.4059
p (same mean)	0.2891	0.0528	0.0093
Significance	ns	ns	**

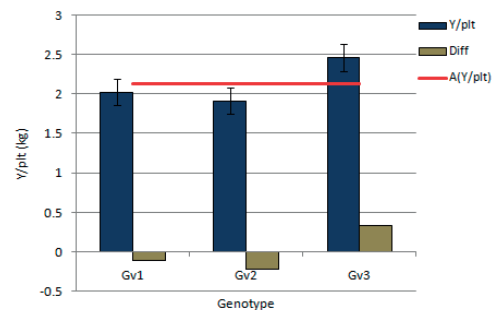


Figure 5. Graphical representation of the Y/plt parameter by genotypes and experimental average

Coefficient of absolute fertility (Cfa), coefficient of relative fertility (Cfr), index of absolute productivity (Ipa), and index of relative productivity (Ipr) were calculated for the three grapevine genotypes (Table 10). The Cfa coefficient recorded values between 1.34 (Gv1) and 1.82 (Gv2). The Cfr coefficient recorded values between 1.00 (Gv1) and 1.64 (Gv2). The Ipa index recorded values between 186.30 (Gv1) and 232.70 (Gv3), and the Ipr index recorded values between 139.00 (Gv1) and 203.40 (Gv2).

Table 10. Values of productivity coefficients and indices

Grape variety	Cfa	Cfr	Ipa	Ipr
Gv1	1.34	1.00	186.30	139.00
Gv2	1.82	1.64	225.70	203.40
Gv3	1.79	1.48	232.70	192.40
SE	±0.16	±0.19	±14.44	±19.89

Quality indices, total soluble solids (TSS), total acidity (TA), and anthocyanins (Ant) were determined on must samples from the experimental variants (Table 11). The TSS index

recorded values between 218.00 ± 2.96 °Brix (Gv2) and 228.00 ± 2.96 °Brix (Gv3). The TA index recorded values between 5.10 ± 0.12 g/L (Gv3) and 5.50 ± 0.12 g/L (Gv2). The Ant index recorded values between 0.34 ± 0.02 mg/L (Gv2) and 0.42 ± 0.02 mg/L (Gv3).

Table 11. Quality indices of the studied grape varieties

Grape variety	TSS	TA	Ant
	(°Brix)	(g tarttric acid/L)	mg/L
Gv1	225.00	5.30	0.39
Gv2	218.00	5.50	0.34
Gv3	228.00	5.10	0.42
SE	± 2.96	± 0.12	± 0.02

In the case of the TSS index, compared to the calculated mean ($TSS = 223.67$), the positive difference ($p < 0.05$, *) generated the Gv3 genotype, and the negative difference ($p < 0.05$, o) was recorded for the Gv2 genotype (Table 12, Figure 6).

Table 12. Comparative analysis results for TSS parameter

Statistical parameters	Gv1	Gv2	Gv3
Given mean	225.00	218.00	228.00
Sample mean	223.67		
95% conf. interval	(219.72 227.61)	(219.72 227.61)	(219.72 227.61)
Difference	1.33	5.67	4.33
95% conf. interval	(-2.6102 5.2769)	(1.7231 9.6102)	(0.38979 8.2769)
t	-0.7797	3.3136	-2.5339
p (same mean)	0.4580	0.0106	0.0350
Significance	Ns	o	*

The content of total soluble solids (TSS) is important for the quality of red wines, and some studies have reported a threshold of 22 °Bx in relation to the variation of mannoprotein content, and its influence on the composition and colour modulation of red wines (Galaz-Torres et al., 2025).

In the case of the TA index, compared to the calculated average ($TA = 5.30$), the positive difference ($p < 0.05$, *) generated the Gv2 genotype, and the negative difference ($p < 0.05$, o) was recorded in the Gv3 genotype (Table 13, Figure 6).

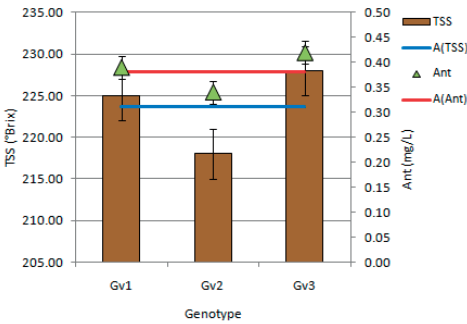


Figure 6. Graphical representation of TSS and Ant parameters by genotypes and experimental average

Table 13. Results of the comparative analysis for the TA parameter

Statistical parameters	Gv1	Gv2	Gv3
Given mean	5.30	5.50	5.10
Sample mean	5.30		
95% conf. interval	(5.1447 5.4553)	(5.1447 5.4553)	(5.1447 5.4553)
Difference	0.00	0.20	0.20
95% conf. interval	(-0.15526 0.15526)	(0.044738 0.35526)	(0.044738 0.35526)
t	0.0000	-2.9704	2.9704
p (same mean)	1.0000	0.0179	0.0179
Significance	ns	*	o

In the case of anthocyanin content (Ant), compared to the calculated average ($Ant = 0.38$ hg/L), the positive difference ($p < 0.05$, *) generated the Gv3 genotype, and the negative difference ($p < 0.05$, o) was recorded in the Gv2 genotype (Table 14, Figure 6).

Table 14. Comparative analysis results for the Ant parameter

Statistical parameters	Gv1	Gv2	Gv3
Given mean	0.39	0.34	0.42
Sample mean	0.38		
95% conf. interval	(0.35332 0.41335)	(0.35332 0.41335)	(0.35332 0.41335)
Difference	0.01	0.04	0.04
95% conf. interval	(-0.02335 0.036684)	(0.013316 0.07335)	(0.0066495 0.066684)
t	-0.5122	3.3290	-2.8168
p (same mean)	0.6224	0.0104	0.0226
Significance	Ns	o	*

"Terroir" parameters, in the form of the content of mineral elements (especially nitrogen), climatic parameters (soil temperature and

humidity, climatic regime), presented specific influence in relation to the yield, the quality of grapes, and the quality of wine products (van Leeuwen et al., 2018). Grape berry size has been found to be a good indicator for estimating grape ripeness, as well as for certain quality indices, such as anthocyanins, phenolic compounds, and berry sugar (Wisdom & Considine, 2022). Different values of grape quality traits for the "Cabernet Sauvignon" variety were reported in

relation to the growing regions, and the main quality indices associated with the region were anthocyanins, titratable acids, and total phenols (Ren et al., 2023). The correlation between shoot parameters, productivity elements, yield, productivity coefficients and indices, and quality indices was evaluated based on the values recorded for the three grapevine varieties (Figure 7).

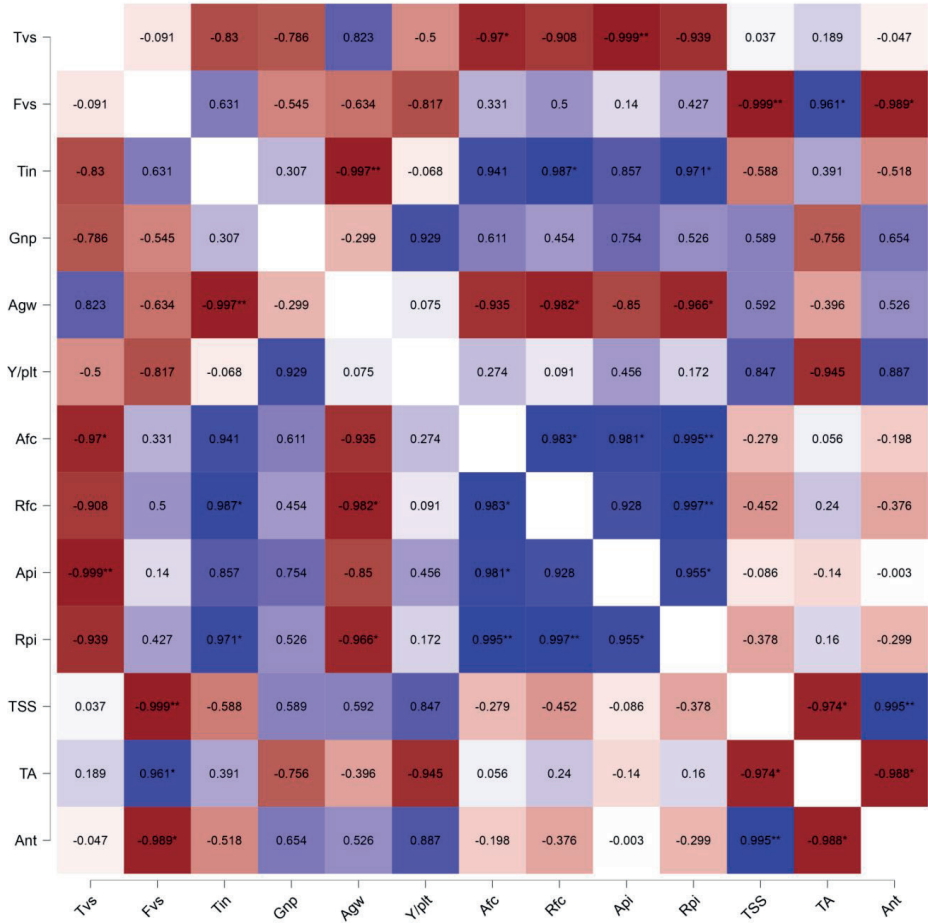


Figure 7. Correlation matrix between parameters determined in the vineyard

Positive and negative correlations of different intensity levels were recorded between the parameters considered. Certain values of the correlation coefficient are of interest, with high implication in the cultivation practice. The parameter Agw showed a positive correlation with Tvs ($r = 0.823$) and a negative correlation

with Tin ($r = -0.997^{**}$). The yield (Y/plt) showed a positive correlation, of very strong intensity, with Gnp ($r = 0.929$). The TSS quality index showed a negative correlation with Fvs ($r = -0.999^{**}$), with TA ($r = -0.974^{*}$) and with Tin ($r = -0.588$) and a positive correlation with Gnp ($r = 0.589$), with Agw ($r =$

0.592), with Y/plt ($r = 0.847$) and with the Ant quality index ($r = 0.995^{**}$).

Total acidity (TA) showed a negative correlation with Gnp ($r = -0.756$), with Y/plt ($r = -0.945$), with TSS ($r = -0.974^{*}$) and with Ant ($r = -0.988^{*}$). Positive correlation was recorded between TA and Fvs ($r = 0.961^{*}$). The Ant quality index showed a negative correlation with Fvs ($r = -0.989^{*}$), with Tin ($r = -0.518$), and with TA ($r = -0.988^{*}$).

Based on the results recorded in the determined parameter values, and the differences calculated for each genotype, in relation to the average values, a performance diagram of the grapevine genotypes was generated (Figure 8).

	Gv1	Gv2	Gv3
Tvs	**	ns	ns
Fvs	ns	**	o
Tin	o	*	ns
Gnp	ns	ns	**
Agw	*	o	ns
Y/plt	ns	ns	**
TSS	ns	o	*
TA	ns	*	o
Ant	ns	o	*

Figure 8. Performance diagram of grapevine genotypes in relation to the parameters considered
 Notes: green colour - significant positive differences; yellow colour - significant negative differences; gray - insignificant differences

Information associated with the type of correlation and the level of intensity is important for maintenance and fruiting pruning in the vine. A negative correlation ($R = 0.704$) was reported between shoot number and shoot length in grapevines, with effects on leaf area and berry quality indices (Kliwer & Dokoozlian, 2005). Negative relationships were reported between bud number (controlled by pruning), shoot number (controlled by maintenance work) and leaf area in grapevines, and between the change in the ratio of fruit weight to leaf area (Myers et al., 2008). A strong negative correlation ($r = -0.70$; 0.84) was reported during the study period

between TSS and TA in grapevines (Williams et al., 2023).

In relation to the parameters Tvs, Fvs, and Tin, Gv1 and Gv2 showed advantages. In relation to the parameters Gnp and Y/plt, the genotype Gv3 showed an advantage, and in relation to Agw, the genotype Gv1 showed an advantage. In relation to Tss and Ant, the genotype Gv3 showed an advantage, and in relation to TA, the genotype Gv2 showed an advantage.

CONCLUSIONS

The grapevine varieties studied generated differentiated values for the productivity, yield, and quality indices considered in the study.

The Gv1 genotype was highlighted in the case of the Tvs parameter, with a positive difference from the experiment average ($p < 0.01$, **). The Gv2 genotype was highlighted in the case of the Fvs parameter, with a positive difference from the experiment average ($p < 0.01$, **) and in the case of the Tin parameter, with a positive difference from the experiment average ($p < 0.05$, *).

In the case of the Gnp parameter, the Gv3 genotype was highlighted, with a positive difference from the experiment average ($p < 0.01$, **). The Gv1 genotype was highlighted in the case of the Agw parameter, with a positive difference from the experiment average ($p < 0.05$, *). In the case of the Y/plt yield, respectively Y/ha, the Gv3 genotype was highlighted, with a positive difference from the experiment average ($p < 0.01$, **).

In the case of quality indices, the Gv3 genotype was highlighted in the case of TSS, with a difference above the average value of the experiment ($p < 0.05$, *) and in the case of Ant, with a difference above the average value of the experiment ($p < 0.05$, *). The Gv2 genotype was highlighted in the case of the TA index, with a difference above the average of the experiment ($p < 0.05$, *).

A variable level of correlation was recorded between the parameters and indices determined for the three grapevine varieties. It is worth considering the correlation of the Gnp, Agw, and Y/plt parameters with the values of the shoot parameters (Tvs, Fvs, Tin) and the quality indices (TSS, TA, Ant) with the other parameters of the grapevine, the genotypes

studied, in order to optimize the cultivation technology and ensure performance at the level of yield and quality indices.

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INFLUENCE OF ORGANIC FERTILIZATION WITH VITIVINICULTURAL BY-PRODUCTS ON VINE GROWTH AND FRUIT BEARING IN THE CODRU WINE-GROWING REGION

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Abstract

This study investigates the influence of organic fertilization using vitivinicultural by-products on vine growth and fruit bearing in the Codru wine-growing region. The research was conducted in the locality of Cricova, Republic of Moldova, using the wine grape variety Pinot Noir. The experimental design included three treatment variants: an unfertilized control, application of dried grape seeds, and organic fertilization with compost obtained from grape pomace mixed with cattle manure at a 1:1 ratio. Throughout the vegetation period, vine growth and fruiting parameters, overall plant condition, and cluster development were systematically monitored in relation to soil characteristics and agroclimatic conditions. The results revealed noticeable differences among the tested variants, indicating that the use of vitivinicultural by-products as organic fertilizers may support vine growth dynamics without negatively affecting vine productivity. The study highlights the potential of valorizing these by-products in sustainable viticulture and supports the implementation of circular economy principles within wine-growing systems.

Key words: fruit bearing, organic fertilization, Pinot Noir, sustainable viticulture, vitivinicultural by-products.

INTRODUCTION

Global wine production generates millions of tons of winery by-products annually, highlighting a significant waste management challenge (OIV, 2023). Within this context, the European Green Deal promotes resource efficiency and waste reduction across agricultural systems (European Commission, 2019). Vitivinicultural by-products, particularly grape pomace, are increasingly recognized as valuable resources within circular economy models (Beres et al., 2017; Provenzano et al., 2024). Composting has been identified as an effective approach for recycling these residues and improving soil fertility (Carmona et al., 2022). Grape pomace is rich in organic carbon, nutrients, and bioactive compounds such as phenolics, while winery residues also contain structural components including cellulose and hemicelluloses (Buchmann et al., 2025; Rockenbach et al., 2011; Spigno et al., 2008). Grape pomace is increasingly recognized as a sustainable source of bioactive compounds with potential applications in agriculture and other value-added sectors (Karastergiou et al., 2025). Its application enhances soil organic matter,

microbial activity, and nutrient availability (Teixeira et al., 2014; Galantini et al., 2021; Bhatta et al., 2021). Consequently, organic fertilization supports sustainable viticulture and may improve vine growth and productivity (Pascual et al., 2022; Riva et al., 2023; Ferrara et al., 2020; Santos et al., 2020), with similar approaches also reported under local vineyard conditions (Visanu, 2022).

MATERIALS AND METHODS

Study area and experimental design

The study was carried out during the 2025 vegetation period in the Codru wine-growing region of the Republic of Moldova.

The experimental vineyard was planted with Pinot Noir under local production conditions. The site was located at an altitude of approximately 160-195 m (Figure 1), with a slope of 3-6° and north-east/east exposure. The soil was classified as typical clay-loam chernozem, with pH 8.4, 38-40% physical clay, mobile phosphorus 2.5 mg 100 g⁻¹ soil, and mobile potassium 15.4 mg 100 g⁻¹ soil. The planting scheme was 2.5 × 1.2 m (about 3333 vines ha⁻¹).

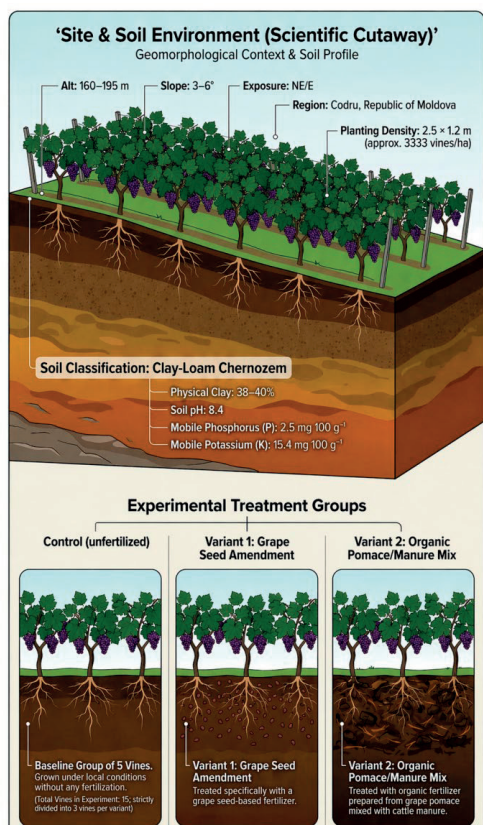


Figure 1. Experimental design and site characteristics of the Pinot Noir vineyard, including soil profile and treatment structure in the Codru wine-growing region (Republic of Moldova)



Figure 2. Application of organic fertilizer derived from grape pomace at a depth of 25-30 cm in the experimental vineyard

The experiment included 75 vines, distributed into three treatments with 25 vines per variant: Control (unfertilized) - without fertilization; Variant 1 - fertilization with grape seed-based fertilizer;

Variant 2 - fertilization with organic fertilizer prepared from grape pomace mixed with cattle manure.

Agroclimatic conditions

The agroclimatic conditions during the 2025 vegetation period were characteristic of the temperate continental climate of the Codru wine-growing region. Meteorological data were obtained from the vineyard weather monitoring system used for disease-climate monitoring.

Air temperature gradually increased from early spring, with mean values rising from approximately 10-12°C in April to peak summer temperatures exceeding 25-30°C during July and August. From September onward, temperatures progressively declined, reaching approximately 8-12°C by the end of October.

Relative air humidity showed notable fluctuations throughout the vegetation period, frequently exceeding 70-80%, particularly during late spring and early summer. Several precipitation events occurred mainly between May and September, contributing to moderate soil moisture conditions during the main stages of vine development.

Soil temperature followed a seasonal pattern similar to that of air temperature, increasing from approximately 8°C in early spring to about 24-26°C during midsummer before gradually decreasing in autumn. These climatic conditions provided a suitable thermal regime for vine vegetative growth and reproductive development during the study period.

Preparation of organic fertilizer

The organic fertilizer used in Variant 2 was prepared from grape pomace obtained from Mirești Mici winery, originating from the cultivars Rara Neagră, Fetească Neagră, and Merlot. A 100 g reference sample showed the following composition: 65.43 g skins, 23.3 g seeds, and 8.82 g stems.

Before composting, preliminary tests were carried out to evaluate the extractability of soluble and phenolic compounds. Distilled water was used to observe the release of water-soluble components and to adjust the material moisture. A second test was performed using Histanol 100 (an ethanol-based solution), which produced a dark red extract, indicating a stronger dissolution of phenolic compounds.

The material was also heated to approximately 68°C to facilitate the release of active components.

After this preliminary stage, the pomace was mixed with cattle manure at an approximate 1:1 ratio and composted under natural outdoor conditions during the early vegetation period. During this period, the heap was manually turned about four times, and water was added twice. Due to the relatively short composting period, the resulting material was considered a partially stabilized organic fertilizer.

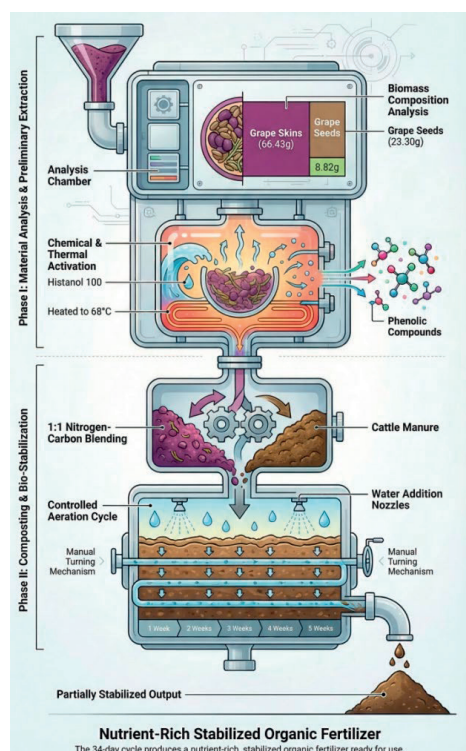


Figure 3. Processing pathway for the transformation of grape pomace into organic fertilizer, including preliminary extraction, thermal activation, and composting stages

Fertilizer application and measured indicators

The treatments were applied manually during the vegetation period at an approximate depth of 25-30 cm (Figure 2), then covered with soil. The application dose was about 3-4 kg vine⁻¹, corresponding to approximately 10-13 t ha⁻¹. To evaluate the effect of the treatments, a set of vegetative and reproductive parameters was recorded during the vegetation period. These

included structural indicators related to vine load (number of spurs, number of canes, number of buds per spur, and number of buds per cane), vegetative development indicators (number of buds that started growth and number of shoots from dormant buds), and reproductive indicators (number of fertile shoots and inflorescences from winter buds and dormant buds).

At harvest, grape yield was also recorded and expressed as kg vine⁻¹. The mean yield values were 1.71 kg vine⁻¹ for the control, 1.59 kg vine⁻¹ for Variant 1, and 1.73 kg vine⁻¹ for Variant 2.

The processing steps of grape pomace transformation into organic fertilizer are illustrated in Figure 3.

Statistical analysis

The data were processed using descriptive statistics and one-way ANOVA. For each indicator, the mean, standard deviation, F value, and p value were calculated. Differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSIONS

The vegetative and reproductive indicators of Pinot Noir under different fertilization treatments are presented in Table 1, while fertility coefficients, fertility indices, and yield data are summarized in Table 2 and illustrated in Figure 4.

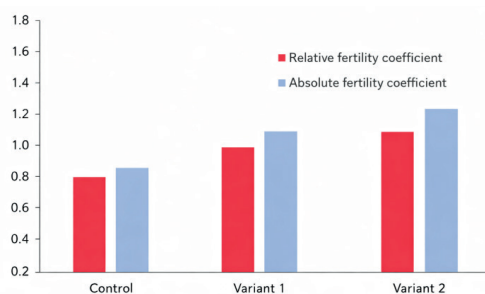


Figure 4. Relative and absolute fertility coefficients of Pinot Noir under different fertilization treatments in the Codru wine-growing region

In general, the structural parameters of the vines, including the number of spurs, number of canes, number of buds per spur, and number of buds per cane, showed only moderate variation among treatments. No statistically significant

differences were observed ($p > 0.05$), indicating that the tested fertilization strategies did not disturb the vine structural balance during the first year after application.

A similar pattern was recorded for the number of buds that started growth and the number of shoots from dormant buds, which remained relatively stable across treatments.

Slight differences were observed in the reproductive indicators among treatments. The number of fertile shoots from winter buds increased from 11.60 in the control to 16.00 in Variant 1 and 18.25 in Variant 2. The number of inflorescences on shoots from winter buds followed the same tendency, rising from 14.20 in the control to 23.60 in Variant 1 and 30.25 in Variant 2. Although these differences were not

statistically significant ($p > 0.05$), Variant 2 consistently showed the highest reproductive values. A similar tendency was observed for the indicators associated with dormant buds, where Variant 2 recorded higher values for both fertile shoots and inflorescences. The fertility coefficients and indices further supported this tendency. As shown in Figure 4, the relative fertility coefficient increased from 0.798 in the control to 1.372 in Variant 1 and 1.476 in Variant 2, while the absolute fertility coefficient increased from 1.224 to 1.475 and 1.658, respectively. The fertilized treatments generally showed higher fertility indices compared with the control, indicating a favorable tendency in reproductive potential.

Table 1. Effect of fertilization treatments on vegetative and fruit-bearing indicators of Pinot Noir in the Codru wine-growing region

Indicator	Control (Mean $\pm$ SD)	Variant 1 (Mean $\pm$ SD)	Variant 2 (Mean $\pm$ SD)	F value	P value
Number of spurs:	4.40 $\pm$ 2.07	3.80 $\pm$ 1.30	4.00 $\pm$ 1.63	0.175	0.842
Number of canes:	4.00 $\pm$ 1.22	2.60 $\pm$ 0.55	3.50 $\pm$ 1.29	2.476	0.126
Number of buds per spur:	8.80 $\pm$ 4.97	8.80 $\pm$ 3.90	9.00 $\pm$ 4.24	0.004	0.996
Number of buds per cane:	19.40 $\pm$ 5.03	13.60 $\pm$ 3.36	18.25 $\pm$ 7.93	1.690	0.226
Number of buds that started growth:	17.80 $\pm$ 6.76	17.20 $\pm$ 6.87	20.50 $\pm$ 9.40	0.294	0.750
Number of fertile shoots from winter buds:	11.60 $\pm$ 10.41	16.00 $\pm$ 7.11	18.25 $\pm$ 7.54	0.857	0.449
Number of inflorescences on shoots from winter buds:	14.20 $\pm$ 11.43	23.60 $\pm$ 12.50	30.25 $\pm$ 15.92	2.058	0.170
Number of shoots from dormant buds:	6.40 $\pm$ 3.05	6.20 $\pm$ 2.28	6.50 $\pm$ 3.51	0.015	0.985
Number of fertile shoots from dormant buds:	3.20 $\pm$ 3.27	3.40 $\pm$ 2.07	6.25 $\pm$ 6.40	0.964	0.409
Number of inflorescences on fertile shoots from dormant buds:	4.20 $\pm$ 4.38	3.80 $\pm$ 3.49	4.50 $\pm$ 3.42	0.046	0.955

Overall, the fertilized treatments demonstrated a more favorable reproductive tendency than the control, particularly the treatment based on composted grape pomace mixed with cattle manure.

Yield data supported this interpretation. The mean yield per vine was 1.71 kg in the control, 1.59 kg in Variant 1, and 1.73 kg in Variant 2. Thus, the highest productivity was recorded in Variant 2, which corresponded with the highest fertility coefficients and indices. Although yield differences were relatively small, the pomace-based treatment showed a slightly improved fruit-bearing potential compared with the control.

Table 2. Fertility coefficients, fertility indices, and yield of Pinot Noir under different fertilization treatments

Parameter	Control	Variant 1	Variant 2
Relative fertility coefficient	0.80	1.37	1.48
Absolute fertility coefficient	1.22	1.48	1.66
Relative fertility index	97.26	155.00	152.12
Absolute fertility index	149.24	166.63	170.88
Mean yield (kg vine ⁻¹)	1.71	1.59	1.73

These results may be related to the expected effects of organic amendments on soil fertility. Grapevines annually remove significant quantities of nutrients from the soil through biomass production and fruit development from the soil, including nitrogen, phosphorus, and potassium (Visanu, 2022). Previous studies have shown that grape pomace-based materials can improve soil organic matter, stimulate microbial activity, and enhance the availability of essential nutrients, which may indirectly support vine vigor and reproductive performance. In the present study, the pomace-based fertilizer did not negatively affect vine growth and showed the most favorable tendency in reproductive indicators and yield.

However, the compost applied in this experiment was only partially stabilized, as it was produced under natural outdoor conditions and composted for 34 days. Therefore, strong short-term responses were not necessarily expected. In addition, although the number of

vines per treatment was limited, the experiment still provided useful preliminary observations on the effect of organic fertilization, which may have limited the statistical power of the analysis. Nevertheless, the absence of negative effects and the favorable tendency observed in Variant 2 suggest that grape pomace mixed with cattle manure can represent a feasible organic fertilization strategy for vineyards in the Codru wine-growing region.

CONCLUSIONS

This study evaluated the influence of organic fertilization using vitivinicultural by-products on the vegetative growth and fruit-bearing performance of Pinot Noir cultivated in the Codru wine-growing region of the Republic of Moldova.

The results showed that the applied fertilization treatments did not significantly affect the structural vegetative parameters of the vines during the first year after application. Indicators such as the number of spurs, canes, and buds per spur and cane remained relatively stable across treatments, suggesting that the applied organic fertilizers did not disrupt the vegetative balance of the vines.

However, some reproductive indicators showed a favorable tendency in the fertilized variants. In particular, the treatment based on composted grape pomace mixed with cattle manure (Variant 2) recorded higher values for the number of fertile shoots and inflorescences compared with the control treatment.

Yield data followed a similar trend, with Variant 2 presenting the highest mean yield per vine ($1.73 \text{ kg vine}^{-1}$). Although the differences among treatments were not statistically significant, the results indicate that the application of grape pomace-based organic fertilizer did not negatively affect vine productivity and may contribute to improved fruit-bearing potential.

Overall, the findings suggest that composted grape pomace mixed with cattle manure can represent a viable organic fertilization strategy for vineyards in the Codru wine-growing region. Further research conducted over multiple growing seasons and larger experimental plots is necessary to better understand the long-term

effects of these fertilization practices on soil fertility, vine physiology, and grape yield.

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INTEGRATED ASSESSMENT OF WATER STRESS IN TABLE GRAPE VINES: PHYSIOLOGICAL, BIOMETRIC AND SATELLITE INDICATORS AND THE IMPACT OF IRRIGATION ON YIELD AND HARVEST QUALITY

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Abstract

The integrated assessment of water stress in grapevines for table grapes, using physiological, biometric, and satellite indicators, highlighted the significant influence of irrigation and fertilization on yield and quality. Moderate irrigation (75% Active Humidity Index) combined with complex C4 fertilization proved most effective, enhancing both quantitative and qualitative production. The cultivar Țăța caprei neagră showed the highest performance, with the largest number of clusters per vine, the highest average cluster weight, and 100-berry mass, reaching yields of over 4,000 kg/vine under optimal conditions. Țăța caprei albă exhibited stable results, while Coarnă albă showed lower potential, requiring more careful management. Water stress analysis using CWSI and $\Delta T_c - T_a$ indicated high deficit tolerance in Țăța caprei neagră, moderate tolerance in Țăța caprei albă, and sensitivity in Coarnă albă. Selecting a well-adapted cultivar and applying optimal irrigation and fertilization strategies are essential for achieving stable, high-quality yields under variable climatic conditions.

Key words: cultivar selection, CWSI, fertilization, irrigation, water stress.

INTRODUCTION

Water is one of the most important factors for vine growth and grape quality. In recent years, its importance has become even more evident as climate change brings more frequent droughts and unpredictable weather conditions. Lack of water limits table grape varieties by reducing berry size, grape weight, and their appearance for marketing (Chaves et al., 2010).

Water deficit affects the physiological processes of the vine: gas exchange is reduced, transpiration is limited, and the temperature at the leaf level increases, making it necessary to evaluate the water status in the correct establishment of irrigation regimes. In practice, thermal indicators such as the Crop Water Stress Index (CWSI) or the difference between canopy temperature and air temperature ($\Delta T_c - T_a$) are frequently used; these provide a quick assessment of the stress levels (Idso et al., 1981; Jones, 2004), and can signal stress before symptoms are visible in the vineyard.

In addition to physiological measurements, biometric indicators of production, such as the number of grapes per vine, the average weight

of a grape, the mass of berries, and the weight of 100 berries, reflect how the vine has responded to the availability of water throughout the growing season.

Studies show that a moderate deficit can have positive effects by improving water use efficiency without negatively affecting the quality of the production. When water stress becomes severe, production decreases are evident and berry development is affected (Keller, 2015). Recent research confirms that careful irrigation management can support both yield and quality, even under drought conditions (El-Sayed et al., 2024; Strydom, 2025).

Satellite imagery, especially thermal and multispectral imagery, is used in water stress forecasting to analyze variations within vineyards, highlighting differences in vegetation vigor and water status (Zarco-Tejada et al., 2013). Integrating these data with field measurements leads to more accurate assessments and better informed irrigation decisions (Cohen et al., 2024).

Although the literature is rich in studies on wine varieties, information on table varieties grown in Romanian conditions is still limited.

Furthermore, approaches that simultaneously analyze irrigation, fertilization, and their effects on yield and quality are relatively few.

Starting from these aspects, the present work aims to assess water stress in several Romanian table grape varieties (Țâța caprei neagră, Țâța caprei albă și Coarnă albă), using a combined approach. Physiological measurements, production parameters, and vegetation indices obtained from satellite data are used to understand how water availability influences both quantity and quality. The aim is to propose practical strategies for obtaining stable and quality harvests, even under increasingly unpredictable climatic conditions.

MATERIALS AND METHODS

The experiment was conducted in 2025 at the ampelographic collection of the Institute for Research and Development for Viticulture and Winemaking Valea Călugărească (ICDVV Valea Călugărească). Three Romanian table grape varieties were included: Coarnă albă, Țâța caprei neagră and Țâța caprei albă (Figure 1).

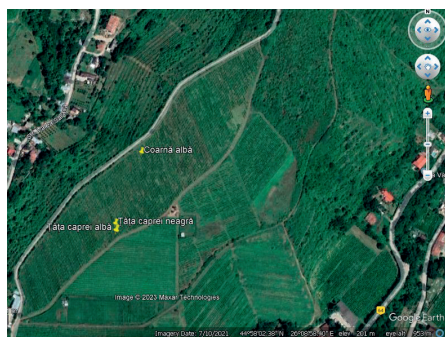


Figure 1. Location in the GIS system of the experimental plot

The study was designed as a three-factorial experiment to evaluate the effects of variety, irrigation level, and mineral fertilization on the physiological responses and productive performance of the vine.

Irrigation treatments were determined based on the active soil moisture index (IUA), representing the percentage of water available in the soil. Three irrigation regimes were applied: un-irrigated control, IUA 50% and IUA 75%.

Water was administered through a drip irrigation system, allowing precise regulation of the

applied quantities according to evapotranspiration requirements and soil moisture levels.

Fertilization treatments were applied at different phenological stages using the following products: NPK 15:15:15 at 200 kg/ha, Atonik at 5 L/ha by foliar application, Solfert 20:20:20 enriched with microelements at 10 kg/ha and Novatec Classic at 300 kg/ha.

Physiological measurements were carried out around noon, when water stress usually reaches its peak. Photosynthesis rate (A) and respiration (R) were measured using the portable LCi photosynthesis system ($\mu\text{mol CO}_2/\text{m}^2/\text{s}$). Simultaneously, transpiration rate (E) and stomatal conductance (gs) ($\text{mmol H}_2\text{O}/\text{m}^2/\text{s}$) were recorded. Leaf temperature (Tc) and air temperature (Ta) were also measured to calculate $\Delta T_c - T_a$ and the crop water stress index (CWSI), following the methods of Idso et al. (1981) and Jones (2004). Vegetation dynamics and canopy vigor were monitored at the vegetative phenophases of flowering (June 02), veraison (July 13 vs August 12) and ripening (September 10) using Sentinel-2 satellite images processed through the EO Browser platform.

The Normalized Difference Vegetation Index (NDVI) was calculated using the standard formula: $\text{NDVI} = (\text{NIR} - \text{RED}) / (\text{NIR} + \text{RED})$. At harvest, biometric parameters, grape yield per vine, and average grape weight were measured. Data were analyzed using three-way ANOVA to evaluate the effects of cultivar, irrigation, fertilization, and their interactions. Mean comparisons were performed using the Least Significant Difference (LSD) test at $p \leq 0.05$.

RESULTS AND DISCUSSIONS

The soil water regime during the growing season was influenced by rainfall, evapotranspiration and the application of differentiated irrigation norms. In April and May, soil water reserves were sufficient due to moderate rainfall (41-172 mm) and low water consumption (82-160 m^3/ha), which is why irrigation was not necessary. In contrast, during June-September, due to low rainfall (5-49 mm) and high evapotranspiration (166-220 m^3/ha), the application of differentiated irrigation was

essential to maintain the water balance. Two irrigation levels were used: 280 m³/ha at 50% IUA and 420 m³/ha at 75% IUA. Three irrigations were applied in July (420-630 m³/ha), four irrigations in August (560-840 m³/ha) and a single irrigation in September (140-210 m³/ha). Physiological processes: photosynthesis, respiration, transpiration, and stomatal conductance, represent functional indicators of the physiological status and photosynthetic efficiency of the grapevine, and provide information on the mechanisms of adaptation to water deficit. Monitoring of physiological processes was carried out in the flowering and fruiting phenophases. Statistical analysis (ANOVA) showed that both the variety and the irrigation norms, as well as their interaction, significantly influenced the values of physiological parameters ($p \leq 0.05$). Of the three varieties analyzed, the Țâța caprei neagră variety showed the highest values, with an average of 11.13 $\mu\text{mol CO}_2/\text{m}^2/\text{s}$, followed by the Țâța caprei albă variety with intermediate values of 10.17 $\mu\text{mol CO}_2/\text{m}^2/\text{s}$ and the Coarnă albă variety with the lowest photosynthesis rate, respectively (9.53 $\mu\text{mol CO}_2/\text{m}^2/\text{s}$), (Table 1).

Table 1. The influence of irrigation regime on photosynthesis ($\mu\text{mol CO}_2/\text{m}^2/\text{s}$)

AXB Interaction	FACTOR B – irrigation regime			Average Factor A
Factor A (Variety)	b1-un irrigated	b2- 50% IUA	b3- 75% IUA	
Coarnă albă	8.8	9.7	10.1	9.53c
Țâța caprei albă	9.4	10.3	10.8	10.2b
Țâța caprei neagră	10.3	11.3	11.8	11.1a
Average Factor B	9.5c	10.4b	10.9a	

Both the variety and the level of irrigation influence the intensity of photosynthesis; the best results were obtained with the Țâța caprei neagră variety at an irrigation level of 75% of the active soil moisture index.

In the case of respiration, the highest level was recorded for the Țâța caprei neagră variety (1.34 $\mu\text{mol CO}_2/\text{m}^2/\text{s}$), followed by the Țâța caprei albă variety (1.23 $\mu\text{mol CO}_2/\text{m}^2/\text{s}$), and the lowest level was recorded for the Coarnă albă variety (1.12 $\mu\text{mol CO}_2/\text{m}^2/\text{s}$). The respiration level increased with the irrigation rate applied; thus, the irrigation variant with 75% IUA determined the highest respiration value (1.34 $\mu\text{mol CO}_2/\text{m}^2/\text{s}$), followed by the variant with 50% IUA (1.23 $\mu\text{mol CO}_2/\text{m}^2/\text{s}$), and the lowest

value was recorded in the case of the unirrigated vineyard plot (1.12 $\mu\text{mol CO}_2/\text{m}^2/\text{s}$), (Table 2).

Table 2. The influence of irrigation regime on the respiration ($\mu\text{mol CO}_2/\text{m}^2/\text{s}$)

AXB Interaction	FACTOR B – irrigation regime			Average Factor A
Factor A (Variety)	b1 - un irrigated	b2 - 50% IUA	b3 - 75% IUA	
Coarnă albă	1.00	1.12	1.23	1.12c
Țâța caprei albă	1.12	1.23	1.34	1.23b
Țâța caprei neagră	1.23	1.34	1.45	1.34a
Average Factor B	1.12c	1.23b	1.34a	

Regarding the AXB interaction (Varieties×Irrigation), the highest response was recorded for the Țâța caprei neagră variety at an irrigation level of 75% IUA (1.45 $\mu\text{mol CO}_2/\text{m}^2/\text{s}$), while the lowest response was observed for the Coarnă albă variety under unirrigated conditions was observed in Coarnă albă (1.00 $\mu\text{mol CO}_2/\text{m}^2/\text{s}$), these results indicate that the variety has a significant influence on the respiration process. Among the three varieties analyzed, the Țâța caprei neagră variety stands out with the highest transpiration rates, with values ranging between 3.05 and 4.02 mmol H₂O/m²/s, depending on the irrigation level, followed by Țâța caprei albă variety with 3.25 mmol H₂O/m²/s and Coarnă albă variety with 3.05 mmol H₂O/m²/s. Analyzing the effect of the irrigation regime, a clear and consistent increase in transpiration can be observed with the intensification of water supply. The overall average transpiration increased from 2.77 mmol H₂O/m²/s in the unirrigated variant, to 3.29 mmol H₂O/m²/s in the variant with 50% IUA, reaching 3.79 mmol H₂O/m²/s under irrigation conditions at 75% IUA (Table 3).

Table 3. The influence of irrigation regime on transpiration (mmol H₂O/m²/s)

AXB Interaction	FACTOR B – irrigation regime			Average Factor A
Factor A (Variety)	b1 - un irrigated	b2 - 50% IUA	b3 - 75% IUA	
Coarnă albă	2.52	3.05	3.57	3.05c
Țâța caprei albă	2.73	3.25	3.78	3.25b
Țâța caprei neagră	3.05	3.57	4.02	3.57a
Average Factor B	2.77c	3.29b	3.79a	

This phenomenon can be explained by the wider opening of the stomata in the presence of sufficient water availability, which facilitates

gas exchange, including the release of water vapor, and contributes to cooling the plant. The Coarnă albă variety appears to be the most adaptable to water stress, making it a suitable choice for areas with limited water availability. The results obtained in the experiment clearly show a significant influence of both the variety (A factor) and the irrigation regime (B factor) on stomatal conductance. The Țâța caprei neagră variety presented average stomatal conductance values ranging between 32.02 and 36.20 mmol/m²/s, reflecting wide and efficient stomatal opening, followed by the Țâța caprei albă with intermediate values of (29.38–33.00 mmol/m²/s) and Coarnă albă with values between (25.96–29.20 mmol/m²/s). The irrigation regime significantly influenced the opening of the stomata. Stomatal conductance progressively increased from 29.12 mmol/m²/s in the non-irrigated variant to 30.87 mmol/m²/s in the variant irrigated with 50% IUA, reaching a maximum of 32.80 mmol/m²/s at 75% IUA, an evolution closely correlated with increased water availability (Table 4).

Table 4. Influence of experimental factors on stomatal conductance (mmol H₂O/m²/s)

Intercation AxB	FACTOR B - irrigation regime			Average Factor A
	b1 - un irrigated	b2 - 50% IUA	b3 - 75% IUA	
Coarnă albă	25.96	27.50	29.20	27.55c
Țâța caprei albă	29.38	31.10	33.00	31.16b
Țâța caprei neagră	32.02	34.00	36.20	34.07a
Average Factor B	29.12c	30.87b	32.80a	

The NDVI, NDWI, and Moisture Index (MI) values during the 2025 growing season highlight the evolution of vine water status and vine vigor of the vine depending on water availability. In

general, a high NDVI during the flowering phenophase (0.87) indicates relatively good photosynthetic activity, even though a moderate water deficit was recorded (Figure 3). The decrease in NDVI values to 0.78 during the veraison phenophase indicates a moderate decline in photosynthetic efficiency, and the decrease in NDWI (-0.69) and MI (-0.15), leads to stomatal closure to limit water loss due to the onset of water stress, which may affect shoot growth, berry size, and sugar accumulation in grapes (Figure 2).

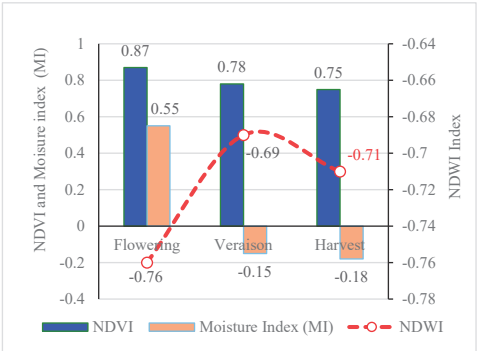


Figure 2. NDVI, NDWI, and Moisture Index (MI) values during the main vegetative phenophases

During the harvest phenophase, NDVI (0.75), NDWI (-0.71) and MI (-0.18) indicate a significant reduction in soil water reserves, with direct implications for grape production and its quality. Thus, the combined analysis of these indices NDVI, NDWI, and MI confirms the importance of efficient irrigation management to maintain vine vigor, hydration status, and productive potential of the vine.

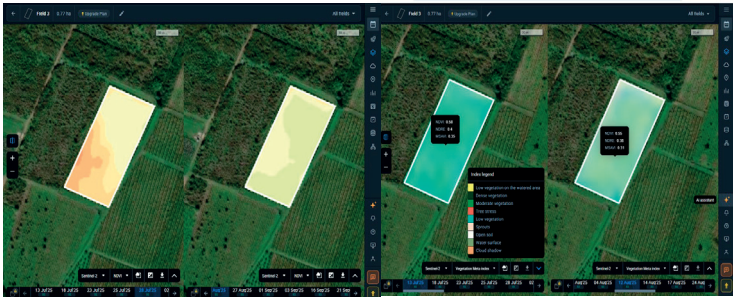


Figure 3. July-August NDVI index (left), July-August vegetation index (right)
*Note (image from July 13 vs August 12, periods with high temperatures)

The analysis of the Crop Water Stress Index (CWSI) and the leaf air temperature difference ($\Delta T_c - T_a$) highlights the different physiological responses of the three grapevine varieties to irrigation regimes during the flowering and veraison phenophases. In both phenophases, a consistent decrease in CWSI and $\Delta T_c - T_a$ values is observed as the irrigation level changes from un-irrigated to 50% and 75% of the active soil moisture index (IUA), indicating the reduction of water stress and the improvement of physiological conditions. During the flowering phenophase, the *Țâța caprei neagră* variety presented the lowest CWSI and $\Delta T_c - T_a$ values under all irrigation variants (0.55/+2.9 in the un-irrigated variant, 0.36/+1.7 in 50% IUA, and 0.19/+0.9 in 75% IUA), which reflects an efficient capacity for stomatal regulation and transpiration, even under water deficit conditions. The *Țâța caprei albă* variety showed an intermediate behavior, with moderate values (0.61/+3.2 in the un-irrigated variant and +0.22/+1.0 at 50% of IUA). The *Coarnă albă* variety recorded the highest values across all irrigation regimes, indicating a higher sensitivity to drought (Table 5).

Table 5. CWSI and $\Delta T_c - T_a$ of grapevine varieties under irrigation regimes

Varieties/Irrigation regimes	CWSI/ $\Delta T_c - T_a$ Flowering	CWSI/ $\Delta T_c - T_a$ Veraison
Coarnă albă control	0.64a/+3.4a	0.85a/+4.7a
Coarnă albă 50% IUA	0.44b/+2.1b	0.52b/+2.6b
Coarnă albă 75% IUA	0.24c/+1.1c	0.30c/+1.4c
Țâța caprei albă control	0.61a/+3.2a	0.88a/+5.0a
Țâța caprei albă 50% IUA	0.41b/+2.0b	0.57b/+2.8b
Țâța caprei albă 75% IUA	0.22c/+1.0c	0.33c/+1.5c
Țâța caprei neagră control	0.55a/+2.9a	0.76a/+4.3a
Țâța caprei neagră 50% IUA	0.36b/+1.7b	0.46b/+2.3b
Țâța caprei neagră 75% IUA	0.19c/+0.9c	0.27c/+1.1c

Note: Different letters (a, b, c) indicate statistically significant differences between irrigation regimes ($p < 0.05$, Tukey HSD).

During the veraison phenophase, when evapotranspiration is more intense and soil water reserves are reduced, the differences among the three varieties become more evident. *Țâța caprei neagră* continues to have the lowest values (0.76/+4.3 in the un-irrigated variant and 0.27/+1.1 at 75% of IUA). The *Țâța caprei albă* and *Coarnă albă* varieties show higher values, especially in the un-irrigated variant, which indicates a pronounced water stress (CWSI 0.88-0.85 and $\Delta T_c - T_a$ +5.0-+4.7°C). Overall,

the application of irrigation norms at 50% and 75% of IUA reduces water stress for all varieties.

The combined effects of variety and irrigation were also evident in yield parameters. Regarding yield per vine, *Țâța caprei neagră* recorded the highest values (4.394 kg at 75% IUA), followed by (4.394 kg at 75% IUA), followed by *Țâța caprei albă* and *Coarnă albă*. A similar hierarchy was observed for the average berry weight of a grape: *Țâța caprei neagră* produced heavier berries (338 g at 75% IUA), followed by *Țâța caprei albă* and *Coarnă albă*. Increasing the irrigation level from un-irrigated to 75% IUA led to progressive increases in both production and grape weight, demonstrating the importance of an optimal water supply for supporting grape development.

The statistical significances (letters a, b, c) show that the differences between the combinations of variety $\times$ irrigation regime are significant, which confirms that both variety genetics and irrigation influence the yield parameters and average berry weight. Therefore, combining a productive variety, such as *Țâța caprei neagră*, with an adequate irrigation level (75% IUA) optimizes both the yield per vine and the maximization of the variety's performance (Table 6).

Table 6. The grape yield and average berry weight

Varieties/Irrigation regimes	Grape yield (kg/vine)	Average berry weight (g)
Coarnă albă control	1.325c	265c
Coarnă albă 50% IUA	1.776b	296b
Coarnă albă 75% IUA	2.844a	316a
Țâța caprei albă control	1.944c	312c
Țâța caprei albă 50% IUA	2.808b	324b
Țâța caprei albă 75% IUA	3.696a	336a
Țâța caprei neagră control	2.584c	323c
Țâța caprei neagră 50% IUA	4.277b	329b
Țâța caprei neagră 75% IUA	4.394a	338a

Note: Different letters (a, b, c) indicate statistically significant differences between irrigation regimes ($p < 0.05$, Tukey HSD).

The analysis of the interaction between irrigation level and fertilization type (B $\times$ C) showed a significant influence on average grape weight and yield per vine. The average grape weight values increased progressively with increasing irrigation intensity, from 305 g in un-irrigated, 315 g at 50% IUA, and 325 g at 75% IUA, regardless of the type of fertilization applied. In terms of fertilization, Novatec classic fertilizer resulted in the highest average weights

(330 g), followed by Solfert (320 g), Atonik (310 g) and NPK 15:15:15 (300 g) (Table 7).

Table 7. Effect of irrigation and fertilization on average berry weight (g) and yield per vine (kg)

Fertilization	Unirrigated	50% IUA	75% IUA
NPK 15:15:15	290 g/2.000 kg (c/d)	300 g/2.717 kg (c/c)	310 g/3.367 kg (d/a)
Atonik	300 g/2.150 kg (c/d)	310 g/2.850 kg (b/c)	320 g/3.500 kg (c/a)
Solfert	310 g/2.300 kg (b/c)	320 g/2.983 kg (b/b)	330 g/3.633 kg (b/a)
Novatec classic	320 g/2.433 kg (a/c)	330 g/3.117 kg (a/b)	340 g/3.767 kg (a/a)

The average grape yield per vine followed the same trend: increasing irrigation levels led to average values of 2.221 kg/vine in the unirrigated variant, 2.917 kg/vine at 50% IUA, and 3.567 kg/vine at 75% IUA. Fertilization with Novatec classic led to the highest yield (3.106 kg/vine), while Solfert, Atonik, and NPK 15:15:15 recorded average values of 2.972, 2.833 and 2.695 kg/vine, respectively. The significant differences (letters a-d) confirm that both the irrigation level and the type of fertilization determine changes in the average grape weight and grape yield per vine, with the combined effect of the two factors being most pronounced at the 75% IUA irrigation level associated with the Novatec classic fertilization, representing the optimal strategy for maximizing grapevine performance.

CONCLUSIONS

Grapevine physiological processes were strongly influenced by both irrigation and fertilization. The application of 75% IUA irrigation ensured optimal soil water availability and mitigated water stress, as reflected by decreased CWSI values (from 0.64-0.85 to 0.24-0.33) and consistently high NDVI values (up to 0.87), indicating active photosynthesis and an overall favorable physiological status. In terms of yield, the variety Tâta caprei neagră consistently outperformed the others, producing 4.394 kg per vine and 372 g per berry at 75% IUA, followed by Tâta caprei albă, while Coarnă albă exhibited the lowest performance. Fertilization also had a significant effect on yield, with Novatec Classic resulting in the highest average yield and berry weight.

These findings highlight that the combination of an optimal irrigation regime (75% IUA) with complex fertilization effectively enhances grapevine physiological status and maximizes grape yield.

Additionally, NDVI and CWSI proved to be reliable indicators for monitoring vine water status and guiding irrigation strategies. Among the varieties studied, Tâta caprei neagră demonstrated the highest efficiency in water and nutrient utilization, making it the most suitable choice under water deficit conditions and for adaptation to climate change.

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CARBOHYDRATE RESERVES AND GRAPEVINE RESILIENCE UNDER CLIMATIC VARIABILITY: A TWO-YEAR STUDY FROM BLAJ VITICULTURAL CENTER

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Abstract

This study investigates how carbohydrate reserves vary in both grapevine and rootstock canes over two consecutive years (2023–2024) and explores their relationship with environmental conditions, assessing cultivar performance and resilience patterns. Seven grapevine cultivars (Fetească albă, Fetească regală, Fetească neagră, Italian Riesling, Neuburger, Traminer roz and Sauvignon blanc) and three rootstocks (C-26, C-71 and SO4-4) were analyzed, quantifying non-structural carbohydrate (NSC) reserves in one-year-old canes. The results revealed significant differences between cultivars, vintages, and their interaction. Total NSC levels varied between 11.59 ± 0.95 % and 17.94 ± 0.41 %. Generally, fruit-bearing cultivars accumulated more NSC reserves than rootstocks. Almost all cultivars showed higher NSC reserves in 2023 than in 2024. Starch content remained relatively stable across years, while soluble sugars were more sensitive to environmental variation. In terms of performance and resilience, Traminer roz and Fetească regală emerged as the top fruit-bearing cultivars, and Crăciunel 71 proved to be the most resilient rootstock.

Key words: grapevine, rootstock, carbohydrate reserves, climatic resilience, cultivar performance.

INTRODUCTION

With a decreasing trajectory of area harvested and grape production (Figure 1), illustrated by

the latest available statistics and time-series trends over the past two decades (FAOSTAT, 2025), Romania maintains a significant role within the European viticulture.

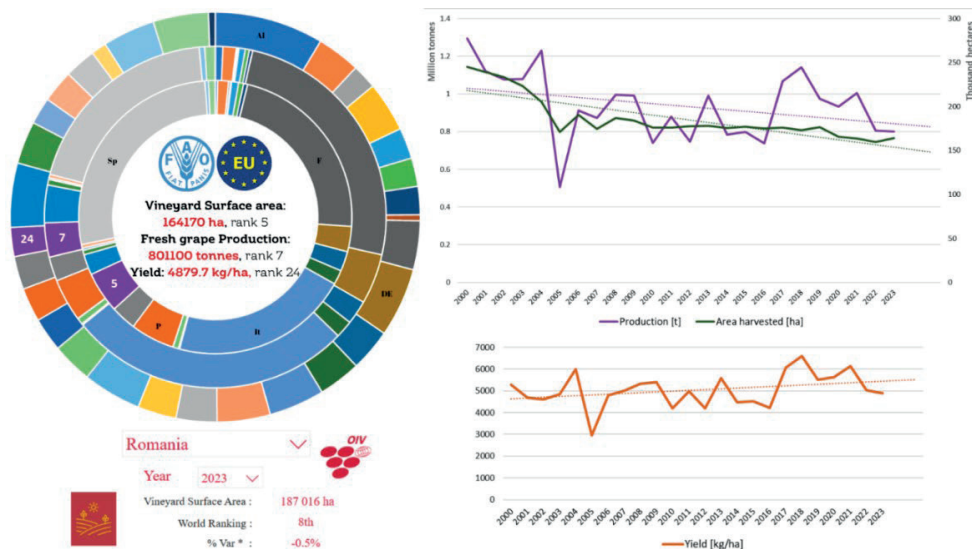


Figure 1. Statistics of Romania's viticulture

Recent FAOSTAT data show that Romania ranks 5th in vineyard area and 7th in fresh grape production among the European Union (EU) countries (Figure 1) (FAOSTAT, 2025). Also, Romania ranked in 8th globally in 2023, with more than 187,000 hectares of vineyard area (Figure 1) (Country Statistics | OIV, 2025). Despite Romania's extensive vineyard area, productivity metrics reveal a relatively low average yield of 4879.7 kg/ha, positioning it only in 24th place in the EU (Figure 1) (FAOSTAT, 2025). Such discrepancies between vineyard potential and realized yield point to underlying physiological and management-related constraints.

Among the key internal factors influencing grapevine performance is the status of non-structural carbohydrate (NSC) reserves, particularly in perennial organs such as one-year-old canes. Research indicates that higher carbohydrate content in grapevine canes can significantly enhance grapevine resilience serving as vital energy sources during periods of stress, such as after pruning, during adverse weather conditions, or in response to pest attacks (Căpruciu et al., 2024; Falchi et al., 2019; Vuerich et al., 2023; Zufferey et al., 2012). These reserves support critical functions such as healing wounds, regenerating tissues, and maintaining vital metabolic processes. Specifically, increased carbohydrate reserves improve overall grapevine vigor and resilience by facilitating rapid recovery from stress-related damage (Ferrandino et al., 2023; Pellegrino et al., 2014; Rogiers et al., 2011). Additionally, they contribute to better tolerance of environmental stress such as drought or extreme temperatures, as the stored energy provides a buffer to sustain metabolic activities when photosynthetic capacity is compromised (Gambetta et al., 2020).

The NSC reserves serve as critical energy sources during early-season growth phases, especially budburst and shoot development, and are essential for buffering the grapevine against environmental stress and variable annual conditions (Zufferey et al., 2012).

Studies have shown that the seasonal dynamics of carbohydrate storage - largely in the form of starch and soluble sugars - are influenced by climatic variables, source-sink relations, and post-harvest management (Călugăr et al., 2022;

Greven et al., 2016). Moreover, variations in starch levels during dormancy have been correlated with bud viability and cane maturity (Calugar et al., 2010; Călugăr et al., 2019), both of which are key indicators of grapevine resilience.

This study focuses on a two-year evaluation of carbohydrate reserves in one-year-old canes from selected cultivars in Blaj Viticultural Center, part of the prestigious Târnave vineyard in Romania.

By tracking interannual variations in NSC accumulation during the dormant season, our study aims to elucidate patterns that may explain grapevine adaptation and performance under temperate continental conditions. Such data not only deepen our understanding of grapevine physiology in Transylvanian terroirs but also support targeted vineyard management strategies that align with climate resilience and sustainable yield optimization.

MATERIALS AND METHODS

Experimental design

The present study was conducted in Blaj Viticultural Center, a cool-climate region favorable for grapevine cultivation and the production of quality white wines with PDO and PGI. The specific ecoclimatic conditions of this area are suitable for producing dry, semi-sweet or sweet wines, semi-aromatic and aromatic wines, and also sparkling wines (Călugăr et al., 2018; Chedea et al., 2021).

The research aimed to evaluate carbohydrate reserves across diverse grapevine genotypes over two consecutive years (2023-2024) and explore their relationship with environmental conditions. For this purpose, eleven cultivars were selected for analysis, including fruit-bearing grapevine cultivars (*Vitis vinifera* L.) and rootstock cultivars (*Berlandieri x Riparia*) (Table 1), which are representative of the Blaj region and among the most widely cultivated in local vineyards.

The selected cultivars were distributed across three vineyard plots (P1, P2, P3), in two different locations (Crăciunelu de Jos and Blaj). Table 1 provides detailed information on cultivar identity, and Table 2 summarizes the vineyard plots characteristics.

Table 1. Sample details

Crt no.	Cultivar Name	Type	Loc.	Plot	Planting year	Sample code
1	Italian Riesling	G	Cr	P ₁	2010	RI_P ₁
2	Traminer roz	G	Cr	P ₁	2007	TR_P ₁
3	Sauvignon blanc	G	Cr	P ₁	2014	SB_P ₁
4	Fetească neagră	G	Cr	P ₁	2010	FN_P ₁
5	Fetească regală	G	Cr	P ₂	2012	FR_P ₂
6	Fetească albă	G	Cr	P ₂	2007	FA_P ₂
7	Neuburger	G	Cr	P ₂	2012	Nb_P ₂
8	Italian Riesling	G	Cr	P ₂	2012	RI_P ₂
9	Crăciunel 26	R	Bj	P ₃	2008	C26
10	Crăciunel 71	R	Bj	P ₃	2008	C71
11	Oppenheim 4sel	R	Bj	P ₃	2008	SO4-4

*Notes: Loc. = location; G = fruit-bearing grapevine cultivar; R = rootstock cultivar; Cr = Crăciunelu de Jos; Bj = Blaj.

Table 2. Experimental plots characteristics

Plot	GPS coord.	Soil type	Soil manag.	Planting Distance	Training sist.
P ₁	46.1733 23.8484	Alluvial soil	Grass strips	2.0 x 1.0	Demi-high Guyot system with periodic replacement arms
P ₂	46.1782 23.8187	Coluvioisil		2.0 x 1.2	
P ₃	46.1732 23.9350	Saline alluvial soil	Alt. grass strips	2.0 x 1.6	Diagonal support system

*Notes: Alt = alternative; Coord. = coordinates; Sist. = system; Planting distance is expressed in meters.

One-year-old canes were collected during winter dormancy (on 16.01.2023 and 11.12.2024) from both fruit-bearing grapevine cultivars and rootstock cultivars. Due to their greater length, each rootstock cane was divided into three distinct segments: base, middle, and tip. Each segment was treated and analyzed as an individual sample to accurately assess the distribution of non-structural carbohydrate reserves along the cane. Fruit-bearing cultivar canes were analyzed as whole samples without segmentation.

The collected samples were cut into convenient sizes (15-20 cm) and oven-dried at 55°C to a constant mass (approx. 48 h). The dried material was finely ground until the cellular structure was destroyed and passed through a 0.7 mm sieve. The samples thus prepared were stored at room temperature until analysis (NSC quantification). Assessment of non-structural carbohydrate (NSC) reserves.

Standards and reagents preparation

Glucose standard: 0.1 g glucose dissolved in 1000 mL distilled water; diluted to 50 µg/mL for use; freshly prepared for each assay.

The assessment of non-structural carbohydrate (NSC) reserves was done by determining the total content of hydrocarbons (soluble sugars + starch) using the spectrophotometric method with the anthrone reagent described by Cimpoi et al. (2020). The method is based on the soluble sugars and starch extraction and their quantification after anthrone dosing.

Sample preparation and carbohydrates extraction

A precisely weighed amount of 0.25 g (± 0.01 g) dried powder was mixed with approximately 0.25 g of quartz sand and 2.5 ml of 80% ethanol. The mixture was homogenized with a mortar and pestle to form a uniform paste. Then, 15 ml of distilled water (5 ml initially, followed by 10 ml for rinsing) was added, and the mixture was transferred to a centrifuge tube. After centrifugation at 6000 rpm for 20 minutes, the supernatant was collected into a 100 ml volumetric flask.

The residue was re-extracted three times with warm 80% ethanol (2.5 mL, then 10 mL in each cycle) followed by centrifugation, and all supernatants were combined. The final volume was adjusted to 100 ml with distilled water. From this solution, 50 mL were transferred to another 100 mL volumetric flask, treated with 0.5 mL of lead acetate, allowed to react (for 5 min), brought to volume with distilled water and filtered. Then, 50 ml of the filtrate was neutralized with 0.5 ml sodium sulfate (saturated solution), brought to volume with distilled water (100 ml) and filtered again. The obtained sugar extract can be stored at 4°C up to 5 days.

The centrifuge residue from the sugar extraction was homogenized with 2.5 mL of distilled water, and then treated with 3.3 ml of 52% perchloric acid (dropwise) under stirring for 15 minutes. After adding 10 mL of distilled water and centrifugation (6000 rpm, 20 minutes), the supernatant was collected. This extraction step was repeated once more. The combined supernatants were brought to 250 mL with distilled water, forming the starch extract (which can be stored at 4°C up to 5 days).

Anthrone reagent (0.2%): 0.2 g anthrone dissolved in 100 mL 95% sulfuric acid, freshly prepared at least 4 hours before use and protected from light.

Carbohydrate quantification by anthrone dosing

In labeled test tubes (14 mm diameter), the following solutions were prepared:

- (a) Blank control: 2 mL distilled water;
- (b) Standard: 1 mL distilled water + 1 mL glucose standard;
- (c) Sugar extract: 1 mL distilled water + 1 mL sugar extract;
- (d) Starch extract: 1 mL distilled water + 1 mL starch extract.

To each tube, 4 mL anthrone reagent was added dropwise in an ice-water bath. Tubes were gently mixed, heated in a boiling water bath for 10 minutes, and then cooled in an ice bath for 15 minutes. Absorbance was measured at 620 nm using a ScanDrop2 (AnalytikJena AG, Germany) UV-Vis spectrophotometer.

Calculations

Glucose concentrations in extracts were calculated by:

$$Z = \frac{E_c - E_a}{E_b} \times 50 \quad [\mu\text{g/mL}] \quad (1)$$

$$A = \frac{E_d - E_a}{E_b} \times 50 \quad [\mu\text{g/mL}] \quad (2)$$

Where E_a , E_b , E_c , E_d are the absorbance values of blank, standard, sugar extract, and starch extract solutions, respectively.

Final sugar and starch contents, expressed as glucose equivalents in percent dry weight (% w/w DW), were calculated as follows:

$$\text{Sugars} = \frac{Z \times V_s \times DF \times 100}{m \times 10^6} \quad [\%] \quad (3)$$

$$\text{Starch} = \frac{A \times V_p \times 100}{m \times 10^6} \quad [\%] \quad (4)$$

Where:

Z = sugar content in the extract [$\mu\text{g/mL}$];

A = starch content in the extract [$\mu\text{g/mL}$];

m = dry mass of the sample used for analysis;
 $DF = 4$ (dilution factor used in the sugar extraction protocol);

$V_s = 100$ = volume of sugar extract [mL];

$V_p = 250$ = volume of starch extract [mL].

Carbohydrate contents reported as percentages (%) correspond to grams of glucose equivalents per 100 grams of dry sample, thus normalizing for sample moisture and allowing direct comparison. The analysis was conducted in duplicate to ensure statistical validity.

Agroclimatic context and weather data

Weather data were recorded daily by an iMetos 3.3 weather station (Pessl Instruments GmbH; Weiz, Austria), located between P1 and P2 in

Crăciunelu de Jos at the following coordinates: 46.174372 N latitude and 23.85257 E longitude. The viticultural years 2023 and 2024 were characterized in comparison with the reference period between 1990-2022. The following parameters were recorded and analyzed:

- Vegetation period length (VP): Number of days from budbreak to leaf fall.
- Average annual temperature ($^{\circ}\text{C}$): Mean temperature over the entire calendar year.
- Average temperature during the vegetation period ($^{\circ}\text{C}$): Mean temperature recorded during the active growing season.
- Global heat units ($\sum t^{\circ}\text{G}$, $^{\circ}\text{C}$): The sum of daily positive mean (>0 $^{\circ}\text{C}$) temperatures, representing total heat accumulation.
- Active heat units ($\sum t^{\circ}\text{A}$, $^{\circ}\text{C}$): Calculated as the sum of daily mean temperatures above the base threshold of 10°C .
- Useful heat units ($\sum t^{\circ}\text{U}$, $^{\circ}\text{C}$): Portion of heat units effectively utilized by grapevines, calculated as the sum of differences between daily average temperature $> 10^{\circ}\text{C}$ and the biological threshold required for the vegetation start (10°C).
- Absolute minimum temperature ($^{\circ}\text{C}$): Lowest recorded temperature during the study period.
- Average minimum temperature ($^{\circ}\text{C}$): Mean of daily minimum temperatures.
- Absolute maximum temperature ($^{\circ}\text{C}$): Highest recorded temperature during the study period.
- Average maximum temperature ($^{\circ}\text{C}$): Mean of daily maximum temperatures.
- Relative humidity (RH, %): Average atmospheric moisture content.
- Annual precipitation ($\sum \text{Ppt}$, mm/m^2): Total rainfall over the year.
- Precipitation during the vegetation period ($V_p \sum \text{Ppt}$, mm/m^2): Total rainfall accumulated during the active growing season.
- Sunlight during the vegetation period (hours): Total hours of solar radiation received during the growing season.
- Huglin Index ($^{\circ}\text{C}$): Provides a measure of heat accumulation over the growing season, integrating temperature and day length to assess climatic suitability. Was calculated according to Irímia et al. (2013).

Statistical analysis

The data were analysed using Minitab 17 (Minitab Inc., State College, PA, USA). One-way ANOVA with Fisher's LSD test ($p < 0.05$) was applied to assess differences between cultivars. Main effects analysis evaluated factor influences. A General Linear Model examined cultivar performance and resilience. Two-sample t-tests compared each vintage's climatic data to the historical reference period (1990-2022). Normality and variance assumptions were verified prior to testing. Statistical significance was set at $p < 0.05$.

RESULTS AND DISCUSSIONS

Overview of NSC accumulation

A general overview of NSC accumulation is presented in Table 3 as mean $\pm$ standard deviation. The results are expressed as grams of

glucose equivalents/100 grams of dry matter. Total carbohydrate levels varied between 12.55% and 17.94% with an average of 15.23% in 2023 and between 11.59% and 15.53% with an average of 13.89% in 2024. Almost all cultivars showed higher average reserves in 2023 exceptions being Neuburger (Nb_P2), Crăciunel 71 (C71_P3) and Oppenheim 4sel (SO4-4). This trend indicates favorable reserve accumulation during the 2023 season. Soluble sugars maintained the same trend, declining from an average of 9.32% in 2023 to an average of 8.12% in 2024. Starch content showed the greatest stability across years, registering an average of 5.92% in 2023 and 5.76% in 2024. The relative stability of starch across vintages suggests a buffering role in reserve dynamics, while the decline in soluble sugars reflects higher sensitivity to environmental variation (Nawaz et al., 2025; Zepeda et al., 2023).

Table 3. Results regarding the carbohydrate content

Crt no.	Sample code	2023			2024		
		Sugars	Starch	Total NSC	Sugars	Starch	Total NSC
1	RI P ₁	10.20 $\pm$ 0.97 ^{ab}	6.31 $\pm$ 0.24 ^c	16.51 $\pm$ 0.74 ^{abc}	7.94 $\pm$ 0.66 ^{cd}	5.67 $\pm$ 0.63 ^{de}	13.61 $\pm$ 0.11 ^{cd}
2	TR P ₁	8.79 $\pm$ 1.19 ^{cd}	6.88 $\pm$ 0.37 ^{bc}	15.67 $\pm$ 0.82 ^{bc}	7.81 $\pm$ 0.26 ^{cd}	7.29 $\pm$ 0.26 ^b	15.10 $\pm$ 0.44 ^{ab}
3	SB P ₁	8.96 $\pm$ 0.71 ^{bcd}	6.31 $\pm$ 0.43 ^c	15.27 $\pm$ 1.13 ^c	8.39 $\pm$ 0.33 ^{bc}	5.74 $\pm$ 0.30 ^d	14.13 $\pm$ 0.03 ^{bc}
4	FN P ₁	9.53 $\pm$ 0.78 ^{bc}	7.34 $\pm$ 0.11 ^b	16.87 $\pm$ 0.68 ^{ab}	7.64 $\pm$ 0.47 ^{cde}	6.80 $\pm$ 0.60 ^{bc}	14.44 $\pm$ 1.06 ^{abc}
5	FR P ₂	8.19 $\pm$ 0.98 ^d	8.48 $\pm$ 0.25 ^a	16.67 $\pm$ 1.21 ^{abc}	8.04 $\pm$ 0.44 ^{cd}	6.22 $\pm$ 0.45 ^{cd}	14.26 $\pm$ 0.87 ^{abc}
6	FA P ₂	10.97 $\pm$ 0.76 ^a	6.97 $\pm$ 0.35 ^{bc}	17.94 $\pm$ 0.41 ^a	7.40 $\pm$ 0.46 ^{de}	4.61 $\pm$ 0.34 ^f	12.01 $\pm$ 0.80 ^{ef}
7	Nb P ₂	9.24 $\pm$ 0.95 ^{bcd}	3.39 $\pm$ 0.27 ^f	12.63 $\pm$ 0.68 ^e	6.81 $\pm$ 0.43 ^e	8.72 $\pm$ 0.65 ^a	15.53 $\pm$ 1.08 ^a
8	RI P ₂	10.89 $\pm$ 0.18 ^a	5.24 $\pm$ 0.12 ^{de}	16.13 $\pm$ 0.30 ^{bc}	7.18 $\pm$ 0.72 ^{de}	4.40 $\pm$ 1.25 ^f	11.59 $\pm$ 0.95 ^f
9	C26 P ₃	8.41 $\pm$ 0.59 ^d	5.52 $\pm$ 0.46 ^d	13.93 $\pm$ 0.86 ^d	9.48 $\pm$ 0.64 ^a	3.27 $\pm$ 0.44 ^g	12.76 $\pm$ 0.76 ^{de}
10	C71 P ₃	8.69 $\pm$ 0.62 ^{cd}	4.79 $\pm$ 0.44 ^e	13.40 $\pm$ 1.00 ^{de}	9.72 $\pm$ 0.64 ^a	4.94 $\pm$ 0.34 ^{ef}	14.66 $\pm$ 0.80 ^{abc}
11	SO4-4 P ₃	8.66 $\pm$ 0.87 ^{cd}	3.89 $\pm$ 0.49 ^f	12.55 $\pm$ 1.20 ^e	8.91 $\pm$ 0.47 ^b	5.74 $\pm$ 0.77 ^d	14.65 $\pm$ 0.96 ^{abc}

Note: *Different letters indicate statistically significant differences according to Fisher's LSD test ($p < 0.05$).

Similar carbohydrate contents are reported by Călugăr et al. (2010, 2019) and by Iliescu et al. (2012) in the same area.

The main effect analysis shows significant influences of studied factors on the sugar (Figure 2), starch (Figure 3), and total NSC levels (Figure 4). Sugar content was consistently higher in rootstocks than in fruit-bearing cultivars, and in samples from the Blaj location (Bj). Among the plots, P3 exhibited the highest average sugar concentration, while P2 showed the lowest (Figure 2).

Fruit bearing cultivars accumulated more starch than rootstocks, and Crăciunel location (Cr) registered higher starch levels than Blaj (BJ). Among the plots, P1 recorded the highest starch content, while P3 had the lowest starch content

(Figure 3). In terms of total NSC, fruit bearing cultivars accumulated more reserves than rootstocks; the Crăciunel location (Cr) and P1 plot registered the highest average carbohydrate values (Figure 4).

A consistent reduction from 2023 to 2024 was observed across all three parameters, reinforcing the strong influence of the meteorological conditions of each vintage on the grapevines' reserve dynamics.

Cimpoi et al. (2020), reports a total carbohydrate content in dormant canes of both rootstock and fruit bearing cultivars ranging from 12.02% to 15.64%. Soluble sugars were the major fraction (8.63-11.38%), with starch contributing 2.44-5.41%. Lower levels of total NSC were determined in the rootstock cultivars.

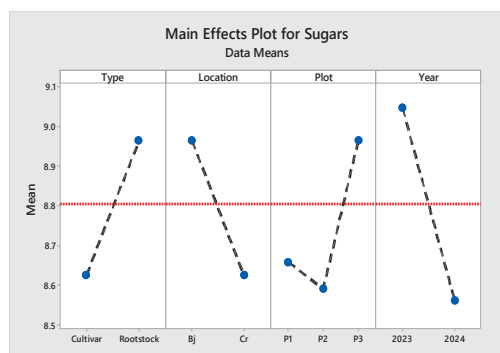


Figure 2. Main effect plot for soluble sugars content

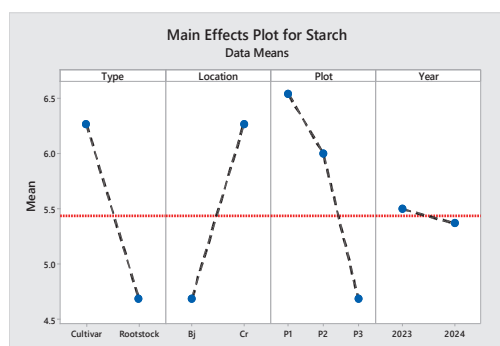


Figure 3. Main effect plot for starch content

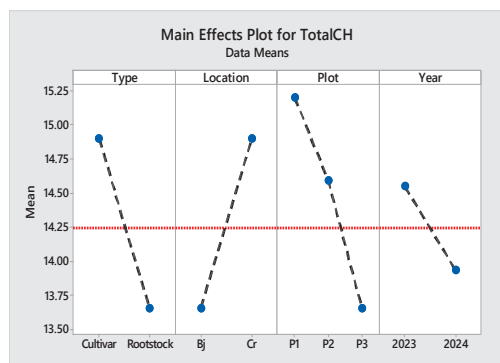


Figure 4. Main effect plot for total carbohydrate content

Cultivar performance and resilience patterns

Grapevine resilience refers to a cultivar's ability to maintain physiological performance despite seasonal variability (e.g., heat, drought, and yield stress). In our study, resilience is indicated by stable total NSC levels across 2023 and 2024, the ability to recover or maintain reserves regardless of climate conditions, and smaller carbohydrate declines in 2024 under more stressful conditions.

Fruit bearing cultivars

In evaluate how each cultivar performed and how performance changed depending on the year, a General Linear Model (GLM) was applied. This allows the evaluation of both main effects and interactions while adjusting for environmental variability. The General Linear Model (GLM) including cultivar, year, and their interaction explained 84.2% of the variance in total NSC content and confirmed significant effects of cultivar ($P = 0.022$), year ($P < 0.001$), and their interaction ($P < 0.001$).

Traminer roz and Fetească regală emerged as the top cultivars. Traminer roz maintained the most stable carbohydrate reserves across years, with only a minimal decrease from 15.67% in 2023 to 15.10% in 2024 (Table 3 and Figure 5). Fetească regală consistently accumulated high carbohydrate levels, with means of 16.67% and 14.26%, respectively, reflecting strong performance with moderate resilience (Table 3 and Figure 5). Fetească neagră also showed solid performance, decreasing from 16.87% to 14.44%, and retained relatively stable reserves (Table 3 and Figure 5). Sauvignon Blanc showed moderate performance and stability with 15.27% total NSC content in 2023 and 14.13% total NSC content in 2024 (Table 3 and Figure 5).

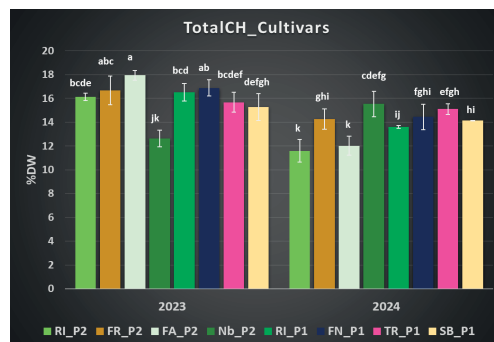


Figure 5. Graphical representation of the fruit-bearing cultivars (RI - Italian Riesling, FR - Fetească regală, FA - Fetească albă, Nb - Neuburger, FN - Fetească neagră, TR - Traminer roz, SB - Sauvignon Blanc; *Different letters indicate statistically significant differences according to Fisher's LSD test, $p < 0.05$)

Italian Riesling, while reaching high total NSC content in 2023, registered a significant drop in 2024 in both plots (Table 3 and Figure 5). This decline indicates a strong year effect and

positions it as a vintage-responsive cultivar, potentially vulnerable under adverse conditions. Fetească albă, despite having the highest carbohydrate content in 2023 (17.94%), showed a sharp decline in 2024 (12.01%), underscoring its sensitivity to climatic or physiological stress (Table 3 and Figure 5). Neuburger is the only fruit bearing cultivar that showed a moderate increase from 12.63% to 15.53% (Table 3 and Figure 5), suggesting a recovery dynamic possibly linked to physiological depletion from prior yield (Muntean et al., 2023). Similar NSC levels are reported in the literature. Holzapfel & Smith (2012), and Zufferey et al. (2015), report non-structural carbohydrate (NSC) reserves in dormant grapevines canes ranging between 10 and 15% DW, with slight variation depending on cultivar, and stress exposure. Also, the study of Călugăr et al. (2022), analyzing eight *Vitis vinifera* varieties (Muscat de Hamburg, Napoca, Cardinal, Perla de Csaba, Fetească regală, Muscat Ottonel, Pinot noir, Fetească neagră) during dormancy (October-February 2019-2020) reveals total NSC levels ranging from 12.77% (Cardinal cultivar) to 16.03% (Fetească regală cultivar). Higher total NSC levels were observed in October (12.93-16.03%) while minimum levels were highlighted in January (11.70-13.70%) (Călugăr et al., 2022).

Rootstock cultivars

Usually, the canes of the studied rootstock cultivars reach 5 m long therefore we analyzed them into three longitudinal segments: base, middle and tip. This helps to better understand the distribution and dynamics of carbohydrate reserves in grapevine rootstocks and may also provide insights into how environmental stressors or previous crop loads influence reserve partitioning. A uniform carbohydrate distribution might suggest sustained metabolic activity and a good vascular connectivity, while pronounced differences could indicate localized storage or transport limitations. Interannual variation in segmental carbohydrate levels may reflect rootstock specific physiological strategies or responses to climatic conditions (Chandra Dahal, 2019; Falchi et al., 2019; Martínez-Lüscher & Kurtural, 2021). The General Linear Model for the rootstock cultivars included cultivar, cane part, year and

their interaction. The model explains 63% of the total variation in NSC levels, which is moderate but typical in field-based viticultural studies. According to the GLM, the effect of year was highly significant ($P = 0.006$), with 2023 generally recording lower carbohydrate levels than 2024. The cane part factor was also significant ($P = 0.026$), with the base section consistently showing higher carbohydrate content than middle and tip. This reflects the natural gradient of reserve accumulation along the cane, the base of the cane, being closest to the main vascular connection and older lignified tissues, is typically richer in stored reserves, while the tip often represents the youngest, least lignified segment with lower reserve density (Chandra Dahal, 2019; Frioni et al., 2016; Rogiers et al., 2011; Toolo, 2022). The cultivar effect approached significance ($P = 0.085$), suggesting meaningful but slightly variable differences among rootstocks. The Cultivar $\times$ Year interaction was highly significant ($p < 0.001$), confirming that total NSC responses differed across years depending on the cultivar (Figure 6).

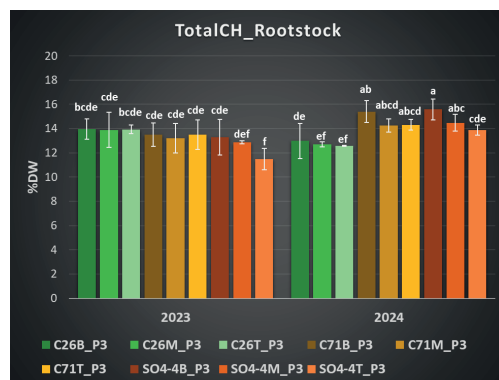


Figure 6. Graphical representation of the rootstock cultivars performance (C26 - Crăciunel 26, C71 - Crăciunel 71, SO4-4 - Oppenheim 4sel; B - base cane segment, M - middle cane segment, T - tip cane segment; *Different letters indicate statistically significant differences according to Fisher's LSD test, $p < 0.05$)

In terms of performance and resilience, Crăciunel 71 proved to be the top-performing and most resilient rootstock cultivar. It maintained relatively high carbohydrate reserves in both years (13.40% in 2023, 14.66% in 2024) and a balanced carbohydrate distribution across cane segments. SO4-4 showed the most

pronounced increase (+2.10%), after registering the lowest total NSC level in 2023 (12.55%). This indicates strong recovery potential but greater interannual variability.

Crăciunel 26 began with moderately high carbohydrate reserves in 2023 (13.93%), but declined to 12.76% in 2024, suggesting greater sensitivity to annual environmental or physiological stressors.

Our findings are in agreement with other studies from the available literature. Cimpoi et al. (2020) studied pre-grafting dormant canes of three rootstocks cultivars: Riparia Gloire, SO4-4 and Crăciunel 2 Selection. They found the highest total NSC content in Riparia gloire (13.49%), followed by Crăciunel 2 (12.78%) and SO4-4 (12.02%). Iliescu et al. (2012) studied SO4-4 rootstock canes, sampled from plantation (autumn, winter) and from storage (spring) and found total NSC levels between 11-13% observing that the NSC content remained stable across seasons and storage, with a slight gradient from base to tip segments.

Meteorological Conditions and Climatic Stressors

The key indicators for the meteorological conditions of the two studied vintages are presented in Table 4. The 2023 and 2024 growing seasons in Blaj Viticultural Center were marked by statistically significant deviations from the 1990-2022 climatic reference period, with 2024 in particular standing out as an extreme vintage.

Both years showed significantly higher average annual temperatures (11.6 °C in 2023 and 12.1 °C in 2024, $p < 0.001$), with 2024 also exhibiting a significantly extended vegetation period (205 days, $p < 0.001$) and the highest cumulative global heat units ($\Sigma t^{\circ}G = 3763.9^{\circ}C$, $p < 0.001$). Sunshine hours during the vegetation period increased dramatically in 2024 (1987 hours, $p < 0.001$).

These climate patterns, especially the combination of increased temperature, longer growing seasons, and greater solar radiation, are known to elevate grapevine respiration and accelerate phenological stages, often at the expense of reserve accumulation (Colibaba et al., 2024; Dominguez et al., 2024; Hall et al., 2016).

The observed reduction in Tmax. combined with an increase in Tmin. clearly shows a narrowing

of the thermal amplitude during recent vintages. This combination results in a lower carbon surplus available for allocation to perennial storage organs such as roots and canes (Smith et al., 2009). Cooler daytime temperatures can reduce photosynthetic carbon assimilation, while warmer nights elevate respiration rates, increasing carbon demand (D. Greer & Weedon, 2014; Zufferey et al., 2015).

Table 4. Meteorological conditions of the studied vintages

Parameter	Average		
	1990-2022	2023	2024
January	-1.5	3.3	0.9
February	1.0	1.1	6.7
Mars	5.4	6.9	8.8
April	11.2	9.1	13.2
May	16.0	15.8	16.1
June	19.6	19.2	22.3
July	21.2	21.9	23.0
August	21.0	21.9	22.5
September	15.9	18.5	17.7
October	10.7	12.8	9.6
November	4.7	6.3	2.8
December	-0.2	2.4	2.2
Vegetation period length, VP [days]	187	187 ^{n.s}	205 ^{***}
Average annual temperature [°C]	10.4	11.6 ^{***}	12.1 ^{***}
Average annual temperature during VP [°C]	17.5	17 ^{***}	17.7 ^{n.s}
Global heat unit, $\Sigma t^{\circ}G$ [°C]	3456.7	3649.3 ^{***}	3763.9 ^{***}
Active heat unit, $\Sigma t^{\circ}A$ [°C]	3312.4	3517.6 ^{***}	3676.3 ^{***}
Usefull heat unit, $\Sigma t^{\circ}U$ [°C]	1464.8	1577.6 ^{***}	1736.3 ^{***}
Absolute minimum temperature [°C]	-24.7	-14.8 ^{***}	-12.2 ^{***}
Average minimum temperature [°C]	-9.3	-0.3 ^{***}	-0.2 ^{***}
Absolute maximum temperature [°C]	41.6	36.5 [*]	38.5 ^{***}
Average maximum temperature [°C]	30.1	24.9 ^{***}	26.7 ^{***}
Relative humidity, RH [%]	80.6	77.5 [*]	79.2 ^{n.s}
Annual precipitations ΣPpt [mm/ m ²]	619.9	547.6 ^{**}	606.4 ^{n.s}
Precipitations during the vegetation period, $Vp \Sigma Ppt$ [mm/m ²]	430.1	385.2 [*]	442.2 ^{n.s}
Sunlight during the vegetation period [hours]	1355	1563.0 ^{***}	1987.0 ^{***}
Huglin Index	-	2590.2	3023.0

Although total precipitation in 2024 was not significantly different from the historical mean,

2023 exhibited significantly lower annual and vegetation period rainfall ($p < 0.05$), paired with a significant drop in relative humidity. Moderate water deficit and optimal temperature regimes during the growing season promote carbohydrate accumulation in grapevines (Chaves et al., 2007; Holzzapfel et al., 2010). In contrast, prolonged periods of high precipitation and humidity tend to favor vegetative growth, reducing the allocation of resources to carbohydrate reserves (Palliotti & Poni, 2016). The Huglin Index, which measures heat accumulation relevant for grapevine development, was 2590.2 in 2023 and increased to 3023.0 in 2024, indicating a shift towards warmer and potentially more stressful conditions. Table 5 presents the climatic stressors recorded in Blaj Viticultural Center during the two studied vintages. The frequency of critical stress events in 2023 and 2024 was evaluated using thresholds relevant to grapevine damage and physiological disruption. Critical winter frost (minimum temperatures below -15°C) did not occur in any of the core winter months (December, January, or February) in either 2023 or 2024 (Table 5). This contrasts with historical patterns in Blaj Viticultural Center, where extreme lows of -24.7°C have previously been recorded (Table 4). In 2023, five days in March fell below the late frost threshold of -2°C , accounting for 16% of March days. No spring frost events occurred in 2024.

Table 5. Stress factors frequency recorded in 2023 and 2024

Risk/stress factor	Critical level	Month	Days with critical levels/month		Risk factor frequency (%)	
			2023	2024	2023	2024
Winter frost	Minimum temperature $< -15^{\circ}\text{C}$	Dec.	0	0	0	0
		Jan.	0	0	0	0
		Feb.	0	0	0	0
Late spring frost	Minimum temperature $< -2^{\circ}\text{C}$	March	5	0	16	0
		April	0	0	0	0
		May	0	0	0	0
Drought	Maximum temperature $> 30^{\circ}\text{C}$	June	3	17	10	56.7
		July	18	16	58	51.6
		Aug.	17	24	55	77.4
		Sept.	3	7	10	23.3

Heat stress, defined here as days with maximum temperatures exceeding 30°C , occurred with greater frequency in 2024, especially during June and August (Table 5). In total, 2024 recorded 64 days over 30°C , compared to 41

days in 2023. These high temperatures are likely to have reduced stomatal conductance and limited photosynthetic activity during key periods of carbohydrate accumulation (Escalona et al., 2000; D. H. Greer & Weedon, 2013; D. Greer & Weedon, 2012), especially if combined with limited soil moisture (Soar et al., 2006; Tombesi et al., 2015). The stress is particularly significant in June, when shoot growth is still vigorous and the grapevines carbohydrate demand is high (Kizildeniz et al., 2021; Lebon et al., 2008).

Taken together, the results indicate that 2024 posed the highest level of climatic stress over the two-year study period, with probable consequences on the NSC reserves accumulation affecting the grapevines capacity for resilience and recovery.

CONCLUSIONS

This two-year study provides insight into dormant-season non-structural carbohydrate (NSC) accumulation in grapevine canes highlighting the influence of climatic variability on grapevine reserves status and cultivar-specific resilience patterns.

Across both years, non-structural carbohydrate (NSC) levels in dormant canes fell within ranges previously reported. Generally, 2023 - significantly warmer and drier than the reference period (1990-2022) - favored higher total NSC accumulation, whereas 2024 - characterized by even higher average temperatures, increased frequency of days $>30^{\circ}\text{C}$, and a narrower thermal amplitude - led to a consistent decline in total reserves in most cultivars. This confirms that interannual climate variability is a major driver of perennial reserve accumulation.

Soluble sugars were more sensitive to climate variability than starch. Sugars declined substantially from 2023 to 2024, while starch content remained relatively stable, supporting its role as a buffering reserve pool. Warmer night temperatures in 2024 likely increased respiration rates, contributing to reduced sugar availability for storage.

In terms of cultivar-specific resilience the following patterns emerged: Traminer roz and Fetească regală displayed the highest combined performance and stability, maintaining high NSC levels across both vintages. In contrast,

Fetească albă and Italian Riesling showed large interannual declines, indicating greater sensitivity to climate stress. Neuburger was the only fruit-bearing cultivar to increase NSC in 2024, suggesting a recovery dynamic after potential depletion in 2023. Rootstock cultivars exhibited clear reserve gradients along the cane. Carbohydrate content was consistently highest in the basal cane segments and lowest at the tips, reflecting storage physiology and vascular connectivity. Crăciunel 71 showed the highest and most stable NSC levels across years, while SO4-4 demonstrated strong recovery potential despite low reserves in 2023.

The climatic stressors from 2024 that most likely restricted NSC reserve accumulation are the following: intensified heat, extended vegetation period, increased solar radiation, and high cumulative temperatures. The combination of these climatic stressors, is known to elevate grapevine respiration and accelerate phenological stages, suggesting an intensified metabolic load, likely leading to greater consumption of non-structural carbohydrates (NSCs) during the ripening and post-harvest phases at the expense of reserve accumulation. Reduced NSC reserves can compromise budburst, early shoot growth, and flowering in the following season, potentially influencing yield and fruit composition. Cultivars with higher resilience (e.g., Traminer roz, Fetească regală) may better sustain consistent wine quality under warming scenarios, whereas more sensitive cultivars (e.g., Fetească albă) may require targeted management - such as moderated crop loads, optimized irrigation, and postharvest leaf area maintenance - to protect reserve status.

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CLIMATE CHANGE IN THE VALEA CĂLUGĂREASCĂ VITICULTURAL CENTER

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Abstract

This study evaluates how soil management practices influence the physical and biological properties of the soil, grapevine productivity, and the adaptive capacity of vineyards to climate change in the Valea Călugărească wine region. Compared with black fallow, permanent grassing cover cropping increased soil organic matter content to 1.6%, while grass reached 1.7%. Mixtures of dwarf white clover with grass and vetch with grass enhanced organic matter by 38% (1.8%), reduced soil compaction to 255–250 kPa, and increased water-holding capacity to 44–47%. Erosion was limited to 2.2–2.1 t/ha/year, and biodiversity increased to 55–59%. Legumes and grass mixtures supported yields 50% higher than the control (3.960 kg/vine), with grapes containing 240 g/L sugars, allowing the production of wines with 14° alcohol content. These results highlight that such practices significantly contribute to soil fertility, biodiversity, and the sustainability of viticulture.

Key words: biodiversity, cover crops, climate change, soil fertility, sustainable viticulture.

INTRODUCTION

Global climate change is exerting increasing pressure on agricultural systems, with viticulture being particularly vulnerable to alterations in temperature and water regimes, increased frequency of extreme events, and soil resource degradation (Fraga et al., 2018). In wine-growing regions, these changes affect grapevine physiological processes, grape ripening dynamics, compositional balance, and yield stability, with direct consequences for wine quality (van Leeuwen & Darriet, 2016).

Soil represents a fundamental component of vineyard agroecosystems, playing a crucial role in regulating water availability, nutrient supply, and biological activity - key factors for grapevine adaptation to climatic stress (Brillante et al., 2016; Lal, 2020). In this context, soil management practices are recognized as effective tools to enhance vineyard resilience by improving soil structure, increasing organic matter content, reducing compaction, and limiting erosion processes (Celette & Gary, 2013; Novara et al., 2019).

Alternative soil management systems, such as spontaneous cover cropping or the use of legume-grass mixtures, are increasingly

promoted in sustainable viticulture due to their ability to enhance soil biodiversity, improve water-holding capacity, and contribute to carbon sequestration in the soil (Pardini et al., 2002; García-Díaz et al., 2018). Compared with conventional bare-soil systems, these practices can reduce soil vulnerability to degradation and support yield stability and grape quality under conditions of pronounced climatic variability (Morlat & Jacquet, 2003; Wolff et al., 2018). Considering the importance of the Valea Călugărească viticultural region and its vulnerability to the effects of climate change, it is necessary to analyze the influence of different soil management systems on soil physical and biological properties, grapevine productivity, and vineyard resilience in order to establish sustainable solutions for viticulture.

MATERIALS AND METHODS

The study was conducted in vineyards located in the Valea Călugărească wine-growing center, on a chernozem soil, fertile, with neutral pH and a moderate organic matter content (approximately 3-3.5%). The experiments were carried out over a two-year period and aimed to evaluate the influence of different soil maintenance systems

on productivity and grape quality. The experimental factor was the soil maintenance system, with six variants: a1 - black field (conventional tillage system); a2 - permanent grass consisting of spontaneous flora; a3 - lawn mixture (40% *Lolium perenne*, 33% *Festuca rubra*, and 27% *Poa pratensis*); a4 - *Phacelia tanacetifolia*; a5 - white dwarf clover combined with a lawn mixture (40% *Lolium perenne*, 33% *Festuca rubra*, and 27% *Poa pratensis*); a6 - vetch combined with a lawn mixture (40% *Lolium perenne*, 33% *Festuca rubra*, and 27% *Poa pratensis*).

The black field system, currently practiced in the vineyard, consists of autumn and spring plowing, five mechanical inter-row hoeings, and four manual hoeings along the vine row.

The experiment was organized in a randomized block design with three replicates per soil maintenance system, on 100 m² plots, in a vineyard with planting distances of 2.5 m between rows and 1.2 m between vines within the row. All maintenance operations, irrigation, fertilization, and phytosanitary treatments were applied uniformly in order to isolate the effect of the soil maintenance system on the analyzed parameters.

The monitored parameters included soil characteristics, biological indicators, and production traits. Soil analyses were performed according to the standard procedures described in Sparks (1996). Total nitrogen was determined by the Kjeldahl method; available phosphorus and potassium were determined by the Egner-Riehm-Domingo method; Zn, Cu, F, and Mn were extracted with DTPA (pH 7.3); water-soluble boron was determined by hot-water extraction; and molybdenum was extracted with acid ammonium oxalate (pH 3.3). Micronutrients in the extracts were quantified by atomic absorption spectrophotometry (Zn, Cu, Fe, Mn) and by colorimetric methods (B, Mo). Regarding vine performance, yield per vine and grape quality parameters were assessed. Sugar content was determined by refractometry (OIV, 2021a), and total acidity was determined by titration with NaOH (OIV, 2021b). The gluco-acidimetric index was calculated as the ratio between sugar content and total acidity (expressed as g/L tartaric acid). Measurements were performed annually at harvest, and

chemical analyses were conducted according to standard OIV methodology.

To evaluate soil quality as influenced by the soil maintenance system, a synthetic soil quality index was calculated. This index integrates the normalized values of the main physical and chemical soil indicators (field capacity, bulk density, humus content, pH, total nitrogen, available phosphorus and potassium, and micronutrients). Data normalization was performed using the min-max method, and the final soil quality index score was calculated as the arithmetic mean of the normalized indicators, providing a single comparative parameter among soil maintenance systems.

Statistical analysis was performed using analysis of variance (ANOVA) for a randomized complete block design. When significant differences among soil maintenance systems were detected, mean values were compared using Tukey's Honestly Significant Difference (HSD) test at a significance level of $p \leq 0.05$. The presented results represent the mean values calculated over the two-year experimental period and are expressed as mean $\pm$ standard deviation. Significant differences are indicated in the tables as follows: $p < 0.05$, $p < 0.01$, and ns = not significant.

RESULTS AND DISCUSSIONS

The analysis of the results highlighted that soil maintenance systems significantly influenced organic matter content, vegetative growth of the vines, as well as the quantitative and qualitative parameters of grape production. The control treatment, represented by the black field (a1), served as the reference for evaluating the effects of the other experimental variants.

Compared to the control, all treatments with vegetative cover exhibited higher organic matter content. Permanent grass composed of spontaneous flora (a2) and the lawn mixture (a3) led to moderate increases of +10-18%, which were statistically significant ($p < 0.05$). In the case of the annual *Phacelia tanacetifolia* (a4), the increase was lower (+8-12%) and did not reach statistical significance (ns). The highest organic matter content was recorded in treatments combining legumes and grasses, namely white dwarf clover + lawn (a5) and vetch + lawn (a6), where increases ranged from +18-

28% compared to the control, showing highly significant differences ($p < 0.01$) (Table 1).

Table 1 Effect of soil maintenance systems on organic matter content

Variant	Description	Organic matter content
a1	Black field (Mt)	1.30
a2	Permanent grass with spontaneous flora	1.43-1.50* (+10-15%)
a3	Lawn mixture	1.46-1.54* (+12-18%)
a4	<i>Phacelia tanacetifolia</i>	1.40-1.45 ns (+8-12%)
a5	White dwarf clover +lawn	1.54-1.63** (+18-25%)
a6	Vetch + lawn	1.56-1.66** (+20-28%)

Significance levels: $p < 0.05$; $p < 0.01$; ns = not significant

The results indicate that permanent soil cover, particularly through the inclusion of nitrogen-fixing legume species in mixtures with grasses, promotes organic matter accumulation within a relatively short period (two years), contributing to improved soil fertility, structure, and the overall sustainability potential of the vineyard system.

Furthermore, a positive effect can be anticipated on other soil properties, such as reduced compaction and enhanced water-holding capacity. Soil maintenance systems significantly influenced the physical and chemical properties of the soil. Compared to the control (black field, a1), all treatments with vegetative cover resulted in decreased soil compaction and increased water-holding capacity.

Regarding soil compaction, the greatest reductions were observed in treatments combining legumes and grasses, namely white dwarf clover + lawn (a5) and vetch + lawn (a6), where compaction decreased by 25-26% relative to the control, showing highly significant differences ($p < 0.01$). Treatments with lawn only (a3) and permanent grass with spontaneous flora (a2) exhibited intermediate reductions of 21-24%, significant at $p < 0.05$, while annual cultivation with *Phacelia tanacetifolia* (a4) led to a smaller reduction (15%), which was not statistically significant (ns), (Table 2).

Water-holding capacity was also positively affected. The highest increases, of 22-26%, were observed in the legume-grass mixtures (a5 and a6), with differences highly significant ($p < 0.01$). Intermediate increases were recorded in a2 and

a3 (+12-18%, $p < 0.05$), while the a4 treatment showed a more modest increase (+8-11%, ns).

Table 2. Effect of soil maintenance systems on soil compaction and water-holding capacity (% compared to the control)

Variant	Description	Compaction reduction (%)	Water holding capacity increase (%)
a1	Black field (Mt)	340	36
a2	Permanent grass with spontaneous flora	270* (-21%)	40-41* (+12-15%)
a3	Lawn mixture	260* (-24%)	41-42* (+15-18%)
a4	<i>Phacelia tanacetifolia</i>	290 ns (-15%)	39-40 ns (+8-11%)
a5	White dwarf clover +lawn	255** (-25%)	43-44** (+20-24%)
a6	Vetch + lawn	250** (-26%)	44-47** (+22-26%)

Significance levels: $p < 0.05$; $p < 0.01$; ns =not significant

These results highlight the essential role of permanent soil cover in improving soil structure by reducing compaction and enhancing water-holding capacity, key factors determining vineyard resilience under water-limited conditions.

Soil nutrient values for the control (a1) and the ameliorative treatments (a2-a6) were compared as percentage increases relative to the control. Total nitrogen, phosphorus, and potassium showed the largest increases, ranging from 26% to 31%, while micronutrients (Fe, Mn, Cu, Zn, and B) exhibited moderate increases of 1-15%. These results highlight the positive impact of permanent grass cover and legume incorporation on soil fertility and nutrient availability, supporting both vine productivity and grape quality under sustainable vineyard management systems. The observed effects suggest that careful selection of ameliorative species can optimize nutrient supply in the soil, reducing reliance on chemical fertilizers (Table 3).

In addition, these practices contribute to maintaining the ecological balance of agricultural lands and increasing vineyard resilience to climate variability. Overall, the use of ameliorative species can significantly support the maintenance of fertile and balanced soils in viticultural systems.

Table 3. Effect of soil maintenance systems on soil nutrients (% compared to the control)

Indicator	a1	Soil maintenance systems (a2-a6)
Total nitrogen (IN %)	1.61 (0%)	a2: 1.77 (+9.9%), a3: 1.81 (+12.4%), a4: 1.86 (+15.5%), a5: 1.94 (+20.5%), a6: 2.03 (+26.1%)
Available phosphorus (ppm)	65 (0%)	a2: 71 (+9.2%), a3: 75 (+15.4%), a4: 78 (+20.0%), a5: 82 (+26.2%), a6: 85 (+30.8%)
Available potassium (ppm)	145 (0%)	a2: 160 (+10.3%), a3: 164 (+13.1%), a4: 170 (+17.2%), a5: 179 (+23.4%), a6: 185 (+27.6%)
Fe (ppm)	4.30 (0%)	a2: 4.43 (+3.0%), a3: 4.52 (+5.1%), a4: 4.60 (+7.0%), a5: 4.64 (+7.9%), a6: 4.73 (+10.0%)
Mn (ppm)	5.60 (0%)	a2: 5.88 (+5.0%), a3: 6.05 (+8.0%), a4: 6.16 (+10.0%), a5: 6.27 (+11.9%), a6: 6.44 (+15.0%)
Cu (ppm)	11.8 (0%)	a2: 11.92 (+1.0%), a3: 12.04 (+2.0%), a4: 12.15 (+2.9%), a5: 12.27 (+4.0%), a6: 12.39 (+5.0%)
Zn (ppm)	5.0 (0%)	a2: 5.05 (+1.0%), a3: 5.10 (+2.0%), a4: 5.15 (+3.0%), a5: 5.20 (+4.0%), a6: 5.25 (+5.0%)
B (ppm)	1.60 (0%)	a2: 1.62 (+1.25%), a3: 1.63 (+1.88%), a4: 1.63 (+1.88%), a5: 1.64 (+2.5%), a6: 1.65 (+3.1%)

Soil loss measurements demonstrated a significant effect of soil maintenance systems on the intensity of erosional processes in the vineyard. The control treatment (black field, a1) exhibited the highest soil loss, reaching 5.8 t/ha/year. In comparison, all vegetative cover systems reduced the amount of soil removed by erosion, demonstrating the effectiveness of cover vegetation in limiting soil degradation.

The lowest anti-erosion efficiency was observed for the annual *Phacelia* treatment (a4), where soil losses decreased to 3.4 t/ha/year (-41%), a reduction that was not statistically significant. In contrast, permanent vegetative cover systems showed clearly superior protective capacity. Permanent grass with spontaneous flora (a2) reduced erosion to 2.9 t/ha/year (-50%, $p < 0.05$), and lawn (a3) reduced it to 2.4 t/ha/year (-59%, $p < 0.01$), (Table 4).

Table 4. Effect of soil maintenance systems on soil erosion (% compared to the control)

Variant	Description	Soil loss (t/ha/year)
a1	Black field (Mt)	5.8
a2	Permanent grass with spontaneous flora	2.9* (-50%)
a3	Lawn mixture	2.4** (-59%)
a4	<i>Phacelia tanacetifolia</i>	3.4 ns (-41%)
a5	White dwarf clover +lawn	2.2** (-62%)
a6	Vetch + lawn	2.1** (-64%)

Significance levels: $p < 0.05$; $p < 0.01$; ns =not significant

The greatest reductions in soil loss were observed in treatments combining legumes and grasses: white dwarf clover + lawn (a5) and vetch +lawn (a6) limited erosion to 2.2 and 2.1 t/ha/year, respectively (-62 to -64%, $p < 0.01$).

The superior efficiency of these systems can be attributed to dense and continuous vegetative cover, the development of extensive root systems, and improved structural stability of soil aggregates. These factors reduce the kinetic energy of raindrops, decrease surface runoff, and limit the mobilization of fine soil particles. Overall, the results emphasize the importance of maintaining permanent vegetation to mitigate soil degradation and preserve nutrient reserves in non-irrigated vineyards.

The implementation of different vegetative cover systems also led to increased biological diversity compared to the black field control (a1), although the intensity of this effect depended on the type of vegetation (Table 5).

Table 5. Effect of soil maintenance systems on floral and faunal biodiversity (% compared to the control)

Variant	Description	Biodiversity index (%)
a1	Black field (Mt)	22
a2	Permanent grass with spontaneous flora	28–30* (+25-35%)
a3	Lawn mixture	32* (+35-45%)
a4	<i>Phacelia tanacetifolia</i>	27 ns (+15-20%)
a5	White dwarf clover +lawn	34** (+50-60%)
a6	Vetch + lawn	35** (+55-65%)

Significance levels: $p < 0.05$; $p < 0.01$; ns = not significant

Simple lawn and permanent grass with spontaneous flora stimulated a moderate biodiversity, ranging from 25 to 45% and statistically significant. Treatments combining legumes with grasses (white dwarf clover +lawn and vetch +lawn) had a more pronounced impact, increasing biodiversity by 50-65% ($p < 0.01$) and creating a favorable environment for both soil-associated flora and fauna. Annual *Phacelia* cultivation produced a more modest increase (15-20%), which was not statistically significant, indicating that medium-term promotion of biodiversity is more efficiently achieved through permanent mixed cover with legumes and grasses.

The combination of grasses with nitrogen-fixing species (white dwarf clover or vetch) provides a dual benefit: limiting soil erosion while

promoting a more diverse and balanced ecosystem, thus enhancing the resilience and stability of the vineyard.

Compared to the black field control (a1), permanent grass with spontaneous flora (a2) and the lawn treatment (a3) resulted in minor yield increases of +2.1% and +0.8%, respectively, which were not statistically significant (ns). In contrast, the introduction of ameliorative species led to substantial yield gains. Annual *Phacelia tanacetifolia* (a4) increased yield by +29.2% relative to the control, while the white dwarf clover + lawn mixture (a5) produced a +39.6% increase; both showed statistically significant differences ($p<0.01$). The highest yield gain was observed in the vetch + lawn treatment (a6), which exceeded the control by +50.0%, a highly significant difference ($p<0.001$) (Table 6).

Table 6. Effect of soil maintenance systems on grape yield per vine (kg/vine)

Variant	Description	Grape yield kg/vine
a1	Black field (Mt)	2.640
a2	Permanent grass with spontaneous flora	2.695 (ns)
a3	Lawn mixture	2.662 (ns)
a4	<i>Phacelia tanacetifolia</i>	3.410**
a5	White dwarf clover +lawn	3.685**
a6	Vetch + lawn	3.960***

The observed differences in grape yield were accompanied by notable effects on fruit composition, indicating that soil maintenance systems not only influence vine productivity but also affect grape quality parameters.

Permanent grass with spontaneous flora (a2) and the lawn treatment (a3) did not induce significant changes in must composition compared to the control, maintaining similar sugar levels (228-229.5 g/L) and total acidity (6.20-6.25 g/L), with a stable gluco-acidimetric index (~36-37, ns). In contrast, the introduction of ameliorative species led to marked increases in sugar content (237-240 g/L) along with a concomitant reduction in total acidity (5.30-5.50 g/L), resulting in significantly higher gluco-acidimetric indices (43-45) (Table 7).

These results demonstrate that the use of ameliorative species in permanent grass cover contributes to achieving optimal grape ripeness, favoring the production of balanced must suitable for high-quality wine.

Table 7. Effect of soil maintenance systems on grape quality

Variant	Sugar (g/L)	Total acidity (g/L tartaric acid)	Gluco-acidimetric index
a1	230.0	6.30	36.5
a2	228.0 (ns)	6.25	36.5
a3	229.5(ns)	6.20	37.0
a4	238.0**	5.40	44.1
a5	240.0**	5.30	45.3
a6	237.0***	5.50	43.1

The values of the Synthetic Soil Quality Index revealed a clear gradient in soil quality depending on the soil maintenance system, ranging from the lowest score under the black field control (0.00) to the highest in the vetch+lawn treatment (1.00) (Table 8).

Table 8. Synthetic Soil Quality Index under different soil maintenance systems

Variant	Description	Synthetic Soil Quality Index
a1	Black field (Mt)	0.00
a2	Permanent grass with spontaneous flora	0.33
a3	Lawn mixture	0.47
a4	<i>Phacelia tanacetifolia</i>	0.63
a5	White dwarf clover +lawn	0.79
a6	Vetch + lawn	1.00

Treatments including legumes and legume-grass mixtures (a5 and a6) exhibited the highest scores, reflecting simultaneous improvements in soil structure, water retention, humus accumulation, nitrogen content, and mobilization of macro- and micronutrients. The results of the Synthetic Soil Quality Index confirm that permanent soil cover contributes to the stability and resilience of the vineyard ecosystem, providing favorable conditions for long-term productivity and sustainability.

CONCLUSIONS

The results of this study clearly demonstrate that permanent soil cover in vineyards, particularly through the use of legumes and legume-grass mixtures, substantially improves soil fertility, structure, and overall ecosystem resilience. Treatments incorporating ameliorative species led to significant increases in soil organic matter, enhanced water-holding capacity, and reduced compaction, while simultaneously improving the availability of macro and micronutrients.

These improvements were reflected in the Synthetic Soil Quality Index, which reached its highest values under legume-grass mixtures, confirming the cumulative benefits of these systems on soil physical and chemical properties.

Vegetative cover also played a key role in reducing soil erosion, with legume-grass mixtures limiting soil loss by over 60% compared to the black field control.

In parallel, biodiversity indices indicated that permanent and diverse cover promoted a richer floral and faunal community, contributing to a more balanced and resilient vineyard ecosystem. The positive effects of soil maintenance systems extended to vine performance. While simple lawn or spontaneous grass cover had minimal impact on yield and grape composition, the inclusion of ameliorative species resulted in substantial increases in grape production, coupled with improved must quality, as evidenced by higher sugar content, reduced total acidity, and elevated gluco-acidimetric indices. These outcomes highlight the dual benefit of such practices in enhancing both productivity and fruit quality. Overall, the study confirms that integrating legumes and mixed cover crops in vineyard soil management represents an effective, sustainable strategy to enhance soil health, maintain long-term fertility, mitigate erosion, promote biodiversity, and improve both yield and grape quality.

This approach provides a resilient framework for viticultural systems facing environmental and climatic challenges, supporting sustainable production and long-term ecosystem stability.

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BUNCH THINNING AND ITS EFFECT ON WINE QUALITY IN SYRAH VARIETY CLONES

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Abstract

The experiment was conducted during the period 2020-2022 at the Agricultural University-Plovdiv. A unified technological scheme for the production of red dry wines was used. The object of the study was clones from the Syrah variety numbered 100, 174, 470 and 524 grafted onto SO4 rootstock. After flowering, at a berry size of 4 to 6 mm (BBCH - 75), bunch thinning was applied; the number of clusters was reduced to 60% (8 clusters per vine). The following physicochemical indicators determining wine quality and differences were found in the analysis of relative density (g/dm^3); alcohol (%); sugars (g/dm^3); total extract (g/dm^3); sugar-free extract (g/dm^3); titratable acids (g/dm^3); volatile acids (g/dm^3); free sulfur dioxide (mg/dm^3); total sulfur dioxide (mg/dm^3); total phenols (mg/dm^3). Bunch thinning had a positive impact, expressed in the higher average content of total extract (30.00 g/dm^3), sugar-free extract (28.53 g/dm^3) and total phenols (1884.42 mg/dm^3). Selective bunch thinning during vine vegetative growth is a technological practice that positively affects the wine quality obtained from vines with reduced yield.

Key words: clones, green pruning, Syrah variety, red wine, wine analysis.

INTRODUCTION

Clonal selection allows the control of viral diseases and the selection of genotypes according to agronomic, viticultural, or oenological criteria (Roby et al., 2014).

The clone selection of renowned varieties, more adapted to the predicted climatic conditions, can help maintain the typicality of the wine (Arrizabalaga-Arriazu et al., 2020).

Bunch thinning is a common technique in viticulture to modulate the balance between foliage and yield in order to improve fruit quality and increase the level of secondary metabolites (Alem et al., 2019).

According to Meillon et al. (2010) it affects the reduction of yield, the advancement of grape maturity, the improvement of the phenolic content of the grapes and therefore of the wine. Reducing the number of clusters in the "pea-sized" phase has a beneficial effect on the components determining the technological maturity of grapes - the dynamics of sugar content and titratable acids (Popova, & Angelov, 2024).

Other studies on fruit and wine composition have shown that bunch thinning increases anthocyanin and phenolic content (Guidoni et al., 2002; Keller et al., 2005; Nuzzo & Matthews, 2006), while pH and titratable acidity

results vary (Nuzzo & Matthews, 2006; Intrigliolo & Castel, 2011; Preszler et al., 2013). Various authors have observed an advance in fruit ripeness and increased sugar accumulation (Nuzzo & Matthews, 2006; Reynolds et al., 1994; Bowen et al., 2011). In 1980, Freeman et al. found no consistent effects on wine sensory scores due to yield reduction, while Chapman et al. (2004) reported improvements in wine aromas as a consequence of thinning.

Piarnas et al. (2022), cites authors who have studied the impact of reducing the number of bunches on wine grape ripening, as well as on wine composition and quality (Gil-Muñoz et al., 2009; Gatti et al., 2012; Keller et al., 2005; Diago et al., 2010). In addition to improving the phenolic content of wine grapes, thinning of bunches has the ability to reduce total acidity and increase total soluble solids and pH (Nuzzo & Matthews, 2006).

Nevertheless, Diago et al. (2010) reported that this agronomic method showed a positive effect on wine quality, associated with an increase in berry size, which reduced the skin-to-mesocarp ratio.

The physicochemical analysis of wine is the basis for controlling and monitoring the technological flow of wine production (Răducu et al., 2019).

Syrah can produce high-quality wine with a bluish color, a fine, intense aroma, and low acidity (Hanlin et al., 2011).

The main objective of this study was to evaluate the impact of grape thinning on wine quality in clones 100, 174, 470 and 524 of the Syrah variety, grown in the Brestnik village area.

MATERIALS AND METHODS

The subject of the study was Syrah variety clones numbered 100, 174, 470 and 524, distinguished by valuable economic and technological qualities. Clone 100 is for producing wines with lower sugar content, neutral and plain; clone 174 is for high-quality wines; clone 470 is well-coloured, with excellent sugars and good tannin-structure wines; and clone 524 is balanced with medium to high sugar content (ENTAV-INRA-ENSA-M-ONIVINS, 1995). Vines were grafted onto Berlandieri x Riparia SO4 rootstock, planted in April 2011 in the experimental field of Viticulture at the Agricultural University - Plovdiv.

The vineyard is in full fruiting. The planting distance is 3.0 m between rows and 1.00 m between vines in the row - 3330 pcs per ha. The vines are trained with a high stem on a double sided cordon with the corresponding support trellis. The bud loading in all variants was carried out by spur pruning two buds, a total of 6 spurs/12 buds/ per vine. The rows are grassed, and the soil surface between the vines is kept clean by applying herbicides. The vines are grown under non-irrigated conditions.

The rows' direction in the vineyard is northwest - southeast, with a slope of the terrain to the east of 3.2%/1.8% and an average altitude of -194 m. The following 8 variants were included in the scheme of the experimental work:

V1 - clone 100 - non-controlled yield;
V2 - clone 174 - non- controlled yield;
V3 - clone 470 - non- controlled yield;
V4 - clone 524 - non- controlled yield;
V5 - clone 100 - with controlled yield in the "pea size" phase (8 clusters per vine);
V6 - clone 174 - with controlled yield in the "pea size" phase (8 bunches per vine);
V7 - clone 470 - with controlled yield in the "pea size" phase (8 bunches per vine);
V8 - clone 524 - with controlled yield in the "pea size" phase (8 bunches per vine).

Each variant included 60 vines (4 replicates x 15 vines).

The yield rationing was carried out during the vegetation in the "pea size" phase, leaving 8 bunches per vine.

The grapes were harvested when they reached technological ripeness, and for the controlled variants (V5, V6, V7 and V8), it was carried out one week earlier than for the variants with non-controlled yield (V1, V2, V3 and V4). Wines were produced from each harvest according to the same technological scheme for the production of red quality wines - crushing, sulfitation (50 mg/kg SO₂), two hours after sulfitation, seeding with yeast Excellence XR - 20 g/hl and yeast food Vitaferment - 30 g, fermentation temperature 24-26°C, separation from solids, dosulfitation, storage.

At the winery lab, a physicochemical analysis of the experimental wines was carried out according to the methodology described in the Manual for Exercises in Winemaking (Bambalov, 2009). The following were studied: relative density (g/dm³); alcohol (%); sugars (g/dm³); total extract (g/dm³); sugar-free extract (g/dm³); titratable acids (g/dm³); volatile acids (g/dm³); free sulfur dioxide (mg/dm³); total sulfur dioxide (mg/dm³); total phenols (mg/dm³).

The climatic data for the experimental period were taken from the meteorological station located in the vineyard. The indicators are average monthly air temperature (°C) and the amount of precipitation (mm), which characterize the climatic features of the studied area. The graphs show their changes during the experimental years (Figures 1 and 2).

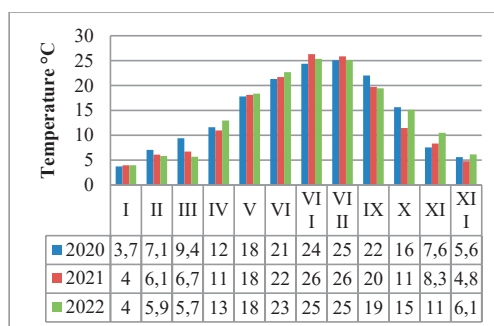


Figure 1. Average monthly air temperature, 2020-2022

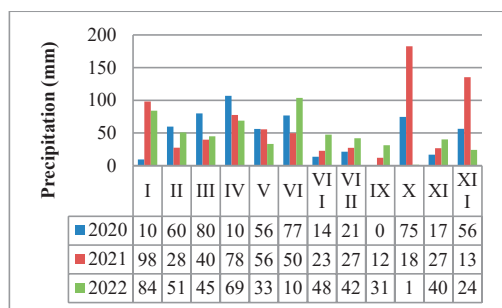


Figure 2. Precipitation, 2020-2022

The correlation analysis was performed using MS Excel.

RESULTS AND DISCUSSIONS

Physicochemical analysis show significant differences in their analytical composition, depending on both the agricultural cultivation technique (standardized and non-standardized variants) and the grape variety clone. The alcohol and titratable acid content are within optimal limits (Figure 3), with the differences between the variants with controlled and non-controlled yield being minimal, since all experimental variants were harvested at approximately the same level of ripeness, which was reached earlier in the variants with standardized yield.

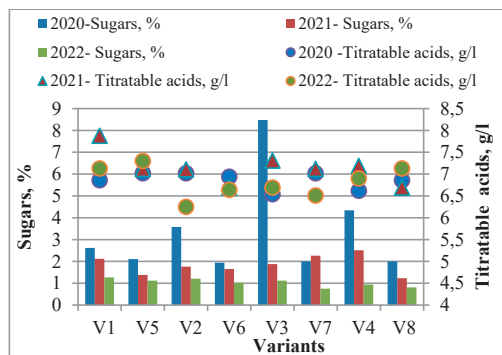


Figure 3. Sugars and titratable acids in wine, 2020-2022

The wines from the 2020 harvest have the highest alcohol content and the lowest titratable acidity, which is due to the very high initial sugar content in the berries.

A higher content of total and sugar-free extract was found in the wines from the 2020 and 2022 harvests (Table 1), compared to 2021, especially in the variants with controlled yield.

Table 1. Relative density, alcohol, total and sugar-free extract, 2020-2022

Variants / Clone	Period (Year)	Relative density	Alcohol %	Total extract, g/dm ³	Sugar-free extract, g/dm ³
V ₁ 100	2020	0.9917	15.10	28.70	26.08
	2021	0.9928	14.30	29.20	27.08
	2022	0.9927	14.30	28.90	27.63
	Average	0.9924	14.56	28.93	26.93
V ₂ 174	2020	0.9931	14.30	30.00	26.42
	2021	0.9930	14.12	29.20	27.44
	2022	0.9939	13.15	28.70	27.49
	Average	0.9933	13.85	29.30	27.11
V ₃ 470	2020	0.9937	15.91	36.20	27.72
	2021	0.9932	14.12	29.70	27.82
	2022	0.9936	13.41	28.70	27.58
	Average	0.9935	14.48	31.53	27.71
V ₄ 524	2020	0.9927	15.91	33.60	29.26
	2021	0.9932	14.12	29.70	27.19
	2022	0.9933	13.67	28.70	27.76
	Average	0.9931	14.56	30.66	28.07
V ₅ 100	2020	0.9924	15.28	31.00	28.89
	2021	0.9932	13.85	28.90	27.52
	2022	0.9931	14.56	30.70	29.58
	Average	0.9929	14.56	30.20	28.66
V ₆ 174	2020	0.9920	14.92	28.90	26.96
	2021	0.9936	13.59	29.20	27.55
	2022	0.9946	13.24	30.70	29.68
	Average	0.9934	13.92	29.60	28.06
V ₇ 470	2020	0.9920	15.37	30.20	28.20
	2021	0.9933	13.67	28.70	26.44
	2022	0.9943	13.50	30.70	29.95
	Average	0.9932	14.18	29.86	28.19
V ₈ 524	2020	0.9926	15.19	31.30	29.86
	2021	0.9935	13.32	28.10	26.87
	2022	0.9946	13.59	31.80	30.99
	Average	0.9935	14.03	30.40	29.24

This result is due to the significant rainfall during the grape ripening period in 2021, which led to waterlogging of grape juice and are the reason for the lower content of total and sugar-free extract in the wines from the 2021 harvest. Wines from clone 174 V2 (13.85%) and V6 (13.92%) have the lowest alcohol content. Wines from clone 524 in variants V4 and V8 from the 2020 and 2022 harvests have a higher content of total extract and sugar-free extract. Wines from clone 100 (V1 and V5) report a higher alcohol content (14.56%), which correlates with a higher sugar content of the grapes, both for the controlled and non-controlled variants. This is most valid for wines from the 2022 harvest and allows us to make the assumption that grapes from this clone have the highest sugar accumulation energy.

The wines from clone 100 had the highest content of total phenolic substances in V1 (1903.76 g/dm³) and V5 (2107.08 g/dm³), compared to wines from all other clones, which makes these wines better structured (Table 2).

Table 2. Content of volatile acids, free sulfur dioxide, total sulfur dioxide and total phenols, 2020-2022

Variants / Clone	Period (Year)	Volatile acids, g/dm ³	Free sulfur dioxide, g/dm ³	Total sulfur dioxide, g/dm ³	Total phenols, g/dm ³
V ₁ 100	2020	0.73	34.24	127.19	2514.40
	2021	0.42	8.11	35.13	1580.48
	2022	0.49	17.96	53.38	1616.40
	Average	0.54	20.10	71.90	1903.76
V ₂ 174	2020	0.77	30.58	133.92	2083.36
	2021	0.36	13.51	41.88	1495.17
	2022	0.37	16.47	53.14	1477.21
	Average	0.50	20.18	76.31	1685.24
V ₃ 470	2020	0.64	30.58	126.58	2370.72
	2021	0.44	16.21	59.44	1558.03
	2022	0.37	14.97	59.88	1373.94
	Average	0.48	20.58	81.96	1767.56
V ₄ 524	2020	0.43	39.14	128.42	2375.21
	2021	0.38	14.86	41.21	1454.76
	2022	0.42	13.47	57.63	1257.20
	Average	0.41	22.49	75.75	1695.72
V ₅ 100	2020	0.61	11.07	42.19	2765.84
	2021	0.31	13.51	43.23	1840.90
	2022	0.35	20.96	68.86	1714.50
	Average	0.42	15.18	51.43	2107.08
V ₆ 174	2020	0.51	8.56	38.52	2330.31
	2021	0.32	13.51	40.53	1516.20
	2022	0.35	19.46	59.13	1562.22
	Average	0.39	13.84	46.06	1802.91
V ₇ 470	2020	0.32	12.25	47.70	2440.80
	2021	0.47	14.86	42.56	1620.80
	2022	0.35	19.46	60.63	1484.30
	Average	0.38	15.52	66.20	1848.63
V ₈ 524	2020	0.48	10.40	46.11	2406.64
	2021	0.34	8.11	40.53	1544.20
	2022	0.36	17.96	59.88	1386.40
	Average	0.39	12.16	48.84	1779.08

The content of volatile acids for all wines was in the normal range, which is an indication of the conduct of pure alcoholic fermentation and their proper storage.

Summarized results from all years report differences in the physicochemical indicators of the wines from the studied clones in each harvest.

The interrelationships between chemical indicators that determine wine quality are important for optimizing the technological processes and for predicting the organoleptic wine properties.

Correlation analysis allows assessing the strength and direction of the dependencies between the different indicators.

A negative correlation was established between the alcohol content and the relative density in both variants (Table 3). The relationships vary from very strong to weak. Changes in relative density are due to variations in the alcohol content in which the accumulation of ethanol leads to a decrease in relative density.

Table 3. Correlations between wine indicators

Indicators	Variants	
	Non-controlled	Controlled
Clone 100		
alcohol ↔ relative density	r = - 0.90	r = - 0.59
alcohol ↔ residual sugar	r = + 0.77	r = + 0.72
alcohol ↔ sugar-free extract	r = - 0.59	r = + 0.81
phenols ↔ total extract	r = - 0.71	r = + 0.43
alcohol ↔ phenols	r = + 0.97	r = + 0.80
Clone 174		
alcohol ↔ relative density	r = - 0.25	r = - 0.20
alcohol ↔ residual sugar	r = + 0.76	r = + 0.90
alcohol ↔ sugar-free extract	r = + 0.47	r = - 0.56
phenols ↔ total extract	r = + 0.34	r = - 0.53
alcohol ↔ phenols	r = + 0.62	r = + 0.94
Clone 470		
alcohol ↔ relative density	r = - 0.27	r = - 0.32
alcohol ↔ residual sugar	r = + 0.98	r = + 0.47
alcohol ↔ sugar-free extract	r = + 0.98	r = + 0.08
phenols ↔ total extract	r = + 0.99	r = + 0.11
alcohol ↔ phenols	r = + 0.99	r = + 0.97
Clone 524		
alcohol ↔ relative density	r = - 0.61	r = - 0.35
alcohol ↔ residual sugar	r = + 0.92	r = + 0.87
alcohol ↔ sugar-free extract	r = + 0.97	r = + 0.56
phenols ↔ total extract	r = + 0.97	r = + 0.29
alcohol ↔ phenols	r = + 0.99	r = + 0.95

The results obtained are in accordance with the technological laws of alcoholic fermentation, in which an increase in alcohol content leads to a decrease in the relative density of the medium. The low value of the coefficients suggests that the relationship is not very pronounced and is probably influenced by additional factors.

A moderate to strong positive correlation was established between the alcohol content and residual sugar. The changes in residual sugar are due to variations in the alcohol content. The differences between the two variants suggest the presence of different biochemical factors affecting the fermentation process.

A different nature of the correlation between the alcohol content and sugar-free extract was established between the two variants in the wines from clones 100 and 174. A moderate to strong negative and very strong positive correlation was observed.

In clones 470 and 524, different results were observed. From a very strong positive correlation to no significant relationship between the indicators (r = 0.06). The variation of the sugar-free extract is due to the changes in alcohol.

Results show different dynamics of extractive substances between the variants, which is due to variations in the primary must.

The correlation analysis between phenols and the total extract in clones 100 and 174 shows a different relationship in the two variants. From a strong negative relationship ($r = -0.71$) to a moderate and positive one ($r = +0.43$). Phenols have an impact on the total extract. In clones 470 and 524 is very strong positive relationship ($r = +0.99$) in the non-controlled variant, compared a non-existent one ($r = +0.11$) in the controlled one.

The must composition and the concentration of phenols have a significant impact on the extractive substances.

The analysis between the alcohol content and phenols shows a strong positive relationship in both variants. Alcohol is the strongest factor determining the concentration of phenols and highlights the importance of alcohol levels for the organoleptic and antioxidant wine properties.

Alcohol content shows the strongest positive correlation with phenols, $r \geq 0.62$, as in all variants; and in the non-controlled variants, the relationship with phenols reaches a value of $r = 0.99$. That is why the alcohol is the best predictor of phenolic concentration and extractives, which is important for the taste and antioxidant potential of the wine.

Residual sugar has a moderate to strong relationship with alcohol, which shows that other factors affect the density, including alcohol content and total extract.

Phenols have a strong relationship with total extract in the non-controlled variant, but in the controlled one this relationship is weak. The grape composition significantly affects the extractive and phenolic components.

CONCLUSIONS

Yield regulation is one of the most important tools in the production of quality wines with a controlled designation of origin, and the results of the study clearly illustrate this.

During the experiment, was found that the variants with controlled yield (V5, V6, V7 and V8), have a higher content of total and sugar-free extract, and total phenolic substances, compared to the variants with non-controlled yield (V1, V2, V3 and V4).

The reduction of bunch numbers has an impact on extremely important indicators like the

dynamics of sugar content and titratable acids in the grapes.

Within the experimental period, all variants reach the technological ripeness phase relatively early (first part of September) for the studied region. The grape harvest from vines with controlled yield precedes those with non-controlled yield by approximately one week. Correlation analysis shows that alcohol and phenols are major indicators of wine quality, while total extract and residual sugar have a more moderate influence on wine density.

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STUDY ON THE CARBOHYDRATE RESERVE OF SOME ROMANIAN ANCESTRAL VINE CULTIVARS IN ODOBEȘTI VINEYARD, VRANCEA COUNTY

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Abstract

The carbohydrates are produced by the leaves and stored in the form of starch and soluble sugars in the perennial organs of the vine. The carbohydrate reserve plays an essential role in the growth process of the vine, being the main source of carbon necessary for the start of vegetation in the following season. This paper evaluates the state of the carbohydrate reserve in the vines during the vegetative dormancy period of some ancestral Romanian grape cultivars in the climatic conditions of the Odobești vineyard, Vrancea. Five old cultivars that were part of the traditional assortment of the vineyard were studied: 'Galbenă de Odobești', 'Cruciuliță', 'Pârciu', 'Gordin' and 'Razachie albă'. The content of soluble sugars, starch content, and the amount of total carbohydrates were determined by the anthrone reagent method. Under the climatic conditions of the 2024 wine year, the analyzed cultivars presented a sufficient carbohydrate reserve for the first vegetation phenophases of the 2025 wine year, two of them being classified as very well matured, having a total carbohydrate content of over 18 g 100 g⁻¹ DM.

Key words: grapevine, total carbohydrates, soluble sugars, starch.

INTRODUCTION

Monitoring carbohydrate reserves (mostly starch, but also soluble sugar) in grapevines during dormancy is important because carbohydrate reserves will influence shoot growth and reproductive development in the following season (Bennett et al., 2005). Carbohydrates are produced by the leaves and stored as starch and soluble sugars (mainly glucose, fructose and sucrose) in perennial organs (Bennet et al., 2005; Pellegrino et al., 2014; Călugăr et al., 2022). In the literature, the state of carbohydrate reserves in different parts of plants has been described by various authors (Warmund et al., 1986; Koblet and Perret, 1990; Candolfi-Vasconcelos et al., 1994; Zapata et al., 2004; Schreiner et al., 2012; Zufferey et al., 2012; Vaillant-Gaveau et al., 2014; Cimpoi et al., 2020; Muntean et al., 2023).

In wood, starch concentrations are higher than those of sugars during the growing season, but some of the starch is converted to sugars before the vegetative dormancy period to help protect the vine from cold damage during winter (Noronha et al., 2018; Călugăr et al., 2019; Popova, 2021). Shoot maturation is a

physiological process of accumulation of reserve substances, which is highlighted in autumn by the change in bark color and marks the transition from the active vegetation period to the vegetative rest period (Alexandrescu et al., 1994). The process of shoot maturation begins in mid-August, with the slowdown in growth, from the base of the shoots and gradually advances towards their tip. Along the shoot, there is a maturation gap, in the sense that the internodes at the base accumulate a greater amount of carbohydrates than those oriented towards the tip of the shoot. The maturation process ends with the fall of the leaves; therefore, at the end of the vegetation period, the tips of the shoots remain unripe, often being affected by early frosts in autumn (Țârdea and Dejeu, 1995). The dynamics of carbohydrate reserves in perennial grapevine organs are mainly determined by growth and maintenance requirements at certain developmental stages. This balance between carbon supply and demand is influenced by manageable factors such as grape yield, trunk size, and water supply, as well as by limiting climatic factors such as temperature during the growing season, which affect photosynthesis and therefore the net

carbon assimilation capacity (Holzapfel et al., 2010). Lighter pruning generally leads to a higher proportion of old wood and higher yields (Clingeffer and Pellegrino, 2006). Climate is a major interactive factor influencing the dynamics of carbohydrate reserves in perennial tissues, in addition to crop load (Holzapfel and Smith, 2012). The concentration of soluble carbohydrates in the trunk has been shown to be negatively correlated with average air temperature (Zufferey et al., 2012). Warm temperatures are likely to have a negative impact on the carbon balance through faster canopy development and improved respiration rates, while the source activity (assimilation rate) may stabilize (Schultz, 2000; Lebon et al., 2004).

This study aims to evaluate the carbohydrate content in the cords (the most reliable laboratory indicator for assessing the degree of wood maturation) of five ancestral grapevine varieties under the conditions of the 2024 wine year in the Odobești wine area, in order to anticipate the evolution of the vegetation period in the 2025 wine year.

MATERIALS AND METHODS

The study was conducted during the vegetative rest period in the winter of 2024-2025. Five ancestral Romanian grape cultivars were investigated: four cultivars for wine grapes ('Galbenă de Odobești', 'Cruciuliță', 'Pârciu', 'Gordin') and one cultivar for table grapes - 'Razachie albă' grafted on the 'Berlandieri x Riparia Teleky 4B SO4-4' rootstock, located within the Ampelographic Collection of the Research and Development Station for Viticulture and Oenology Odobești.

The Ampelographic Collection was established in 2012, within the experimental base no. 1 Odobești (45°45'48"N; 27°04'04"E). The soil is of medium leached chernozem type, with an average humus content (2.0-2.3%) and a good supply of nutrients; the slope of the land is 1-2% with a southeast exposure. The planting density is 4132 vines/ha (2.2 m × 1.1 m), and the form of the trunks is semi-high; the pruning system – mixed; the type of pruning – multiple Guyot, with the load distributed over 3 fruiting rings consisting of 3 canes with 8-9 eyes and 3 fruiting cones with 2-3 eyes.

To determine the carbohydrate content, annual cane samples were collected from each cultivar (in three repetitions) on January 21, 2025. The determined parameters included cane diameter, water content, and cane carbohydrate content (sugars, starch, and total carbohydrate content). The diameter of canes was measured with a caliper; 24 measurements were made for each sample. The water content of canes was determined using the oven-drying method at a temperature of 55°C to constant mass. The canes were cut into pieces of approximately 2 cm and dried in a drying oven (SLN STD 115, POL EKO Aparature sp.j, Poland), at 55°C, for approximately 72 hours (until constant mass). Then, the samples were ground, sieved (sizes of max. 0.7 mm), packaged and labeled accordingly, and stored at room temperature until analysis (Figure 1).



Figure 1. Samples prepared for analysis

The degree of maturation of the samples was assessed by determining the total carbohydrate content (TCH) using the colorimetric method adapted from Dubois et al. (1956). The principle of the method consists in the extraction of sugars with ethyl alcohol and the extraction of starch with perchloric acid, under determined conditions, and measuring the color intensity after treating the extracts with the anthrone reagent.

The measurement of color intensity was performed spectrophotometrically using a UV/VIS spectrophotometer (Specord Plus 200, Analytik Jena, Konrad, Germany) at a wavelength of 620 nm. The total carbohydrate content (TCH) of the samples sums the sugar and starch content, expressed as glucose (in



Figure 2. Aspects regarding the extraction and preparation of starch and soluble sugar solutions for the determination of carbohydrates in canes

grams) and expressed as a percentage of dry mass (DM), and the results obtained were evaluated as follows:

- TCH < 12 g 100g⁻¹DM - very poorly matured
- TCH between 12-14 g 100g⁻¹DM - poorly matured
- TCH between 14-16 g 100g⁻¹DM - matured
- TCH between 16-18 g 100g⁻¹DM - well matured
- TCH > 18 g 100g⁻¹DM - very well matured

The data were analyzed using analysis of variance (Anova) and the Student's t-test ($p \leq 0.05$) via Microsoft Excel; Data Analysis was performed to highlight significant differences among the analyzed cultivars.

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. 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 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 had temperatures above 35°C. The total amount of precipitation recorded during the vegetation period was 222.1 mm, representing only 64.6% of the multiannual amount (343.7 mm). Only 7 days with precipitation greater than 10 mm were recorded during this period. During the vegetative dormancy period 2024-2025, in the Odobești wine-growing area, the lowest temperatures were recorded for very short periods at the end of February (-10.0...-12.0°C), far from the frost resistance of the vine (-15±3°C).

From a thermal point of view, the dormancy period associated with the winter of 2024-2025 benefited from excess average air temperature values in December and January and deficit values for November and February, compared with multiannual values.

Table 1. Main climatic conditions during the grapevine growing season and the dormancy period (Odobești, April 2024 - February 2025)

Climate parameters	2024									2025	
	IV	V	VI	VII	VIII	IX	X	XI	XII	I	II
Average temperature (°C)	15.5	16.6	24.2	26.8	25.4	19.6	12.6	4.6	2.7	3.3	-1.6
Temp. Min. abs.	4.2	6.2	14.3	15.2	16.8	9.1	0.5	-5.0	-4.6	-5.8	-11.7
Temp. Max abs.	32.1	28.8	35.9	40.7	35.8	33.6	25.3	20.7	13.0	15.5	10.9
Precipitation (mm)	33.9	28.8	50.4	51.8	24.0	57.3	19.5	32.9	96.5	9.4	4.3

With the exception of December, the rainfall regime during this period was deficient compared with multiannual precipitation amounts for this interval.

Given the fact that in 2024 the grape production ranged between 2.26 kg/vine for 'Pârciu' and 4.05 kg/vine for 'Razachie albă', the analyzed cane samples recorded a normal humidity level for the period in which the canes were collected, oscillating around 42%. The lowest humidity level, 40.51%, was observed for the 'Pârciu' sample, and the highest humidity level, 44.94%, was recorded for 'Gordin' samples (Table 2).

The canes diameter ranged between 6.72 mm for the 'Razachie albă' sample and 8.12 mm for the 'Pârciu' sample. The total carbohydrate content varied between 12.89 g 100g⁻¹ DM for 'Gordin' variety and 24.39 g 100g⁻¹ DM for 'Cruciuliță' variety; values that are above the threshold of 12 g 100g⁻¹ DM, which, according to STAS 220-

10/85 allows the material to be used for propagation.

Table 2. Results regarding cane water content, diameter and grape yield obtained in 2024

Crt. no.	Ancestral grape cultivar	Humidity [%]	Diameter [mm]	Yield [kg/vine]
1.	'Galbenă de Odobești'	43.12	7.78	3.09
2.	'Cruciuliță'	41.70	7.16	3.93
3.	'Pârciu'	40.51	8.12	2.26
4.	'Gordin'	44.94	6.89	3.76
5.	'Razachie albă'	43.08	6.72	4.05

The values obtained for the total carbohydrate reserve place the samples from 'Gordin' and 'Razachie albă' in the poorly matured category, the canes from 'Galbenă de Odobești' well-matured category, and the samples from 'Cruciuliță' and 'Pârciu' very well-matured category (Table 3).

Table 3. Results regarding the carbohydrate content in canes of Romanian ancestral cultivars (Odobești, 2024-2025)

Crt. No.	Ancestral grape cultivar	Soluble sugars content, [g 100g ⁻¹ DM]	Starch content, [g 100g ⁻¹ DM]	Total carbohydrate content, [g 100g ⁻¹ DM]	Interpretation
1.	'Galbenă de Odobești'	8.94	8.50	17.44	well matured canes
2.	'Cruciuliță'	8.49	15.90	24.39	very matured canes
3.	'Pârciu'	8.59	10.49	19.08	very matured canes
4.	'Gordin'	5.56	7.33	12.89	poorly matured
5.	'Razachie albă'	7.18	6.55	13.73	poorly matured

The distribution of total carbohydrate content along the length of the canes (base, middle, and top) highlights higher total carbohydrate reserves in the middle and base areas.

Regarding the content of soluble sugars, 'Gordin' and 'Razachie albă' samples recorded a lower sugar content ranging between 5.56 g 100g⁻¹ DM and 7.18 g 100g⁻¹ DM (Figure 3).

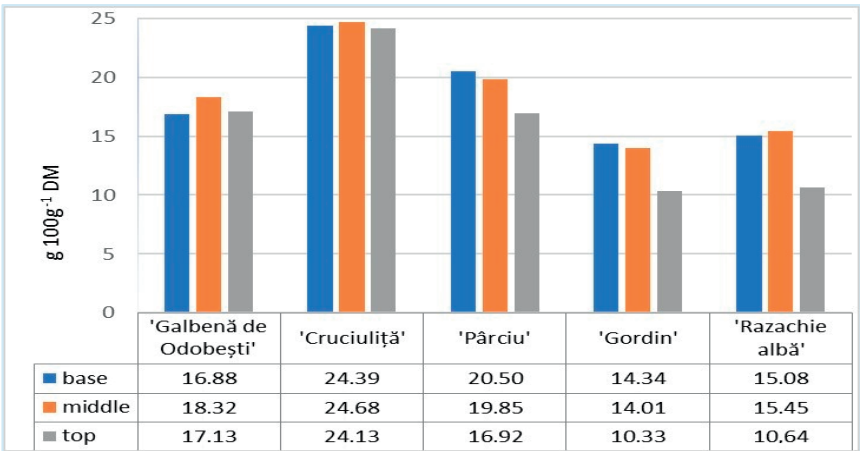


Figure 3. Distribution of total carbohydrate reserves across the canes (Odobești, 2024-2025)

The samples from 'Galbenă de Odobești', 'Pârciu' and 'Cruciuliță' cultivars recorded significantly higher and relatively similar sugar content values ranging between 8.94 and 8.49 g 100g⁻¹ DM.

The distribution of the soluble sugar reserve along the length of the canes shows higher amounts of soluble sugars in the top area for the 'Cruciuliță' and 'Pârciu' samples (11.34 g 100g⁻¹ DM, respectively 8.95 g 100g⁻¹ DM) and in the middle area for 'Galbenă de Odobești' (9.88 g 100g⁻¹ DM), 'Gordin' (6.37 g 100g⁻¹ DM) and 'Razachie albă' samples (8.61 g 100g⁻¹ DM) (Figure 4).

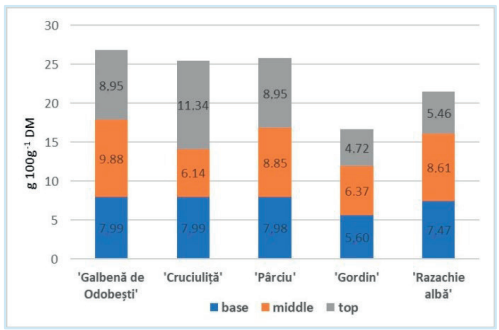


Figure 4. Distribution of the soluble sugar across the canes (Odobești, 2024-2025)

Compared to the soluble sugar reserve, the starch content in the canes varied within wider limits, with values ranging between 6.55 and 15.90 g 100g⁻¹ DM. The differences are significant among most of the analyzed samples, with the highest starch content being recorded for the 'Cruciuliță' cultivar, followed by 'Pârciu',

'Galbenă de Odobești' and 'Gordin'. A lower starch content was determined in the 'Razachie albă' (6.55 g 100 g⁻¹ DM).

The distribution of starch reserves along the canes shows higher amounts accumulated in the basal area for the 'Galbenă de Odobești', 'Pârciu', 'Gordin' and 'Razachie albă' samples (between 7.61 g 100 g⁻¹ DM and 12.52 g 100 g⁻¹ DM) and in the middle area for 'Cruciuliță' sample (18.53 g 100g⁻¹ DM) (Figure 5).

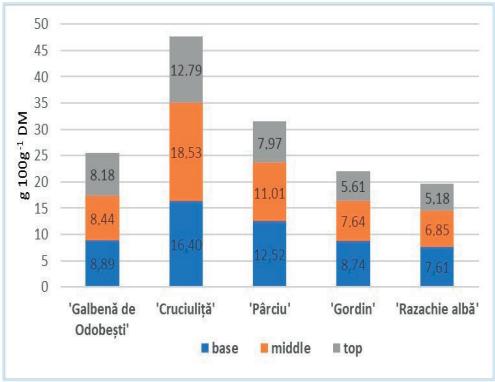


Figure 5. Distribution of the starch across the canes (Odobești, 2024-2025)

The statistical interpretation of the experimental data obtained for the total carbohydrate content (TCH) and for the starch content of the canes, based analysis of variance (Anova test), shows significant and highly significant differences between the analyzed cultivars, the value of the parameter P (0.000102, respectively 0.00086) being much lower than P<0.05 (Table 4).

Table 4. Statistical interpretation of experimental data obtained through analysis of variance (Anova test)

Source of Variation	SS	df	MS	F	P-value	F crit
Carbohydrate content in canes						
Between Groups	256.7518	4	64.18796	19.52082	0.000102	3.47805
Within Groups	32.88179	10	3.288179			
Total	289.6336	14				
Starch content						
Between Groups	168.3687	4	42.09274	11.69843	0.00086	3.47805
Within Groups	35.9810	10	3.59810			
Total	204.349	14				

The significance of the differences between the grapevine cultivars studied regarding the total carbohydrate content in the canes was established with the Student's t-test via

Microsoft Excel - Data Analysis; the calculated P values being presented in Table 5.

Regarding the total carbohydrate content, according to the P > Pcritical value, the sample

from the 'Cruciuliță' presents significant positive statistical differences compared to the other analyzed cultivars, and the 'Pârciu' sample also differs significantly from the 'Gordin' and 'Razachie albă' samples. The 'Galbenă de Odobești' sample also differs significantly from 'Gordin'.

Table 5. Significance of differences in total carbohydrate content (Student's t-test probability value, P critical <0.05)

Cultivar	'Galbenă de Od.'	'Cruciu -liță'	'Pârciu'	'Gordin'	'Razachie albă'
1	0				
2	0.000122*	0			
3	0.238291	0.008841*	0		
4	0.028622*	0.000885*	0.021559*	0	
5	0.081950	0.002361*	0.047602*	0.700872	0

The significance of the differences between the studied grapevine cultivars in terms of total starch content in the canes, established with the Student's t-test, are presented in Table 6.

Table 6. Significance of differences in starch content (Student's t-test probability value, P critical <0.05)

Cultivar	'Galbenă de Od.'	'Cruciu -liță'	'Pârciu'	'Gordin'	'Razachie albă'
1	0				
2	0.011809*	0			
3	0.215391	0.065106	0		
4	0.280145	0.010893*	0.122858	0	
5	0.058944	0.006806*	0.060078	0.538302	0

Regarding starch content, according to the $P > P_{critical}$ value, the sample from 'Cruciuliță' shows significant positive statistical differences compared with the samples 'Galbenă de Odobești' ($P=0.011809$), 'Gordin' ($P=0.010893$) and 'Razachie albă' ($P=0.006806$).

The relationship between the humidity content of the canes and the carbohydrate content was established by calculating the correlation coefficient (r), and the straight line and the regression equation were represented (Figure 6). From the graphic representation, it is observed that the relationship between the two variables is linear and indirect (negative), with an increase in the humidity content of the canes leading to a decrease in the total carbohydrate content. The value of the coefficient of determination R^2 (0.5152) indicates a significant dependence between the two parameters.

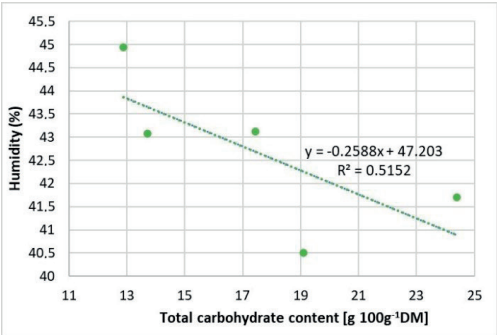


Figure 6. Correlation between total carbohydrate content and cane humidity during the dormancy period

The relationship between the humidity content of the canes and the content of soluble sugars was established by calculating the correlation coefficient (r), and the regression equation shows that the relationship between the two variables is linear and indirect (negative), with an increase in the humidity content of the canes leading to a decrease in the content of soluble sugars. The value of the coefficient of determination, R^2 (0.5982) indicates a significant dependence between the two parameters (Figure 7).

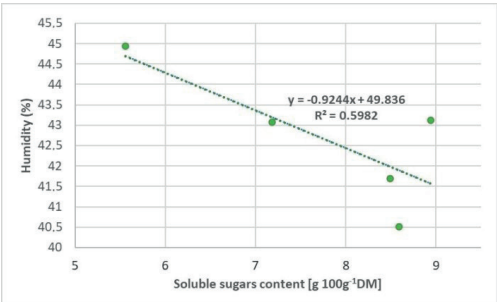


Figure 7. Correlation between soluble sugar content and cane humidity during the dormancy period

Grapevine cultivars that have a genetic tendency to contain more carbohydrates also tend to be more resistant to frost in winter. Scientific studies have shown that low bud viability in several grapevine cultivars is also due to low carbohydrate content, with high bud viability being correlated with a high amount of carbohydrates in the canes (Vasudevan, 1997).

Starch accumulation in grape canes and buds is associated with dormancy. Starch breakdown and the subsequent increase in sugar content are associated with cold hardiness (Rubio et al., 2015). The results of the present study are consistent with the data available in the literature. The shoot or cane is considered mature if, at the end of the vegetation period, the starch content is greater than 10 g 100 g⁻¹DM or the total carbohydrates are greater than 12 g 100 g⁻¹DM (Irimia, 2012). Holzapfel et al. (2010) consider that the shoot is fully mature (reaches a maximum degree of lignification) if, at the time of leaf fall, it has a total carbohydrate content of 19 g 100 g⁻¹DM.

CONCLUSIONS

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.

Under the climatic and technological conditions of the 2024 wine year, during the vegetative dormancy period 2024-2025, the five ancestral Romanian cultivars recorded a total carbohydrate content between 12.89 and 24.39 g 100g⁻¹ DM, with higher values at the base and middle of the cane.

The samples of 'Gordin' and 'Razachie albă' showed a lower carbohydrate reserve (12.89 and 13.73 g 100g⁻¹ DM, respectively), values that indicate poor maturation of the canes.

A very good maturation of the canes was found in the samples of 'Pârciu' and 'Cruciuliță', which accumulated a total amount of carbohydrates of 19.08 g 100g⁻¹ DM and 24.39 g 100 g⁻¹ DM, respectively.

The analyzed cane samples recorded a normal humidity level for the period in which they were collected, which ranged between 40.51%, for the 'Pârciu' samples and 44.94%, for the 'Gordin' samples.

The soluble sugar content recorded lower values in Gordin' samples (5.56 g 100 g⁻¹ DM) and higher values in the samples from 'Galbenă de Odobești', 'Cruciuliță', 'Pârciu' and 'Razachie albă' (between 7.18 and 8.94 g 100 g⁻¹ DM), with higher values at the top and middle of the cane. The starch content recorded high values for the samples from the 'Pârciu' and 'Cruciuliță' (10.49 g 100 g⁻¹ DM, respectively 15.90 g 100g⁻¹ DM) and lower values for the samples from 'Razachie

albă', 'Gordin' and 'Galbenă de Odobești' (between 6.55 and 8.50 g 100 g⁻¹ DM, with higher values at the base and middle of the cane).

The statistical interpretation of the experimental data obtained for total carbohydrate content (TCH) and starch content in the strings shows significant and very significant differences between the analyzed cultivars (P<0.05).

The results of the present study indicate that, between the studied wine and table cultivars, depending on the production direction, there is a significant difference in terms of starch content. The significant differences between the analyzed wine cultivars also show the different potential of genetic determinants for wood ripening, which can be correlated with frost resistance.

A negative correlation was revealed between cane humidity and total carbohydrate and soluble sugar content, while cane diameter and yield had no influence on carbohydrate content.

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THE INFLUENCE OF MACERATION DURATION AND TEMPERATURE ON THE CHARACTERISTICS OF BLACKBERRY WINE

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Abstract

*The production of fruit wines, particularly blackberry wine, is being improved to enhance the quality of the final product. This study aimed to investigate the influence of maceration duration and temperature on the characteristics of blackberry wine. Eight samples were prepared with the addition of a maceration enzyme, potassium metabisulfite, and yeast, with variations in sugar addition (0 and 20%), maceration temperature (20 and 25°C), and maceration duration (5 and 10 days). Analyses were performed on the prepared blackberry juice, must, and wines. Physicochemical properties were analyzed using standard methods recommended by the International Organization of Vine and Wine (OIV). Antioxidative properties included the determination of total phenolics, nonflavonoids, and flavonoids, as well as antioxidant activity (FRAP, DPPH, and ABTS assays). Antimicrobial activity was tested using the agar dilution method to determine the minimum inhibitory and bactericidal/fungicidal concentrations against selected bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes*), pathogenic yeast (*Candida albicans*), and probiotic cultures (*Lactobacillus plantarum* and *Saccharomyces boulardii*). Sensory evaluation of blackberry wines was conducted using the Buxbaum method. The results showed that prolonged maceration at lower temperatures and sugar addition increased the alcohol content, while higher maceration temperatures increased total acidity (6.23-10.60 g/L) and lower temperatures reduced volatile acidity (0.48-1.08 g/L). Shorter maceration at higher temperatures improved color intensity, whereas longer maceration at lower temperatures reduced color quality. The sample without added sugar, macerated for 5 days at 25 °C, showed the highest phenolic content and antioxidant activity, and all samples exhibited antibacterial activity, particularly against *B. cereus* and *P. aeruginosa*, but weak effects against yeast and probiotic cultures. Wines produced without sugar addition during fermentation were quite acidic and weak. By adding sugar, wines of good quality were obtained. Samples produced with sugar addition, shorter maceration, and lower temperature proved superior, and the alcohol content in these samples (>11 vol%) was considered satisfactory.*

Key words: blackberry wine, maceration time, maceration temperature, wine quality.

INTRODUCTION

Blackberries (*Rubus fruticosus* L.) are seasonal fruits that, under the tested climatic conditions, reach their technological ripeness from mid-July to late September. They are grown worldwide due to their delicious taste, pleasant flavor, and excellent nutritional profile. Blackberries contain dietary fibers, vitamin C (ascorbic acid), vitamin A, vitamin E, potassium, and calcium, along with phenolic metabolites that may offer health benefits. Blackberry phenolics have been linked to potential anticancer, antiproliferative, antineurodegenerative, anti-inflammatory, antidiarrheal, antidiabetic, antimicrobial, and antiviral activities. These fruits are enjoyed fresh or processed into various products such as

jam, wine, tea, ice cream, desserts, seedless jellies, and baked goods (Mikulic-Petkovsek et al., 2021; Savic et al., 2021).

Blackberry wine is one of the most common blackberry products and contains high levels of anthocyanins, polyphenols, and aromatic compounds that contribute to its color and aroma, as well as its antioxidant activity and potential to prevent chronic diseases (Wu et al., 2022). Nowadays, wines made from fruits other than grapes are gaining full acceptance in the market. The techniques used for fruit wine production are analogous to those applied for the production of grape wines and include alcoholic fermentation of fruit juice or pulp. The main steps of fruit wine production are: fruit reception, must preparation by crushing and

squeezing (pressing), maceration (pre-fermentation), clarifying, adjusting, spontaneous or inoculated fermentation (using selected wine yeasts), maturation, aging, and bottling (Velic et al., 2018).

The way the production process is carried out clearly affects the quality of the resulting wine. The preparation of fruit musts for wine production varies depending on the type of fruit. To correct imbalances in acidity or sugar levels, it is recommended to dilute the must with water to reduce acidity and to supplement it with sugar to achieve an appropriate balance (Velic et al., 2018). However, differences in raw materials often require special treatment techniques, distinguishing fruit winemaking from grape winemaking and affecting final quality (Tan et al., 2024). The maceration process plays an important role in the composition of the color and sensory properties of wine by facilitating the extraction of phenolic and aromatic compounds from fruits (Şener, 2018). Maceration duration strongly influences the phenolic composition and sensory attributes of red wines. Leaving the pulp in contact with the juice during the first week of maceration and fermentation intensifies the wine's body, character, and color, while extended maceration promotes the formation of polymeric pigments that enhance color stability (Feifel et al., 2025). Although 10-day maceration elevates overall phenolic content, it may also negatively affect sensory quality by intensifying astringency (Gu et al., 2025).

The efficiency of the maceration process is strongly dependent on temperature. Low-temperature prefermentative maceration (cold soaking at 10-15°C) is increasingly used to enhance wine color and aromatic expression (Sener and Yildirim, 2013), whereas increasing the maceration temperature from 15 to 35°C has been reported to raise phenolic concentrations by up to 300-fold (Varo et al., 2024). Although numerous studies have examined the effects of fermentation and maceration temperatures in grape wines, research addressing their influence in fruit-based winemaking remains comparatively limited.

As interest in fruit wines with enhanced functional attributes grows, evaluating technological factors such as maceration time and temperature is increasingly important for ensuring product quality. The optimization of

these parameters is crucial for achieving a well-balanced sensory profile in blackberry wine, with emphasis on the preservation of its natural color, aroma, and antioxidant capacity. The present study aims to evaluate the effects of varying maceration times and temperatures on the quality properties of blackberry wine and to establish the optimal conditions for the production of a premium-quality final product.

MATERIALS AND METHODS

Blackberry fruit samples of the Loch Ness variety came from the Experimental and Educational Center of the Faculty of Agriculture, University of Banja Luka, Bosnia and Herzegovina.

Sample preparation and fermentation. The samples and maceration parameters used: temperature, duration, and percentages of added sugar are presented in Table 1. Eight samples were prepared with the addition of maceration enzyme (40 mg/L), potassium metabisulfite (100 mg/L), and wine yeast (300 mg/L). In four samples (W2, W4, W6, and W8) white sugar was added to make the total sugar content 20%. After the end of the maceration process, decantation and mild sulphiting (potassium metabisulfite - 50 mg/l) were performed, and all samples were further fermented.

Table 1. Samples and maceration parameters

	temperature	added sugar	duration (days)
W1	20	-	5
W2		up to 20%	10
W3		-	
W4		up to 20%	
W5	25	-	5
W6		up to 20%	10
W7		-	
W8		up to 20%	

The physicochemical parameters of blackberry juice (total soluble solids - TSS, °Brix, total titratable acidity (TTA), and pH value) and wines (relative density, alcohol content, total extract, reducing sugars, total acidity, volatile acids, pH value, ash content, wine color intensity, and shade) were carried out using standard methods prescribed by the International Organization for Vine and Wine (OIV, 2015).

The content of total phenolic compounds (TPC) was determined by the Folin-Ciocalteu method (Wolfe et al. 2003). The non-flavonoid content (TNF) was determined by the formaldehyde method (Alberto et al., 2006), whereas the flavonoid content (TF) was calculated as the difference between TPC and TNF. The ability to remove DPPH (1,1-diphenyl-2-picrylhydrazyl) radicals was determined by the method given by Liyana-Pathirana and Shahidi (2005). ABTS radical scavenging activity (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) was evaluated according to Re et al. (1999), while ferric reducing antioxidant power (FRAP) was performed according to the method developed by Benzie and Strain (1996).

The antimicrobial activity against five pathogenic bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Staphylococcus aureus*, and *Listeria monocytogenes*), a pathogenic yeast (*Candida albicans*), and two probiotic strains (*Saccharomyces boulardii* and *Lactobacillus plantarum*) was tested by the agar dilution method according to Jovanović-Cvetković et al. (2025).

Sensory evaluation of blackberry wines was performed using the Buxbaum method (20-point scoring method) (Tešević et al. 2005). A commercial blackberry wine was used as the control sample.

Statistical analysis. The results were expressed as mean values of the individual results of three randomly selected product samples $\pm$ standard deviation (SD). The significance of arithmetic mean differences was determined by analysing the variance with one independent variable (One way ANOVA) and multiple interval tests (Tukey HSD – test) and expressed with 95% probability ($p < 0.05$).

RESULTS AND DISCUSSIONS

The chemical composition of the fruit must play a key role and influence the quality of the wine. Among the most important parameters affecting wine quality are the soluble solids content, total acidity, and actual acidity (pH value). The results of the physicochemical parameters and antioxidative properties of blackberry juice are shown in Table 2.

Table 2. The physicochemical parameters and antioxidative properties of blackberry juice

blackberry juice		
TSS	°Brix	10.00
TTA	g/L	8.80
pH value		3.39
TPC		3.262 $\pm$ 0.175
TNF	g GAE/L	1.988 $\pm$ 0.042
TF		1.274 $\pm$ 0.133
FRAP	mmol Fe ²⁺ /L	20.67 $\pm$ 0.194
DPPH		5.066 $\pm$ 0.166
ABTS	mmol TE/L	10.688 $\pm$ 0.621

The initial Brix was 10, which is in accordance with results obtained by Tosun et al. (2008). The obtained value of total acidity (8.8 g/L) is very high, and it is characteristic for fruits with emphasized freshness and stability (Clark and Finn, 2011). Tosun et al. (2008) stated that high acidity is important for the preservation of sensory characteristics in general, but also for its microbiological stability, because acids act as natural preservatives. Similar values of total acids in blackberry were recorded in the work of Milošević et al. (2012), which confirmed the consistency of the results obtained.

The real acidity (pH value) of blackberry juice indicated the balance between acidity and sweetness. The obtained pH value (3.39) is in accordance with the data of Basaran and Kepenek (2011), who emphasized the importance of the pH value in maintaining the stability and quality of fruit wines.

Blackberry juice used in this work showed good antioxidative properties, based on results obtained by FRAP, DPPH, and ABTS tests. Wang and Lin (2000) tested juice samples from several cultivated blackberry varieties and found higher antioxidant potential than in the tested juice samples from several varieties of strawberries, raspberries, cranberries, and blueberries. Koczka et al. (2018) found high degrees of correlation between the content of total polyphenols, flavonoids, and anthocyanins contained in blackberries and the antioxidant activity determined on the basis of various tests. Also, Monforte et al. (2018) proved high antioxidant activity of blackberry juice samples based on several antioxidant tests, and the activity trend based on potential was ORAC > TEAC > DPPH > FRAP.

The physicochemical parameters, including total soluble solids, total titratable acidity, and pH values, of the prepared blackberry musts are

summarized in Table 3. Based on the results obtained, it can be concluded that the addition of sugar (up to 20%) led to changes in the chemical composition of the blackberry must. The total acidity decreased by 3.8 g/L following sugar addition, while the pH value slightly increased from 3.35 to 3.45.

Table 3. The physicochemical parameters of blackberry musts

		blackberry must	
		after sugar addition	
TSS	°Brix	10.00	20.00
TTA	g/L	8.80	5.00
pH value		3.35	3.45

The results of physicochemical parameters of blackberry wines are presented in Tables 4 and 5. The relative density values varied from 0.9931 (W2) to 1.0094 (W5). Amidžić Klarić et al. (2017) found that the relative density of blackberry wine samples ranged from 1.0017 to 1.0660 g/mL. The same authors stated that higher values might indicate a higher content of sugar, alcohol, and extract, which significantly affected the relative density of the wine. Also, there might be a difference in the raw material itself (e.g. blackberry variety, ripeness), as well as in the production technology (length of time and temperature of maceration).

The highest alcohol content was measured in sample W4 (11.56% vol), which was macerated for 10 days at 20°C, with the addition of sugar up to 20%. The lowest alcohol content was measured in sample W7 (3.49% vol), which was macerated at 25°C for 10 days and to which no sugar was added. Prolonged maceration and lower maceration temperature influenced the increase in alcohol content, but an indispensable factor that influenced the increase in alcohol content is the addition of sugar (up to 20%). Samples to which no sugar was added had a significantly lower alcohol content. Amidžić Klarić et al. (2017) stated that the alcohol content in blackberry wine ranged from 9.37 to 14.78% vol. Johnson and Gonzalez de Mejia (2012) compared blackberry wines from Illinois and found that the ethanol content ranged from 9.5 to 12.4% vol.

Table 4. Relative density, alcohol content, extract without sugar, reducing sugars, and ash content of blackberry wines

	relative density (g/mL)	alcohol content (%vol)	extract without sugar (g/L)	reducing sugars (g/L)	ash (g/L)
W1	1.0087	4.06 ^a ±0.05	34.7 ^a ±0.0	3.75 ^a ±0.00	-
W2	0.9931	11.47 ^b ±0.00	21.4 ^b ±0.0	0.60 ^b ±0.00	1.80 ^a ±0.01
W3	1.0080	3.97 ^a ±0.07	32.9 ^c ±0.1	3.49 ^c ±0.07	3.70 ^b ±0.02
W4	0.9936	11.56 ^b ±0.00	21.2 ^b ±0.0	2.74 ^d ±0.00	1.94 ^c ±0.06
W5	1.0094	3.52 ^c ±0.00	35.1 ^a ±0.0	3.39 ^c ±0.00	-
W6	0.9939	11.26 ^d ±0.06	22.4 ^d ±0.1	1.44 ^c ±0.00	1.95 ^c ±0.01
W7	1.0089	3.49 ^c ±0.05	33.3 ^c ±0.2	3.78 ^a ±0.04	3.87 ^d ±0.03
W8	0.9959	9.99 ^c ±0.06	23.5 ^c ±0.1	1.64 ^f ±0.00	2.19 ^c ±0.02

(a–f) Different letters within the same column indicate statistically significant differences between samples at a significance level of $p < 0.05$.

The obtained results indicated that the addition of sugar affected the extract content of the extract, while the samples without the addition of sugar had a higher extract content of the extract. The content ranged from 21.4 g/L in sample W2 (with added sugar) to 37.5 g/L in samples W1 and W5 (without sugar added). Alepza et al. (2014) stated that the total extract content for blackberry wines ranged from 44.3 to 174.9 g/L.

The content of reducing sugars in all samples was relatively low, and all tested samples belong to the group of dry wines. The lowest content of reducing sugars was measured in sample W2, which was produced at a lower temperature and with a shorter duration of maceration, and was 0.6 g/L. Sample W7 had the highest content of reducing sugars (3.78 g/L), to which no sugar was added. The content of reducing substances (sugar) was relatively low in all tested samples. The average value for the content of reducing sugars was 2.6 g/L, which is in accordance with the OIV definition for the content of reducing sugars for dry wines - from 0.5 to 5 g/L. Petravić-Traminac et al. (2013) found that the content of reducing sugars in blackberry wines after fermentation was 1.416 g/L.

The samples with longer maceration and at a higher temperature had a higher ash content. Sample W7 had the highest (3.87 g/L), and sample W2 had the lowest ash content (1.80 g/L). The average ash content of the examined samples in this research was 2.58 g/L, which is in accordance with the results obtained in research conducted by Amidžić Klarić et al. (2017). On the other hand, higher-quality wine implies a higher content of minerals and ash.

Based on the results presented in Table 5, it can be concluded that the increase in maceration temperature influenced the increase in total acidity. The highest total acidity is found in sample W7, which was macerated at a temperature of 25°C for 10 days and was 10.6 g/L. The lowest total acidity is found in sample W2, which was macerated at a temperature of 20°C for 5 days and was 6.23 g/L. So, it can be concluded that all samples with sugar added had lower total acidity, which varied from 6.23 g/L to 6.69 g/L, regardless of the temperature and duration of maceration.

Table 5. Total acidity, volatile acidity, and pH value of blackberry wines

	total acidity (malic acid g/L)	volatile acidity (g acetic acid /L)	pH value
W1	10.26 ^a ±0.00	0.48 ^a ±0.02	3.20 ^a ±0.00
W2	6.23 ^b ±0.02	0.50 ^a ±0.00	3.32 ^b ±0.00
W3	10.20 ^a ±0.00	0.48 ^a ±0.01	3.25 ^c ±0.00
W4	6.37 ^c ±0.00	0.60 ^b ±0.00	2.99 ^d ±0.00
W5	10.52 ^d ±0.00	0.50 ^a ±0.00	3.55 ^c ±0.00
W6	6.69 ^c ±0.04	0.57 ^b ±0.00	3.38 ^f ±0.00
W7	10.60 ^e ±0.02	1.08 ^c ±0.01	3.42 ^e ±0.00
W8	6.72 ^c ±0.00	0.60 ^b ±0.01	3.48 ^h ±0.01

(a–g) Different letters within the same column indicate statistically significant differences between samples at a significance level of $p < 0.05$.

This is consistent with other studies that reported that the total acidity of blackberry wines ranged from 3.3 g/L to 8.1 g/L (Johnson and Gonzalez Mejia, 2012). Alpeza et al. (2014) reported that the total acidity for all their blackberry wines varied between 8.0 g/L and 16.0 g/L.

The low content of volatile acids is an important indicator of microbiological stability during wine storage. In this regard, all analyzed samples had a satisfactory low volatile acidity, except sample W7, which was subjected to a longer duration and a higher temperature of maceration, and without the addition of sugar. A

lower maceration temperature had a positive effect on reducing the content of volatile acids. Volatile acid content varied from 0.48 g/L to 1.08 g/L, with an average of 0.61 g/L acetic acid. Alpeza et al. (2014) in their study, found that volatile acidity, expressed in g/L of acetic acid, for all wines varied between 0.3 and 1.5 g/L, which is in accordance with the results of this research. Examined wine samples had a rather low pH value. Lower wine pH values were recorded in samples that were subjected to a lower maceration temperature (20°C). The lowest measured pH value was in sample W4, to which sugar was added, and was 2.99. The highest pH value was in sample W5, without added sugar, and was 3.55, which is in line with results obtained by Alpeza et al. (2014) - from 3.11 to 3.99. Chromatic characteristics are one of the main parameters taken into account when evaluating the quality of blackberry wine, and the results are given in Table 6.

Table 6. Chromatic characteristics of blackberry wines

	color intensity	shade
W1	7.09 ^a ±0.01	0.88 ^a ±0.00
W2	5.90 ^b ±0.02	1.06 ^b ±0.01
W3	10.35 ^c ±0.11	0.77 ^c ±0.00
W4	5.20 ^d ±0.11	0.89 ^{ad} ±0.00
W5	10.51 ^c ±0.00	0.82 ^c ±0.00
W6	7.43 ^c ±0.08	0.85 ^f ±0.00
W7	9.32 ^f ±0.04	0.76 ^e ±0.00
W8	6.50 ^e ±0.03	0.90 ^d ±0.00

(a–g) Different letters within the same column indicate statistically significant differences between samples at a significance level of $p < 0.05$.

From the presented results, it can be concluded that shorter duration of maceration (5 days) had a positive effect on increasing the wine's color intensity and shade of the wine. A higher maceration temperature (25°C) also had a positive effect on higher color intensity. In contrast to the color intensity, increasing the maceration temperature negatively affected the color shade. The color intensity of blackberry wines in this study ranged from 5.20 to 10.51. Color intensity values reported in the literature for blackberry wines ranged from 1.80 to 13.19 (Amidžić Klarić et al., 2017), and 2.7–8.4 for Andean blackberry wine (Arozarena et al., 2012). Shades of color ranged from 0.755 (W7) to 1.062 (W2), which was similar to the results obtained in the work of Amidžić Klarić et al. (2017) (0.763–1.740), while lower values are reported by Arozarena et al. (2012) (0.42–0.63).

An overview of the phenolic content and antioxidant activity of blackberry wines is provided in Table 7. Sample W2, which was macerated at a lower temperature and with added sugar, had the lowest content of total phenolics, 0.960 g GAE/L. The highest content of total phenolics is found in sample W5, which was macerated at a higher temperature, without the addition of sugar, and was 1.485 g GAE/L. Ortiz et al. (2013) reported that the content of total polyphenols in blackberry wine varied from 1.122 to 1.400 g GAE/L, which was in line with the results of this study.

Table 7. Phenolic content (total phenolics, non-flavonoids and flavonoids) and antioxidant activity (FRAP, DPPH, ABTS)

	TPC	TNF	TF	FRAP	DPPH	ABTS
	g GAE/L			mmol Fe ²⁺ /L	mmol TE/L	
W1	1.37 ^a ±0.01	0.98 ^a ±0.03	0.39 ^{ab} ±0.02	18.69 ^a ±0.18	4.60 ^{ab} ±0.05	16.94 ^{ab} ±0.29
W2	0.96 ^b ±0.04	0.64 ^b ±0.09	0.32 ^a ±0.05	11.11 ^b ±0.06	3.86 ^c ±0.01	13.38 ^c ±0.23
W3	1.37 ^a ±0.02	0.95 ^a ±0.01	0.42 ^{ab} ±0.011	17.36 ^{ac} ±0.31	5.09 ^{de} ±0.03	19.03 ^{bc} ±0.07
W4	1.07 ^c ±0.02	0.65 ^b ±0.04	0.42 ^{abc} ±0.06	13.52 ^d ±0.78	3.97 ^c ±0.10	13.84 ^{cd} ±1.09
W5	1.49 ^d ±0.02	0.89 ^a ±0.01	0.59 ^d ±0.00	20.98 ^e ±0.76	5.18 ^e ±0.01	19.96 ^e ±0.20
W6	1.051 ^c ±0.029	0.579 ^b ±0.017	0.47 ^{bcd} ±0.012	10.89 ^b ±0.23	3.40 ^c ±0.01	14.47 ^{cd} ±0.27
W7	1.31 ^a ±0.01	0.87 ^a ±0.04	0.44 ^{abc} ±0.05	16.66 ^c ±0.64	4.88 ^{ad} ±0.01	17.61 ^{ab} ±0.17
W8	1.17 ^c ±0.01	0.62 ^b ±0.01	0.55 ^{cd} ±0.02	14.47 ^d ±0.48	4.44 ^b ±0.12	16.02 ^{ad} ±0.14

* a-e mean values with different letters in the same column differ statistically significantly with 95% probability (p <0.05)

The strongest antioxidant activity is shown by sample W5 (20.98 mmol Fe²⁺/L for the FRAP test, 5.18 mmol TE/L for the DPPH test, and 19.96 mmol TE/L for the ABTS test), which was subjected to maceration for 5 days at 25°C. On the other hand, sample W6 showed the weakest antioxidant activity under identical maceration conditions.

The antioxidant activity of wine is directly related to the content of phenolic compounds. This is consistent with findings reported by Amidžić Klarić et al. (2020), who confirmed that the antioxidant activity of blackberry wines is proportional to the concentration of bioactive phytochemicals. Notably, Mudnić et al. (2012) observed that some blackberry wines exhibited lower total phenolic content relative to red grape

wines, yet demonstrated stronger antioxidant capacity, attributing this to a higher content of non-flavonoid compounds, most notably gallic acid, in blackberry wines. Higher maceration temperature of the samples without the addition of sugar had a positive effect on the increase in antioxidant activity. Varo et al. (2022) reported that the maceration process increased the concentration of phenolic compounds and the antioxidant activity values measured by the DPPH assay; however, extended maceration times may reduce these values due to phenolic compound involvement in polymerization, copigmentation, degradation, pyranoanthocyanin formation, and reactions between anthocyanins and tannins.

The results of measuring the antimicrobial activity of blackberry wines, expressed as MIC/MBC/MFC values (%) are presented in Table 8.

Table 8. Antimicrobial activity (MIC, MBC, and MFC) of blackberry wine samples in %

MIC/ MBC/MFC	W1	W2	W3	W4	W5	W6	W7	W8
<i>E. coli</i>	20 30	20 30	20 30	20 30	20 30	20 30	20 30	20 30
<i>P. rugissante</i>	20 30	20 30	20 30	20 30	20 30	10 30	20 30	10 30
<i>B. cereus</i>	10 >30	10 >30	<5 >30	10 >30	10 >30	10 >30	<5 >30	10 >30
<i>S. aureus</i>	20 >30	20 >30	20 >30	20 30	10 20	20 >30	20 >30	20 30
<i>L. monocytogenes</i>	30 >30	30 >30	20 >30	30 >30	20 30	30 >30	20 >30	30 30
<i>C. albicans</i>	>30 >30	>30 >30	>30 >30	>30 >30	>30 >30	>30 >30	>30 >30	>30 >30
<i>S. boulardii</i>	>30 >30	>30 >30	>30 >30	>30 >30	>30 >30	>30 >30	>30 >30	>30 >30
<i>L. plantarum</i>	>30 >30	>30 >30	>30 >30	>30 >30	>30 >30	>30 >30	>30 >30	>30 >30

*MIC - minimum inhibitory concentration; MBC - minimum bactericidal concentration; MFC - minimum fungicidal concentration.

The blackberry wines tested in this work showed pronounced antimicrobial activity against all tested bacteria, except for the probiotic bacterium *L. plantarum*. Also, blackberry wate at the tested concentrations had no effect against yeasts, *C. albicans* and *S. boulardii*. The best inhibitory effect, with the lowest MIC value, was shown by blackberry wine against *B. cereus*, where all samples had an MIC of 10%, and samples W3 and W7 <5%. At the same time, the highest tested concentration of 30% had no bactericidal effect on this bacterium. The best bactericidal activity, according to the tested cforcentrations of wine, was obtained for *P.*

aeruginosa, where two samples had an MIC of 10%, and the bactericidal effect was manifested in all samples at 30%. *E. coli* and *S. aureus* showed similar sensitivity, with sample W5 inhibiting *S. aureus* better, and all samples having a bactericidal effect on *E. coli* at a concentration of 30%. Among the samples, sample W5 exhibited the best inhibitory effect, inhibiting Gram-positive *B. cereus*, *S. aureus* and *L. monocytogenes* better than Gram-negative *E. coli* and *P. aeruginosa*. The samples W3, W4 and W7 also had a more pronounced antimicrobial effect. Czyżowska et al. (2020) investigated the content of phytochemicals and bioactive components in dealcoholized wines from bilberry, blackberry, cranberry, elderberry, raspberry, and strawberry and found that blackberry wine has the best antioxidant activity (by ABTS and FRAP methods), which cannot be associated with the highest content of total phenolics. The authors believe that this activity, in addition to the content of phenolic components, is also related to the content of other non-phenolic components with antioxidant activity, such as ascorbic acid. Similarly, dealcoholized blackberry wine only inhibited the growth of Gram-positive bacteria, *B. cereus* and *L. monocytogenes*, while it had no effect on Gram-negative bacteria, *E. coli* and *Salmonella enteritidis*. Fruit wines are complex systems containing alcohol, various phenolic compounds, organic acids, minerals, and vitamins. The antimicrobial activity of red wines has been extensively researched, and its antimicrobial activity is associated with the combination of organic acids and alcohol (Møretro and Daeschel, 2004), the combination of alcohol, organic acids, pH, and phenolic components (Kim et al., 2014). Boban et al. (2010) concluded that wine is a complex system and that its components act synergistically on the inhibition of microorganisms. This is also confirmed by the comparison of the antimicrobial activity of blackberry wine in this research and dealcoholized wine in the research of Czyżowska et al. (2020).

Sensory analysis revealed that wines produced without sugar addition were characterised by pronounced acidity and a thin body, which is consistent with the naturally high titratable acidity of blackberry must.

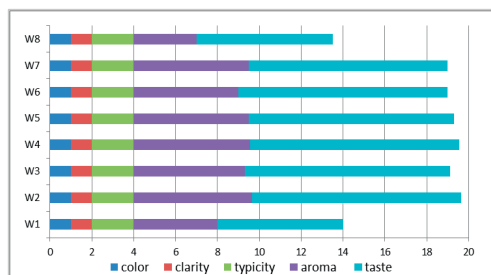


Figure 1. Sensory analysis results

Varo et al. (2022) found that longer maceration or fermentation times decreased the concentration of all compounds, including acids, which resulted in wines with high acidity. The addition of sugar up to 20% yielded wines of markedly improved quality, and samples macerated and fermented at 20°C received superior sensory scores, and the amount of alcohol in the samples (about 12% vol) proved to be satisfactory. With respect to maceration duration, samples subjected to a 5-day maceration period performed better overall - the wines were slightly lighter in color, but exhibited a more acceptable taste profile, characterized by reduced acidity and bitterness. Extended maceration promotes the extraction of high-molecular-weight tannins from seeds, which can lead to pronounced bitterness and astringency in the final product (Varo et al., 2022). Longer maceration can cause a probable negative effect on wine color and sensory properties (Gutiérrez-Escobar et al., 2021).

CONCLUSIONS

The results demonstrated that maceration conditions significantly affect wine composition and quality. Samples macerated at lower temperatures with the addition of 20% sugar exhibited higher alcohol content, whereas higher maceration temperatures combined with sugar addition resulted in increased total acidity. In most cases, lower maceration temperatures were associated with lower volatile acidity. Shorter maceration at higher temperatures enhanced color intensity, while prolonged maceration at lower temperatures reduced color intensity. Wines produced without added sugar showed the highest total phenolic content and the strongest antioxidant activity, with elevated temperatures further enhancing antioxidant

potential. The sample W5, macerated for 5 days at 25°C without sugar addition, exhibited the highest phenolic content and antioxidant capacity. However, wines produced without sugar addition were sensorially less acceptable due to pronounced acidity and low alcohol content. The addition of sugar up to 20% improved overall wine quality, with the best sensory evaluation achieved in the sample macerated for 5 days at 20°C. Overall, the findings confirm that both physicochemical and sensory properties of blackberry wine are strongly dependent on maceration temperature, duration, and sugar addition.

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PERFORMANCE OF SELECTED CLONAL ELITES FROM POPULATIONS OF THE CULTIVARS 'PERLETTE', 'PAULA', AND 'CENTENNIAL SEEDLESS' UNDER THE CONDITIONS OF THREE ROMANIAN VITICULTURAL AREAS

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Abstract

The study analyzes the performance of clonal elites of the cultivars 'Perlette', 'Paula', and 'Centennial Seedless', tested in three representative viticultural areas of Romania: population selections of the cultivar 'Perlette' in Murfatlar, those of 'Paula' in Iași, and those of 'Centennial Seedless' in Ștefănești-Argeș, over two viticultural years (2024-2025). Within this study, eighteen clonal elites were comparatively evaluated, assessing their performance relative to the control variant. The main climatic, phenological, agrobiological, productive, and technological indicators were analyzed in order to identify clonal elites with superior potential for expansion in cultivation. Significant differences among viticultural areas reflect the climatic impact on bud fertility, phenology, yield, and grape quality. During the study period, the clonal elites 'Perlette 7/2/5', 'Paula 5/1/4', and 'Centennial Seedless 5' demonstrated the best performance in terms of productivity and quality, ensuring average yields of 15-24 t/ha. These results recommend the varieties for testing, homologation, and expansion in the studied vineyards and in areas with similar conditions, while also enhancing the profitability of viticultural holdings through the high economic potential of table grapes.

Key words: clonal selection, quality, productivity, stability, variability.

INTRODUCTION

Vitis vinifera is one of the most important horticultural species worldwide, owing to its economic, nutritional, and social significance. In recent decades, the table grape sector has become increasingly important at a global level, driven by consumer demand for fresh, high-quality, seedless fruit with an attractive appearance and superior organoleptic characteristics (Keller, 2022; OIV, 2023). Concurrently, climate change, manifested through rising temperatures, irregular precipitation patterns, and a higher frequency of extreme weather events, has significantly affected grapevine productivity and fruit quality. Under these conditions, stability and

adaptability have become key priorities in viticultural research (IPCC, 2023; van Leeuwen & Destrac-Irvine, 2017).

In Romania, grapevine cultivation has a long-standing tradition, supported by favorable pedoclimatic conditions across several viticultural regions. Table grape production is particularly well developed in areas such as Murfatlar (Dobrogea), Iași (Moldavia), and Ștefănești-Argeș (Muntenia), where thermal resources, solar radiation, and soil characteristics provide optimal conditions for obtaining high-quality fresh grapes (Teodorescu et al., 2021; Oșlobeanu et al., 1991; Cotea et al., 2006). The evolution of the table grape sector in Romania has been shaped by both traditional cultivars and the introduction of new seedless

varieties, which are increasingly preferred by consumers (Olteanu et al., 2015; Dejeu, 2010). Seedless cultivars intended for fresh consumption are highly valued for their earliness, berry size, uniformity, and taste. However, their performance is strongly influenced by genotype-environment interactions, particularly under conditions of thermal and water stress (Keller, 2022). Previous studies (Reynolds & Wardle, 2001), as well as more recent research, have demonstrated that yield stability and fruit quality are closely linked to the adaptability of genotypes to specific environmental conditions. In Romanian viticulture, similar conclusions have been reported regarding the influence of ecological conditions on grape production and quality (Cotea et al., 2006; Oşlobeanu et al., 1991).

Clonal selection represents a fundamental approach for the improvement of grapevine cultivars, aiming to identify elite genotypes with superior productivity, consistent fruit quality, and enhanced adaptability to local environmental conditions (OIV, 2020). In such studies, the focus is placed on assessing the performance stability of individual genotypes across varying environmental conditions, rather than on direct comparisons between cultivars (Becker & Léon, 1988). Romanian research has also emphasized the importance of clonal selection in improving grapevine assortments and ensuring the biological and productive stability of cultivars (Teodorescu et al., 2021; Dejeu, 2010).

In Romania, clonal selection programs have made a significant contribution to the improvement of grapevine assortments, including both wine and table grape cultivars (Teodorescu et al., 2021). Within this context, the present study reports partial results of clonal selection in three seedless table grape cultivars ‘Perlette’, ‘Paula’, and ‘Centennial Seedless’, with the aim of identifying elite genotypes characterized by high productivity, superior fruit quality, and good adaptability to the environmental conditions of the study area.

MATERIALS AND METHODS

The research was conducted over two viticultural years (2024-2025) and involved the study of 18 clonal elites, analyzed in comparison with the control variant, belonging to table grape

cultivars selected for their agronomic and commercial potential. The studied elites belong to the cultivars ‘Perlette’, ‘Paula’, and ‘Centennial Seedless’, located in distinct viticultural areas, namely the Murfatlar vineyard, the Iaşi vineyard, and the Ştefăneşti-Argeş vineyard. Five clonal elites were studied for the ‘Perlette’ variety: 16/5/11, 23/8/2, 14/7/2, 9/3/15, and 7/2/5. For the ‘Paula’ variety, five elites were also studied: 3/4/3, 7/6/3, 9/1/6, 4/2/1, and 5/1/4. For the ‘Centennial Seedless’ variety, eight elites were studied, numbered from 1 to 8.

The objective of the study was to evaluate the stability of these clonal elites originating from different cultivars by analyzing the consistency of their performance across three contrasting pedoclimatic environments.

The analysis of clonal elites originating from different cultivars requires a different methodological approach compared to intravarietal studies. In this case, the aim was not to perform absolute comparisons of yield or quality among clonal elites, but rather to evaluate the coherence of each elite’s response to environmental variability.

The monitored parameters included vegetative and productive indices, phenological stages of vegetation, productivity, and quality of these clonal elites. Since the clones originate from different cultivars, the evaluation focused on the relative stability of their performance across different environments, rather than on direct comparisons of the absolute levels of yield or quality.

The analyzed parameters included meteorological data during the study period, the progression of phenological stages, the relative and absolute fertility coefficients, yield (kg/vine), as well as the analysis of fruit quality (sugar, g/L; acidity, g/L H_2SO_4), together with the determination of the gluco-acidimetric index.

The data were statistically processed using analysis of variance (ANOVA), and the significance of differences was evaluated using the Least Significant Difference (LSD) corresponding to Fisher’s LSD test (Gomez & Gomez, 1984).

The Least Significant Difference was established using the common experimental error obtained from the analysis of variance (ANOVA), by applying the formula corresponding to Fisher’s LSD test. The

significance of the differences between the clonal elites and the control (the original cultivar) was analyzed by calculating the Least Significant Difference (LSD) using Fisher's LSD test. The threshold values of the LSD were established according to the experimental variability and the chosen levels of statistical significance using the following formula:

$$DL = t_{\alpha,df} \cdot \sqrt{\frac{2 \cdot MSE}{r}}$$

where:

$t_{\alpha,df}$ represents the critical value from the Student's t distribution for the chosen significance level (5%, 1%, 0.1%) and the degrees of freedom of the error;

MSE represents the mean square error (experimental error) obtained from ANOVA;

r represents the number of replications, corresponding to the number of observation years.

RESULTS AND DISCUSSIONS

The data analysis presented in Tables 1 and 2, referring to the climatic conditions and the phenological evolution of the grapevine during the viticultural years 2024 and 2025, highlights significant differences among the three analyzed viticultural areas - Murfatlar, Iași, and Ștefănești-Argeș - differences that influenced the development of the vegetative cycle and grape ripening.

The annual mean temperature recorded the highest values in the Ștefănești-Argeș vineyard (15.5°C in 2024) and the lowest in the Iași vineyard (12.2°C in 2024). Comparatively, between the two years, a slight increase in mean temperature was observed in Murfatlar (14.5°C → 14.8°C) and Iași (12.2°C → 12.6°C), while in Ștefănești-Argeș a significant decrease was recorded (15.5°C → 13.6°C). These thermal variations were directly reflected in the dynamics of the vegetation phenophases.

Thus, for the cultivar 'Perlette' cultivated in Murfatlar, budburst occurred later in 2025 (18 April) than in 2024 (2 April), indicating a cooler spring and a slower accumulation of useful temperatures. A similar trend was observed for the cultivars 'Paula' (Iași) and 'Centennial Seedless' (Ștefănești-Argeș), where phenophases were shifted according to the

temperature evolution during the early vegetation period.

The number of days with maximum temperatures above 30°C, an indicator of thermal stress on grapevine, was higher in 2024 than in 2025. In Murfatlar, 75 such days were recorded in 2024 compared with 61 in 2025, while in Ștefănești-Argeș there were 89 days compared with 64 days. The reduction in the frequency of extreme temperatures in 2025 contributed to delays in certain phenophases, particularly flowering and veraison, and was associated with a lower accumulation of effective temperatures.

The pluviometric regime showed important variations between the two years. In Murfatlar, annual precipitation decreased in 2025 (680.8 mm → 342 mm), while precipitation during the vegetation period decreased from 345.6 mm to 165.2 mm, leading to a pronounced reduction of the hydrothermal coefficient (1.2 → 0.5) and the establishment of significant water stress. In contrast, in the Iași and Ștefănești-Argeș vineyards, the pluviometric regime was more balanced, and the hydrothermal coefficient recorded values more favorable for vegetative development and sugar accumulation.

These climatic conditions influenced phenological evolution, particularly in Murfatlar, where for the cultivar 'Perlette' the veraison stage was delayed in 2025 (4 August) compared with 19 July in 2024, suggesting that water deficit slowed berry development.

The Oenoclimatic Aptitude Index (IAOe) and the Huglin Heliothermal Index recorded lower values in most areas in 2025, indicating less favorable conditions for optimal grape ripening. In Murfatlar, for example, the Huglin index decreased from 2,688.5 to 2,434.3, while IAOe decreased from 5,277.4 to 5,197.2.

This reduction explains the phenological shifts observed, particularly the delay in full ripening: the cultivar 'Perlette' reached maturity on 28 August in 2025 compared with 10 August in 2024.

No significant differences in phenology were observed between the studied clonal elites and the control cultivars; therefore, only the data corresponding to the control cultivars were interpreted. Overall, the results demonstrate that interannual climatic variability significantly influences grapevine phenology.

Table 1. Analysis of the climatic conditions during the 2024-2025 period in the three studied viticultural areas

Climatic elements analyzed	Area 1 - Murfatlar vineyard		Area 2 - Iasi vineyard		Area 3 - Stefanesti Arges vineyard	
	2024	2025	2024	2025	2024	2025
Mean annual temperature T, °C	14.5	14.8	12.2	12.6	15.5	13.6
Number of days with maximum temperatures >30°C	75	61	56	42	89	64
Σ Total annual precipitation, mm	680.8	342	509.1	657.4	490.6	572.4
Σ Total precipitation during the vegetation period, mm	345.6	165.2	400.2	313.8	275.8	349.4
Hydrothermal coefficient (CH)	1.2	0.5	1.09	0.97	0.8	1.1
Oenoclimatic aptitude index (IAOe)	5,277.4	5,197.2	5,027.5	4,689.9	3,419	3,217
Huglin heliothermal index (IH)	2,688.5	2,434.3	2,600.5	2,193.7	2,318	2,316.5
Night cooling index (IF)	14.2	12.9	14.1	12.4	11.9	11.9

Table 2. Progression of the vegetation phenophases of the three studied genotypes

Progression of the vegetation phenophases	'Perlette' Area 1 – Murfatlar		'Paula' Area 2 – Iași		'Centennial Seedless' Area 3 – Ștefănești Argeș	
	2024	2025	2024	2025	2024	2025
Budburst (BBCH 08-09)	02 Apr.	18 Apr.	05 Apr.	19 Apr.	18 Apr.	23 Apr.
Flowering (BBCH 65-68)	27 May	12 June	27 May	07 June	05 June	12 June
Veraison (BBCH 85)	19 July	04 Aug.	13 July	23 July	16 July	30 July
Full maturity (BBCH 89)	10 Aug.	28 Aug.	09 Aug.	02 Sept.	14 Aug.	25 Aug.

Analyzing the data presented in Table 3, for the cultivar 'Perlette' (Murfatlar), in the case of CFR, the clonal elites 9/3/15 and 7/2/5 stand out, with increases of +0.15, highly significant (**), indicating a clearly superior relative fertility compared to the control and suggesting a better reproductive potential under the given conditions. The other variants do not differ statistically from the control, which reflects a relatively uniform expression of this parameter among the remaining elites.

For CFA, highly significant differences (**) are recorded for elite 7/2/5 (−0.20), indicating a notable decrease in absolute fertility compared with the control. At the same time, 16/5/11 shows a significant reduction (**), confirming a similar but less pronounced trend. The remaining elites are statistically non-significant, maintaining values close to the control and showing no meaningful deviations.

For the cultivar 'Paula' (Iași), CFR shows no significant differences, except for elite 5/1/4 (+0.10, **), which indicates a moderate but statistically supported increase compared with the control. The other variants remain

statistically similar, suggesting limited variability for this trait within the cultivar.

For CFA, the elites 3/4/3 and 5/1/4 stand out (+0.15, **), indicating improved absolute fertility and a favorable performance. Additionally, elite 4/2/1 records a smaller but significant increase (+0.10, *), suggesting a positive but less pronounced effect. The rest of the variants do not show statistically significant differences and remain comparable with the control.

In the case of the cultivar 'Centennial Seedless' (Ștefănești-Argeș), only elite 5 records a highly significant increase in CFR (+0.20, ***), clearly distinguishing itself from the other variants and highlighting its superior relative fertility. The other elites do not differ significantly from the control, indicating stable values across genotypes.

For CFA, highly significant negative differences are observed for elites 1 (−0.25, ***) and 7 (−0.20, ***), indicating a considerable reduction in absolute fertility compared with the control. The other variants are statistically comparable with the control, maintaining similar values despite minor numerical differences.

Table 3. Comparative analysis of CFR and CFA in clonal elites of the studied cultivars across the three viticultural areas

Genotype	Clonal elite	Relative Fertility coefficient (CFR)			Absolut Fertility coefficient (CFA)		
		Multianual mean	Differences (+/-)	Significance	Multianual mean	Difference (+/-)	Significance
'Perlette' Area 1-Murfatlar	16/5/11	0.50	-0.05	ns	1.05	-0.15	**
	23/8/2	0.50	-0.05	ns	1.25	+0.05	ns
	14/7/2	0.50	-0.05	ns	1.15	-0.05	ns
	9/3/15	0.70	+0.15	***	1.15	-0.05	ns
	7/2/5	0.70	+0.15	***	1.00	-0.20	***
	Control (initial cultivar)	0.55	-	-	1.20	-	-
'Paula' Area 2-Iasi	3/4/3	0.75	+0.05	ns	1.25	+0.15	**
	7/6/3	0.75	+0.05	ns	1.10	0	ns
	9/1/6	0.65	-0.05	ns	1.15	+0.05	ns
	4/2/1	0.75	+0.05	ns	1.20	+0.10	*
	5/1/4	0.80	+0.10	**	1.25	+0.15	**
	Control (initial cultivar)	0.70	-	-	1.10	-	-
'Centennial Seedless' Area 3-Ştefăneşti Argeş	1	0.70	-0.05	ns	1.00	-0.25	***
	2	0.80	+0.05	ns	1.25	0	ns
	3	0.80	+0.05	ns	1.30	+0.05	ns
	4	0.70	-0.05	ns	1.20	-0.05	ns
	5	0.95	+0.20	***	1.30	+0.05	ns
	6	0.80	+0.05	ns	1.20	-0.05	ns
	7	0.70	-0.05	ns	1.05	-0.20	***
	8	0.80	+0.05	ns	1.30	+0.05	ns
	Control (initial cultivar)	0.75	-	-	1.25	-	-
		DL 5% CFR = 0.06			DL 5% CFA = 0.07		
		DL 1% CFR = 0.09			DL 1% CFA = 0.11		
		DL 0.1% CFR = 0.14			DL 0.1% CFA = 0.17		

ns – not significant; *, **, *** – significant for p < 0.05; p < 0.01; p < 0.001.

In the case of the cultivar ‘Perlette’ from Murfatlar, in 2024 all elites exceeded the control, with elite 7/2/5 standing out through the highest yield. In 2025, a general reduction in yield was observed; however, the elites maintained an advantage over the control, indicating a better capacity for adaptation under less favorable conditions. For the cultivar ‘Paula’ from Iaşi, yield was relatively balanced in 2024, while in 2025 an

increasing trend was observed for most elites. Elite 5/1/4 stands out for its consistency and superior performance in both years, suggesting good yield stability. For the cultivar ‘Centennial Seedless’ from Ştefăneşti-Argeş, the year 2024 was characterized by moderate yields, whereas in 2025 a significant increase was recorded for all variants. Elite 5 is distinguished by superior productivity and high adaptability (Figure 1).

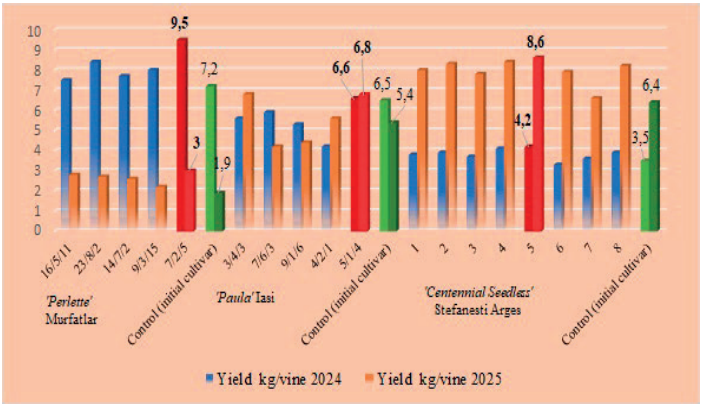


Figure 1. Evaluation of yield (kg/vine) of the elites compared with the control

According to the data presented in Table 4, in the case of the cultivar ‘Perlette’ (Murfatlar), in 2024 clear differences among the elites can be observed regarding sugar accumulation. Elite 7/2/5 (152 g/L) recorded a significant difference compared with the other elites and the control (116 g/L). In 2025, the sugar content decreased and became more uniform (110-114 g/L), indicating a restrictive climatic influence on ripening. In 2024, acidity was low (2.3-3.3 g/L), but in 2025 it increased significantly (5.2-5.4 g/L), exceeding the control in most cases. This evolution indicates slower ripening and reduced sugar accumulation in the second year. Regarding the cultivar ‘Paula’ (Iași), a high level of sugars was observed in both years. In 2025, a significant increase was recorded for most elites (up to 220 g/L in 7/6/3), exceeding the control

(209 g/L). Acidity remained relatively constant, with slight decreases in 2025 for some elites (for example, 7/6/3-3.6 g/L), indicating a favorable balance between sugars and acids and good adaptation to the conditions of the Iași viticultural area. For the cultivar ‘Centennial Seedless’ (Ștefănești-Argeș), sugar values were high and relatively stable in both years (195-210 g/L). Elite 5 stands out with the highest sugar content in 2024 (210 g/L) and maintains a superior level in 2025 as well (206 g/L). Acidity slightly increased in 2025 compared with 2024, but remained within moderate limits (3.1-4.0 g/L). Compared with the control, most elites showed lower acidity in 2025, suggesting more advanced ripening.

Table 4. Crop quality during the study period

Cultivar/vineyard area	Clonal elite	Sugars g/L		Acidity H ₂ SO ₄ g/L	
		2024	2025	2024	2025
‘Perlette’ / Murfatlar	16/5/11	124	113	2.7	5.2
	23/8/2	108	111	2.3	5.2
	14/7/2	112	110	2.4	5.3
	9/3/15	128	112	2.8	5.4
	7/2/5	152	114	3.3	5.3
	Control (initial cultivar)	116	110	2.5	5.8
‘Paula’ / Iași	3/4/3	180	191	4.4	4.4
	7/6/3	182	220	4.4	3.6
	9/1/6	182	207	4.4	4.5
	4/2/1	182	195	4.3	4.4
	5/1/4	186	217	4.2	4.2
	Control (initial cultivar)	186	209	4.2	4.1
‘Centennial Seedless’ / Ștefănești Argeș	1	199	196	3.1	3.9
	2	198	198	3.2	3.6
	3	198	195	3.1	4.0
	4	199	197	3.0	3.6
	5	210	206	2.6	3.1
	6	204	201	3.0	3.3
	7	203	202	3.2	3.1
	8	206	203	2.9	3.2
	Control (initial cultivar)	199	195	3.2	4.1

In the case of the cultivar ‘Perlette’ (Murfatlar), in 2024 the values of the gluco-acidimetric index were relatively uniform (45-47), comparable with the control (46), indicating an appropriate technological balance. In 2025, the index values decreased significantly (20-22), including the control (19), as a result of reduced sugar accumulation and increased acidity. However, the elites still maintained slightly higher values than the control, such as elite 7/2/5, suggesting relatively better adaptation under less favorable conditions.

Regarding the cultivar ‘Paula’ (Iași), in 2024 the values were similar (41-44), close to the control (43), indicating balanced ripening. In 2025, a marked increase in the gluco-acidimetric index was observed for most elites, particularly for 5/1/4 (52), exceeding the control. This evolution reflects superior sugar accumulation and/or a reduction in acidity, confirming good adaptation to the climatic conditions of that year. Analyzing the cultivar ‘Centennial Seedless’ (Ștefănești-Argeș), the values of this index are the highest among the three cultivars. In 2024,

Elite 5 stands out with a very high index (80), well above the control (62), indicating superior quality potential. In 2025, the values decreased slightly but remained high (48-66), with most

elites exceeding the control (47). Elite 5 maintained its superiority (66), followed by elites 7 and 8 (Figure 2).

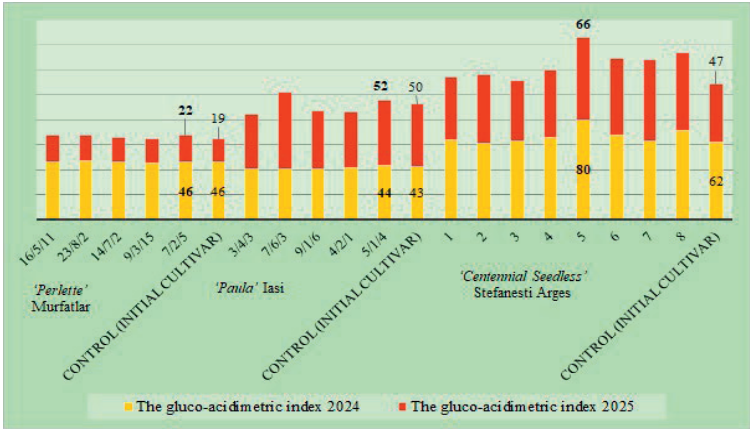


Figure 2. The gluco-acidimetric index

Figure 3 presents original photographs illustrating the clusters of the three initial cultivars

studied in parallel with those of the superior elites.

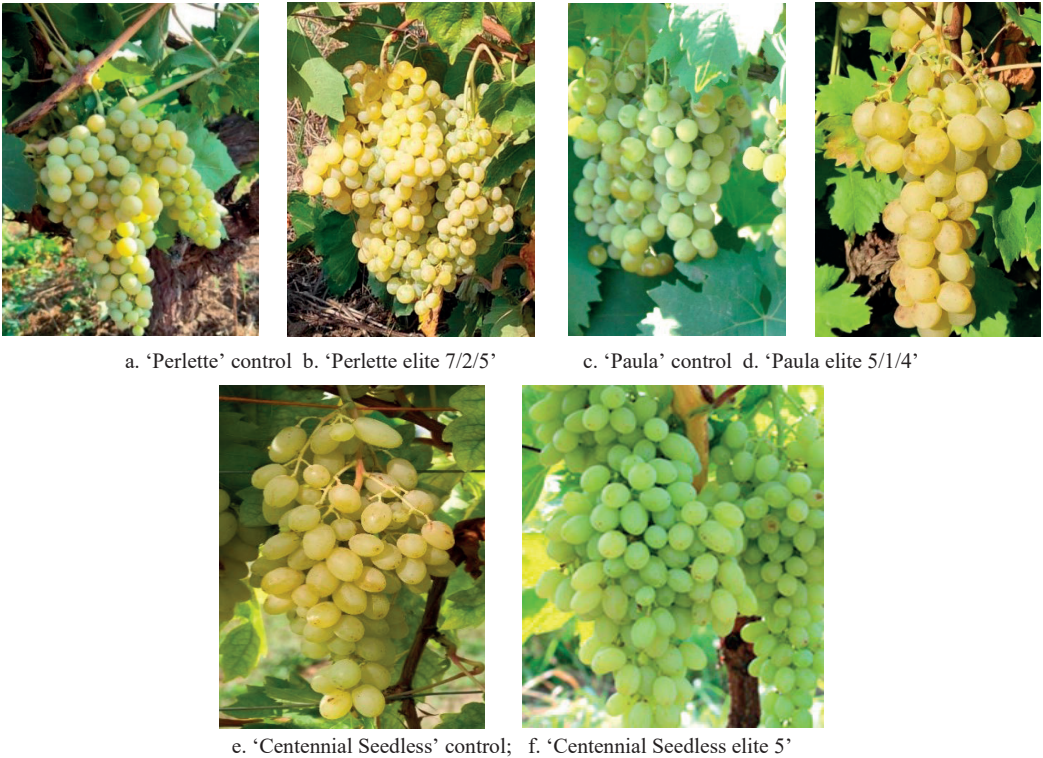


Figure 3. Cluster characteristics of the initial cultivar – control – and the elite (a-b Area 1 - Mufatlar; c-d Area 2 - Iași, e-f Area 3 - Ștefănești Argeș)

CONCLUSIONS

Interannual climatic variability significantly influenced grapevine phenology and grape ripening in all three analyzed viticultural areas. The reduction in mean temperatures and heliothermal indices in 2025 led to phenological shifts and delayed ripening, particularly in the Murfatlar viticultural area.

Regarding the cultivar 'Perlette' (Murfatlar), elites 7/2/5 and 9/3/15 stood out through highly significant increases in the relative fertility coefficient (CFR), while elite 7/2/5 demonstrated relatively superior adaptation under restrictive conditions, maintaining quality values above the control even in the unfavorable year.

For the cultivar 'Paula' (Iași), elite 5/1/4 was distinguished by productive consistency and higher values of the gluco-acidimetric index in both years, confirming good stability and adaptation to the pedoclimatic conditions of the area. For the cultivar 'Centennial Seedless' (Ștefănești-Argeș), elite 5 stood out for high productivity and superior sugar content in both years, recording the highest values of the gluco-acidimetric index, indicating superior qualitative and commercial potential.

The differences between the elites and the control were, in most cases, more evident in productive and qualitative parameters than in phenological ones, suggesting that clonal variability mainly influences technological performance rather than the duration of the vegetative cycle.

The evaluation of relative stability demonstrated that certain elites ('Perlette 7/2/5', 'Paula 5/1/4', and 'Centennial Seedless 5') exhibit a superior capacity to adapt to pedoclimatic variations, recommending them for official registration and multiplication.

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VEGETABLE GROWING



INTERACTION OF ABIOTIC AND BIOTIC STRESS ON PHOTOSYNTHETIC EFFICIENCY AND YIELD IN FABA BEAN (*VICIA FABA* L.)

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Abstract

Faba bean (Vicia faba L.) is an important grain legume whose productivity is increasingly constrained by the combined effects of abiotic and biotic stresses under field conditions. This study evaluated the interaction between these stress factors in relation to photosynthetic efficiency and yield performance in thirteen experimental faba bean breeding lines grown in Romania. The field experiment was conducted in 2025 using a randomized complete block design with three replications. Abiotic stress response was assessed through chlorophyll fluorescence parameters, particularly the maximum quantum efficiency of photosystem II (Fv/Fm), and the chlorophyll content index (SPAD), while biotic stress was evaluated based on natural infestation by the black bean aphid (Aphis fabae). Considerable variability was observed among genotypes, with Fv/Fm values ranging from 0.57 to 0.79 and SPAD values from 10.22 to 23.77. Seed yield varied between 308.2 g m⁻² and 1057.5 g m⁻². Lines L7, L17, L21, and L4 maintained higher photosynthetic efficiency, lower aphid infestation, and superior yield stability under stress conditions. These results highlight the value of integrating physiological indicators and field observations in selecting faba bean breeding material adapted to multiple stress environments.

Key words: *Aphis fabae*, breeding, chlorophyll fluorescence, physiological response, SPAD index.

INTRODUCTION

Faba bean (*Vicia faba* L.) is an important grain legume cultivated worldwide for both human consumption and animal feed due to its high protein content and nutritional value. In addition to its role as a food and feed crop, the faba bean contributes significantly to sustainable agricultural systems through biological nitrogen fixation, improving soil fertility and reducing the need for synthetic nitrogen fertilizers (Jensen et al., 2010; Köpke & Nemecek, 2010). Because of these agronomic and ecological benefits, the crop has gained increasing importance in diversified and climate-resilient cropping systems.

Despite its importance, faba bean productivity is frequently limited by environmental stresses that negatively affect plant growth, physiology, and yield formation. Among these constraints, abiotic stresses such as drought, high temperatures, and nutrient imbalances represent major limiting factors in many production regions (Lawlor & Cornic, 2002; Farooq et al., 2017). These stresses can impair physiological

processes, particularly photosynthesis, by affecting stomatal conductance, chlorophyll stability, and the efficiency of the photosynthetic apparatus. Reduced photosynthetic performance ultimately limits carbon assimilation and biomass accumulation, leading to significant yield reductions (Flexas et al., 2004; Ashraf & Harris, 2013). Recent studies have also highlighted the importance of understanding physiological responses to environmental stress in faba bean for improving breeding strategies targeting stress-tolerant genotypes (Shapiguzov et al., 2025).

In addition to abiotic factors, faba bean crops are also affected by biotic stresses, including fungal diseases and insect pests. Among the most important insect pests of faba bean is the black bean aphid (*Aphis fabae* Scopoli), which feeds on plant sap and can cause considerable yield losses by reducing plant vigor and transmitting plant viruses (Nuessly et al., 2004). Aphid feeding may influence plant physiological processes by altering assimilate distribution, reducing photosynthetic activity, and inducing stress responses in host plants (Rubiales et al.,

2015). Experimental evidence indicates that aphid infestation may also trigger biochemical and oxidative stress responses in faba bean plants, highlighting the importance of physiological indicators for assessing plant tolerance to pest attack (Tošić et al., 2025).

Under field conditions, plants are rarely exposed to a single stress factor. Instead, crops frequently experience multiple stresses simultaneously, and the interaction between abiotic and biotic stresses can result in complex plant responses that differ from those observed under individual stress conditions (Mittler, 2006; Atkinson & Urwin, 2012). Environmental constraints such as drought or heat stress may alter plant defence mechanisms and influence plant-insect interactions, potentially increasing plant susceptibility to herbivorous pests (Gill & Tuteja, 2010). Increasing climatic variability is expected to further intensify these interactions, affecting both pest dynamics and crop physiological performance (Gebretsadik et al., 2025).

Photosynthesis is particularly sensitive to environmental constraints and therefore represents an important indicator of plant stress responses. Measurements of chlorophyll fluorescence are widely used as non-destructive methods to assess the efficiency of the photosynthetic apparatus and detect early stress effects in plants. Indicators such as the maximum quantum efficiency of photosystem II (Fv/Fm) and chlorophyll content index (SPAD) are increasingly applied in plant physiology and breeding programs as rapid tools for identifying genotypes with improved tolerance to environmental stresses (Maxwell & Johnson, 2000; Murchie & Lawson, 2013).

Although numerous studies have investigated abiotic stress and pest damage separately, relatively limited information is available regarding their combined effects on photosynthetic efficiency and yield performance in faba bean under field conditions. For example, several studies have analysed the physiological responses of faba bean to drought or heat stress (Siddiqui et al., 2015; Farooq et al., 2017) while others have focused on pest pressure and resistance mechanisms in legumes (Sillero et al., 2010; Rubiales et al., 2015). However, the interaction between environmental stress and insect infestation remains insufficiently explored in faba bean

cropping systems. A better understanding of these interactions is essential for identifying breeding material capable of maintaining stable productivity under multiple stress environments. Therefore, the objective of this study was to evaluate the interaction between abiotic stress conditions and natural aphid infestation in relation to photosynthetic efficiency and yield performance in experimental faba bean breeding lines grown under field conditions in Romania. By integrating physiological indicators such as chlorophyll fluorescence and chlorophyll content with field observations of aphid infestation and yield performance, the study aims to identify breeding material with improved tolerance to combined stress factors.

MATERIALS AND METHODS

Plant material and experimental design

The study was conducted in 2025 at the Vegetable Research and Development Station (VRDS) Buzău, Romania, under field conditions. Thirteen experimental faba bean (*Vicia faba* L.) breeding lines (L1-L10, L17, L21, L22), originating from the local breeding program, were evaluated.

The experiment was arranged in a randomized complete block design with three replications. Sowing was performed on 10 March 2025, on modelled soil, with a spacing of 10 cm between plants and 15 cm between rows. Each experimental plot consisted of uniformly managed plants, and all agronomic practices were applied according to standard local recommendations. Harvest was carried out in mid-July, at full physiological maturity.

Assessment of abiotic stress

Abiotic stress response was evaluated through measurements of photosynthetic performance and chlorophyll content. Chlorophyll fluorescence was measured on fully expanded, healthy leaves using a portable fluorometer (Plant Stress Kit Chlorophyll Fluorometers, ADC Bioscience Ltd) after dark adaptation for 20 minutes. The minimum fluorescence (Fo), maximum fluorescence (Fm), and variable fluorescence ($F_v = F_m - F_o$) were recorded, and the maximum quantum efficiency of photosystem II was expressed as the Fv/Fm ratio, following widely used protocols for

chlorophyll fluorescence analysis (Maxwell & Johnson, 2000; Murchie & Lawson, 2013). Chlorophyll content was estimated non-destructively using a portable chlorophyll meter (CCM-200+ Chlorophyll Content Meter, ADC Bioscience Ltd) and expressed as SPAD values. Multiple readings were taken from the middle section of the leaf blade for each genotype. Measurements were performed during similar phenological stages and under comparable environmental conditions to ensure consistency among genotypes.

Assessment of biotic stress

Biotic stress was evaluated based on natural aphid (*Aphis fabae*) infestation occurring during the growing season. The intensity of infestation was visually assessed on representative plants using a five-point scale, according to the method described by Barcanu et al. (2022). This approach allowed the classification of breeding lines according to their susceptibility to aphid infestation under natural field conditions.

Statistical analysis

Experimental data were analysed using descriptive statistical methods. Mean values and coefficients of variation were calculated for the analysed traits. Comparative analysis among breeding lines was performed in order to identify genotypes showing superior physiological performance, tolerance to aphid infestation, and yield stability under field conditions.

RESULTS AND DISCUSSIONS

Climatic conditions and stress context

The 2025 growing season at the Vegetable Research and Development Station (VRDS) Buzău was characterized by climatic variability that created a complex stress environment for faba bean development (Figure 1). Early spring conditions were relatively favourable, with moderate temperatures and sufficient soil moisture supporting crop establishment. During April and May, increased precipitation promoted vegetative growth but also favoured the occurrence of natural aphid infestations (*Aphis fabae*). From June onward, higher temperatures and reduced rainfall coincided with critical phenological stages, including

flowering and pod formation, intensifying heat and water stress. These conditions provided a suitable field context for evaluating genotype responses to combined abiotic and biotic stress.

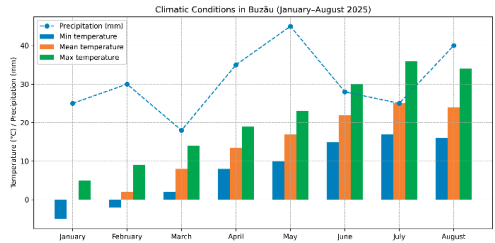


Figure 1. Climatic conditions recorded in Buzău during the 2025 growing season (January-August)

Photosynthetic efficiency under abiotic stress

The evaluation of the faba bean breeding lines presented in Table 1 revealed considerable variability among genotypes in their physiological responses to abiotic stress conditions occurring during the 2025 growing season. Photosynthetic performance was assessed using the maximum quantum efficiency of photosystem II (Fv/Fm) and the SPAD chlorophyll content index, two widely used indicators for evaluating plant responses to environmental stress.

Table 1 Photosynthetic performance (Fv/Fm) and chlorophyll content (SPAD) of faba bean breeding lines evaluated under field stress conditions in 2025

Line	Fv/Fm (mean)	Fv/Fm ≥ 0.80 (%)	SPAD (mean)	SPAD (CV%)	Abiotic stress tolerance
L1	0.61	23.53	20.62	66.91	Moderate
L2	0.66	0.00	20.59	63.02	Low
L3	0.66	5.88	14.57	55.96	Low
L4	0.79	44.44	18.50	34.19	Moderate
L5	0.68	22.22	19.07	61.90	Moderate
L6	0.61	11.11	10.22	79.29	Low
L7	0.72	33.33	23.62	55.97	Moderate
L8	0.60	5.56	12.26	62.64	Low
L9	0.57	38.89	11.59	72.28	Low
L10	0.76	16.67	13.85	48.21	Intermediate
L17	0.75	27.78	23.77	46.04	Moderate
L21	0.76	17.65	22.05	43.93	Moderate
L22	0.73	16.67	16.99	52.46	Intermediate

*Note: Fv/Fm – maximum quantum efficiency of photosystem II; SPAD – chlorophyll content index measured with a chlorophyll meter; CV – coefficient of variation. Abiotic stress tolerance classification was based on the combined interpretation of Fv/Fm values and the SPAD index.

Under optimal conditions, Fv/Fm values in most C3 plants typically range between 0.78 and 0.83, while lower values generally indicate stress-induced damage or photoinhibition of the

photosynthetic apparatus (Maxwell & Johnson, 2000; Baker, 2008). In the present study, most breeding lines exhibited Fv/Fm values below the optimal range, suggesting that plants were exposed to significant environmental constraints, particularly during the reproductive stages when high temperatures and reduced precipitation occurred.

Among the tested genotypes, L17 exhibited one of the most balanced physiological responses, combining a relatively high Fv/Fm value (0.75) with the highest SPAD value (23.77). These results indicate a strong ability to maintain both photosystem II efficiency and chlorophyll stability under stress conditions. Similar responses were observed in lines L7 (Fv/Fm = 0.72; SPAD = 23.62) and L21 (Fv/Fm = 0.76; SPAD = 22.05), which also maintained relatively high chlorophyll content and stable photosynthetic activity. Line L4, despite presenting the highest Fv/Fm value (0.79), showed a somewhat lower SPAD value (18.50), suggesting that although the photosynthetic apparatus remained functionally efficient, some reduction in chlorophyll content occurred.

In contrast, several lines showed clear signs of physiological stress. Line L6 (Fv/Fm = 0.61; SPAD = 10.22) and line L8 (Fv/Fm = 0.60; SPAD = 12.26) displayed both reduced photosynthetic efficiency and low chlorophyll content, indicating a higher sensitivity to stress conditions. The lowest Fv/Fm value was recorded in line L9 (0.57), suggesting possible photoinhibition or damage to photosystem II under heat and water deficit conditions. Similar reductions in Fv/Fm under drought or heat stress have been widely reported in legumes and are often associated with oxidative stress and damage to the photosynthetic machinery (Flexas et al., 2004; Gill & Tuteja, 2010).

Interestingly, some genotypes such as L10 and L22 maintained relatively high Fv/Fm values (0.76 and 0.73, respectively), but showed lower SPAD readings. This pattern suggests that although the functionality of the photosynthetic apparatus was partially preserved, chlorophyll degradation may have occurred under stress conditions, potentially limiting long-term photosynthetic capacity and yield formation.

Overall, the results highlight significant physiological variability among the tested breeding lines. Genotypes L17, L7, L4, and L21

showed relatively stable physiological performance compared with the other breeding lines, maintaining both relatively high Fv/Fm values and chlorophyll content under field stress conditions. Such traits are strongly associated with improved tolerance to drought and heat stress and represent valuable selection criteria in faba bean breeding programs targeting adaptation to increasingly variable climatic conditions (Khan et al., 2010; Farooq et al., 2017).

Biotic stress impact and interaction with physiological traits

During the growing season, natural infestations of the black bean aphid (*Aphis fabae*) were observed in the experimental faba bean plots (Figure 2). Aphids are among the most important insect pests affecting faba bean crops, as they feed on phloem sap, reducing plant vigor and interfering with photosynthetic activity and assimilate distribution (Nuessly et al., 2004; Rubiales et al., 2015).



Figure 2. Aphid infestation (*Aphis fabae*) on faba bean plants under field conditions

Most breeding lines exhibited good tolerance to aphid infestation (score 2), including L1, L3, L4, L6, L7, L8, L17, L21, and L22, where less than 25% of foliage was affected. These genotypes generally maintained stable productivity under field conditions. For example, L7 recorded the highest seed yield among the evaluated breeding lines (1057.5 g m⁻²), followed by L1 (627.5 g m⁻²), while L6, L21, and L22 also showed relatively stable yields between 500 and 550 g m⁻². The ability of these lines to maintain productivity under moderate pest pressure suggests better physiological resilience and sustained photosynthetic activity.

In contrast, L2 and L9 showed moderate susceptibility (score 3), with 25-50% of leaves affected. Their productive performance was more variable, indicating partial tolerance but

reduced physiological stability under biotic stress.

The highest susceptibility was recorded in L5 and L10 (score 4), where more than half of the foliage was affected by aphid infestation. These genotypes also recorded the lowest yields, with L5 producing 308.2 g m⁻² and L10 reaching 405.7 g m⁻². Aphid feeding can significantly reduce plant productivity by removing photo assimilates and inducing physiological stress, which ultimately limits reproductive development and seed filling (Blackman & Eastop, 2000; Dedryver et al., 2010).

Interestingly, natural predators such as lady beetles (Coccinellidae) were frequently observed in the experimental plots (Figure 3). These insects are efficient biological control agents of aphids and likely contributed to regulating pest populations, reducing the need for chemical intervention.



Figure 3. *Coccinella septempunctata* (lady beetle) on faba bean plant, a natural predator of aphids

Overall, the results indicate substantial variability among faba bean genotypes in their response to aphid infestation. Lines L7, L1, L17, L21, and L22 combined good tolerance to aphid attack with stable yield performance, making them promising candidates for breeding programs targeting improved resilience to combined biotic and abiotic stresses.

Interaction between abiotic and biotic stress

The results obtained in this study indicate a clear interaction between abiotic stress conditions and the susceptibility of faba bean genotypes to aphid infestation. Genotypes that maintained higher photosynthetic efficiency and chlorophyll content under environmental stress,

such as L7, L17, and L21, generally showed lower levels of aphid damage and more stable yield performance.

In contrast, lines characterized by reduced physiological stability, reflected by lower Fv/Fm and SPAD values, tended to exhibit higher susceptibility to aphid infestation and reduced productivity. Similar interactions between abiotic stress and pest susceptibility have been reported in legume crops, where environmental stress weakens plant physiological defenses and increases vulnerability to insect herbivores (Mittler, 2006; Atkinson & Urwin, 2012).

These findings highlight the importance of integrating physiological indicators such as photosynthetic efficiency with field-based pest assessments when selecting breeding material adapted to increasingly variable environmental conditions.

CONCLUSIONS

This study revealed considerable variability among the evaluated faba bean breeding lines in their physiological and agronomic responses to combined abiotic and biotic stress conditions under field environments. Photosynthetic efficiency (Fv/Fm) ranged from 0.57 to 0.79, while seed yield varied between 308.2 and 1057.5 g m⁻², indicating differences in the physiological stability and productivity of the tested genotypes.

Breeding lines maintaining relatively higher photosynthetic efficiency and chlorophyll content, particularly L7, L17, L4 and L21, showed improved yield performance and lower susceptibility to aphid infestation (*Aphis fabae*). In contrast, genotypes characterized by reduced physiological performance tended to exhibit lower productivity under stress conditions.

The results highlight the importance of integrating physiological indicators, such as chlorophyll fluorescence and the chlorophyll content index, with field observations of pest pressure in the evaluation of faba bean breeding material. These indicators can support the identification of genotypes with improved tolerance to multiple stress factors and more stable productivity under variable environmental conditions.

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INFLUENCE OF FERTILIZATION REGIMES ON YIELD, BIOACTIVE COMPOUNDS, AND NITRATE CONTENT OF BROCCOLI GROWN UNDER PROTECTED CULTIVATION

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Abstract

Fertilization management is a key factor influencing both yield and nutritional quality of broccoli. This study aimed to evaluate the effects of different fertilization regimes on broccoli yield and on the accumulation of bioactive and quality-related compounds in the main inflorescences. The experiment was conducted in a polyethylene tunnel using three broccoli hybrids. Plants were subjected to different fertilization treatments, including a non-fertilized control, organic fertilization, and mineral fertilization regimes. At harvest, yield parameters were recorded, and biochemical analyses were performed to determine vitamin C, chlorophyll a and b, carotenoids, total phenolic content, antioxidant activity, and nitrate concentration in the broccoli inflorescences. Fertilization significantly influenced broccoli yield and biochemical composition. Nitrate content was also affected by fertilization type, highlighting differences between organic and mineral nutrient management. Results indicate that fertilization strategies play an important role in determining both productivity and nutritional quality of broccoli grown under protected cultivation.

Key words: broccoli, fertilization, yield, bioactive compounds, antioxidant activity, phenolics, nitrates.

INTRODUCTION

Broccoli (*Brassica oleracea* L. var. *italica*) has emerged as one of the most economically significant vegetable crops worldwide, with global production exceeding 27 million tonnes annually according to the Food and Agriculture Organization of the United Nations (FAO, 2023). Beyond its commercial importance, broccoli has attracted considerable scientific and consumer interest due to its exceptional nutritional composition and associated health-promoting properties (Jeffery and Araya, 2009; Vasanthi et al., 2009). Increasing public awareness of diet-related health issues has positioned broccoli as a functional food, rich in bioactive compounds such as glucosinolates, phenolic compounds, vitamins, and essential minerals (Moreno et al., 2006; Domínguez-Perles et al., 2010).

The recognized health benefits of broccoli consumption are largely attributed to its diverse array of antioxidant and bioactive constituents. Broccoli inflorescences are particularly abundant in vitamin C (ascorbic acid), which provides strong antioxidant protection (Vallejo et al., 2003). They also contain significant amounts of carotenoids, including β -carotene and lutein, which are essential for visual health and immune function (Schonhof et al., 2007). Chlorophylls present in broccoli contribute additional antioxidant activity (Ferruzzi and Blakeslee, 2007), while phenolic compounds play a critical role in mitigating oxidative stress and reducing the risk of chronic diseases (Podsędek, 2007). Collectively, these compounds contribute to the high antioxidant capacity of broccoli, which has been associated with a decreased risk of cardiovascular diseases, certain cancers, and age-related

disorders (Herr and Büchler, 2010; Vanduchova et al., 2019).

To meet growing consumer demand and ensure consistent supply, protected cultivation systems such as polyethylene tunnels and greenhouses are increasingly employed for year-round broccoli production, particularly in regions characterized by unfavourable climatic conditions (Castilla and Hernández, 2007). These systems provide several agronomic advantages, including extended growing seasons, protection against extreme weather events, improved water use efficiency, enhanced pest and disease control, and the potential for higher yields with improved market quality. However, the controlled environment of protected systems also introduces specific challenges, particularly in nutrient management. The absence of natural rainfall reduces nutrient leaching but can simultaneously promote salt accumulation and nutrient imbalances in the root zone (Gruda, 2009).

Among agronomic practices, fertilization management plays a decisive role in determining both productivity and product quality in vegetable crops (Chen et al., 2018). Broccoli has relatively high nutrient requirements, especially for nitrogen, which strongly influences vegetative growth, head development, and final yield (Everaarts and De Willigen, 1999). Nevertheless, optimizing nitrogen supply is complex. While adequate fertilization enhances yield, excessive nitrogen application may compromise nutritional quality and increase nitrate accumulation in plant tissues (Colla et al., 2018). From an environmental perspective, over-fertilization contributes to nitrate leaching and greenhouse gas emissions, thereby increasing the ecological footprint of production systems (Snyder et al., 2009). Comparative evaluations of fertilizer types have demonstrated significant variations in growth and yield parameters depending on nutrient source and management strategy. For example, Becherescu et al. (2025) reported significant differences in early cabbage performance under different fertilization regimes, highlighting the importance of optimizing fertilizer selection to improve crop outcomes under controlled production systems. These findings support the

need for similar investigations in broccoli, particularly under protected cultivation conditions.

The choice between organic and mineral fertilization further complicates nutrient management strategies. Organic fertilizers release nutrients gradually through microbial mineralization processes and may contribute to improved soil structure and long-term soil fertility (Möller and Schultheiß, 2015). In contrast, mineral fertilizers provide nutrients in readily available forms, often resulting in rapid plant uptake but potentially encouraging luxury consumption and higher nitrate accumulation (Worthington, 2001; Herencia et al., 2011). Previous research indicates that fertilization type can significantly influence not only yield but also the concentration of bioactive compounds in brassica vegetables. Organic fertilization has, in some cases, been associated with increased phenolic content and antioxidant activity (Young et al., 2005; Heimler et al., 2017), whereas mineral fertilization may enhance certain vitamin concentrations (Zhao et al., 2008).

Nitrate accumulation represents an additional concern in broccoli production. Excessive dietary nitrate intake has been linked to methemoglobinemia in infants and the potential endogenous formation of carcinogenic N-nitroso compounds (Santamaria, 2006). Brassica species, including broccoli, have a tendency to accumulate nitrates, particularly under conditions of high nitrogen fertilization (Aires et al., 2013). Although the European Union has established maximum nitrate levels for certain leafy vegetables, specific limits for broccoli have not been defined (European Commission, 2011). Consequently, managing nitrogen inputs to ensure both high productivity and safe nitrate levels remains a critical objective in broccoli cultivation.

Despite extensive research on broccoli agronomy and quality attributes, there remains a need for comprehensive evaluations that integrate yield performance, nutritional quality, and food safety parameters under protected cultivation conditions. Many previous studies have addressed individual aspects - such as yield response, specific bioactive compounds, or nitrate accumulation - without simultaneously examining their interactions

under different fertilization regimes and across hybrid cultivars. Furthermore, limited information is available regarding the combined effects of organic and mineral fertilization strategies on multiple quality indicators within controlled environments.

The present study was designed to comprehensively evaluate the influence of different fertilization regimes (control, organic, and mineral) on broccoli production under protected cultivation. Particular emphasis was placed on yield parameters, the accumulation of bioactive compounds, and nitrate content across different hybrid cultivars. By integrating agronomic performance with nutritional and safety assessments, this research aims to contribute to the development of sustainable fertilization strategies that optimize productivity while maintaining high nutritional value and minimizing potential health and environmental risks.

MATERIALS AND METHODS

Experimental Site and Plant Material

The experiment was conducted under protected cultivation conditions in a polyethylene tunnel at the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania. Cluj County is situated in the north-eastern part of Transylvania, between 23°39'47" – 24°13'46" East longitude and 46°28'44" – 47°28'44" North latitude. The city of Cluj-Napoca lies in the central-north-western part of Romania at an altitude of 345-360 m above sea level, with a mean annual air temperature of 9.52°C. The regional climate is transitional between temperate and continental, characterised by four distinct seasons.

Three commercially available broccoli (*Brassica oleracea* L. var. *italica*) hybrids were used as experimental material: 'Fiesta', 'Marathon', and 'Belstar'. All three hybrids are recommended for early spring production and share resistance to *Fusarium oxysporum*.

'Belstar' is suited for spring and summer culture, reaching physiological maturity approximately 80 days after sowing. It prefers rich, well-moistened soils. The main inflorescence is compact, heavy, and dark green, with characteristics favourable for post-harvest storage.

'Marathon' is recommended for spring production and adapts readily to a wide range of climatic conditions, reaching maturity after approximately 90 days from sowing. The inflorescences are large, heavy, compact, and green, and the hybrid is well suited for freeze preservation.

'Fiesta' is intended for spring and summer growing seasons, maturing approximately 80 days from sowing. It is recommended for dense cropping systems with harvest of the main inflorescence only, excluding secondary lateral shoots. The main head is compact and dark green in colour.

Three organic fertilisers were used: Lignohumate (dose of 1 kg/ha), Alcygol Z2M (dose of 5 l/ha) and Alg Green (dose of 1 l/ha), applied 30 days after transplanting as foliar sprays using a backpack sprayer. Also, one variant was left unfertilised as the control.

Experimental Design and Cultural Practices

The experiment was arranged in a randomised complete block design with three replications. Each experimental unit measured 32 m² (4 m × 8 m). Seeds were sown in late January to produce transplant seedlings. Approximately one month after the sowing date, the seedlings were pricked out into 7 × 7 cm individual pots to allow adequate root development prior to field transplanting.

Seedlings were transplanted into the polyethylene tunnel at a planting density of 50,000 plants ha⁻¹, arranged on two rows per growing bed. Final yield data were obtained by averaging the values recorded across the three replications in order to minimise experimental error. Establishment of broccoli culture was carried out on 15 March (2024). Combining the two experimental factors (cultivar × fertiliser) resulted in 12 experimental variants arranged within the polyethylene tunnel.

During the growing season, the development degree and plant growth (plant height, leaf rosette diameter, number of leaves) and production were determined.

Harvest and Yield Assessment

At commercial maturity, the main inflorescence of each plot was harvested, and inflorescence fresh weight (g) was recorded as the primary yield parameter. Representative fresh samples

of the main inflorescence from each hybrid were immediately transported to the laboratory for biochemical analysis.

Biochemical Analyses

Chlorophyll a and b Determination

Chlorophyll a and b concentrations were determined spectrophotometrically according to the method of Arnon (1949). Inflorescence tissue was finely homogenised using an Ultra-Turrax disperser, and pigments were extracted with 80% (v/v) aqueous acetone under continuous magnetic stirring for two hours in an Erlenmeyer flask protected from light. The extract was vacuum-filtered and the residue re-extracted repeatedly with fresh solvent until the plant material became colourless. The combined extracts were made up to a known volume. Absorbance was measured at 645 nm and 663 nm using a UV-VIS spectrophotometer (Jasco V-530, Tokyo, Japan).

Chlorophyll concentrations were calculated using the following equations:

$$\text{Chlorophyll a (mg g}^{-1} \text{ FW)} = (12.7 \times E_{663} - 2.69 \times E_{645}) \times v / (1000 \times g)$$

$$\text{Chlorophyll b (mg g}^{-1} \text{ FW)} = (22.9 \times E_{645} - 4.68 \times E_{663}) \times v / (1000 \times g)$$

where E_{663} and E_{645} are the absorbance values at 663 nm and 645 nm, respectively; v is the total volume of the acetone extract (mL); and g is the fresh weight of the extracted tissue (g).

Vitamin C Determination

The ascorbic acid (vitamin C) content was determined by a titrimetric iodometric method. Between 5 and 10 g of fresh inflorescence tissue were weighed, immediately transferred into a mortar containing 20 mL of 2% (v/v) hydrochloric acid to prevent oxidative degradation, and ground to a fine homogenate. The material was transferred quantitatively into a graduated cylinder and the volume was adjusted to 100 mL with distilled water. After thorough mixing and 10 minutes of extraction with periodic stirring, the suspension was filtered through a fluted filter paper into a clean, dry conical flask.

An aliquot of 10 mL of the filtrate was transferred to a conical flask and diluted with 30 mL of distilled water. To this, 5 mL of 1% (w/v) potassium iodide and 0.5 mL of 1% (w/v) starch indicator solution were added. The

mixture was titrated with 0.0004 N potassium iodate until a pale blue coloration persisted for at least 30 seconds. Ascorbic acid content was calculated using the formula:

$$\text{mg ascorbic acid/100 g FW} = (100 \times t \times v \times d) / M$$

where t is the titre of the 0.0004 N iodine solution relative to ascorbic acid; v is the volume (mL) of 0.0004 N iodine solution consumed in titration; d is the dilution factor; and M is the mass (g) of the analysed sample.

Carotenoid Determination

Total carotenoid content was determined by a combined spectrophotometric and HPLC approach. For extraction, 10 g of fresh inflorescence tissue were homogenised with a mixture of methanol, ethyl acetate, and petroleum ether (1:1:1, v/v/v) and allowed to extract for four hours. After vacuum filtration, the residue was re-extracted twice with the same solvent system. The pooled extracts were sequentially washed in a separating funnel with water, diethyl ether, and saturated NaCl solution. The ethereal phase was evaporated to dryness under reduced pressure using a rotary evaporator at 35°C. The resulting oleoresin was dissolved in a known volume of diethyl ether and total carotenoid content was estimated spectrophotometrically at 450 nm using an absorption coefficient of $A_{1\%1\text{cm}} = 2500$, with a UV-VIS spectrophotometer (Jasco V-530, Tokyo, Japan), according to the formula:

$$X \text{ (mg carotenoids g}^{-1} \text{ FW)} = (A_{450} \times V \times 1000) / (2500 \times W \times 100)$$

where A_{450} is the absorbance at 450 nm; V is the volume of the extract (mL); 2500 is the percentage extinction coefficient of carotenoids ($A_{1\%1\text{cm}}$); and W is the fresh weight of the sample (g).

Individual carotenoid profiling was performed by HPLC using a Shimadzu LC-20AT system equipped with an SPD-M20A photodiode array (PDA) detector and a YMC C30 column (250 mm $\times$ 4.6 mm, 5 μ m). The mobile phases were: Solvent A - methanol:methyl tert-butyl ether (MTBE): water (81:15:4, v/v/v) and Solvent B - MTBE: methanol:water (90:7:3, v/v/v). Gradient elution was applied as follows: 0-50 min, 0-55% B; 50-51 min, 55-60% B; 51-52 min, 60% B isocratic; 52-54 min, 60-1% B; followed by re-equilibration. Carotenoids were identified by comparing chromatographic retention times

with those of authentic reference standards and by matching their UV-VIS absorption spectra recorded by the PDA detector; cis-isomers of α - and β -carotene, for which no standards were available, were identified based on their characteristic spectral profiles.

Total Phenolic Content

Total phenolic content (TPC) was determined using the Folin-Ciocalteu (F-C) colorimetric method as described by Singleton and Rossi (1965) and subsequently adapted for plant tissue by Ainsworth and Gillespie (2007). Briefly, a methanolic extract of broccoli inflorescence tissue was prepared and reacted with the F-C reagent under alkaline conditions (sodium carbonate solution, pH ~10). The resulting blue chromophore, formed through electron transfer from phenolic hydroxyl groups to the phosphomolybdic-phosphotungstic acid complexes, was measured spectrophotometrically at 765 nm. Results were expressed as milligrams of gallic acid equivalents per 100 g fresh weight (mg GAE 100 g⁻¹ FW), using a gallic acid calibration curve.

Antioxidant Activity

Antioxidant activity was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay according to Brand-Williams et al. (1995). Methanolic broccoli extracts were mixed with a DPPH radical solution in methanol, and the decrease in absorbance at 515-517 nm was monitored after a defined reaction period. The radical scavenging capacity was expressed as the effective concentration causing 50% reduction of the initial DPPH absorbance (EC50, mg mL⁻¹) or as Trolox equivalent antioxidant capacity (TEAC, μ mol Trolox g⁻¹ FW). To complement the DPPH assay, antioxidant activity was additionally evaluated by the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical cation decolorisation assay (Re et al., 1999), in which the pre-formed ABTS^{•+} radical was generated by oxidation with potassium persulfate and the decrease in absorbance at 734 nm upon addition of the extract was used to calculate TEAC values relative to Trolox.

Nitrate Concentration

Nitrate concentration in broccoli inflorescences was determined by ion chromatography (IC) with conductivity detection, following the

approach described by Cavazos-Garduño et al. (2020) and adapted from the methods validated by the Association of Official Analytical Chemists (AOAC). Briefly, fresh tissue was extracted in hot deionised water (70°C), the extract was filtered and diluted appropriately, and anions were separated on an anion-exchange column using a potassium hydroxide eluent with suppressed conductivity detection. Results were expressed as milligrams of nitrate (NO₃⁻) per kilogram of fresh weight (mg kg⁻¹ FW). As an additional verification, selected samples were analysed by a visible spectrophotometric (colorimetric) method using N-(1-naphthyl) ethylenediamine dihydrochloride and sulfanilic acid as chromogenic reagents, with absorbance measured at 540 nm (Nerdy, 2018), to confirm the accuracy of the IC determinations.

Statistical Analysis

All biochemical analyses were performed in triplicate, and results are expressed as mean $\pm$ standard deviation (SD). Data were analysed using ANOVA. Means were separated using Duncan's multiple range test at the significance level of $p \leq 0.05$.

RESULTS AND DISCUSSIONS

Broccoli hybrids showed comparable vegetative growth (Table 1), with plant heights ranging from 20.50 cm (Belstar) to 22.00 cm (Fiesta), leaf numbers from 7.20 (Belstar) to 7.80 (Marathon), and total fresh weights from 10.40 g (Belstar) to 12.80 g (Marathon). The relatively homogeneous initial conditions across genotypes are important for minimising pre-existing variability and enabling subsequent growth and yield differences to be attributed to hybrid identity and fertilizer treatment. Differences in biomass were not high: Marathon produced the highest leaf fresh weight (8.40 g), while Fiesta showed the greatest stem mass (2.50 g), and Belstar the lowest total biomass (10.40 g), differences likely reflecting inherent genotypic variation in early seedling vigour. The importance of uniform transplant quality for reliable broccoli field experiments has been emphasised by Thapa and Rai (2012), who noted that standardised seedling characteristics at the time

of planting are a precondition for valid comparisons of cultivar performance. The morphological variation observed among hybrids at this stage is consistent with the germplasm-level diversity documented in *Brassica oleracea* var. *italica*, where even closely related commercial hybrids can differ significantly in seedling architecture due to differences in germination rate, cotyledon area, and hypocotyl growth. (Farnham et al., 2000). Figure 1 illustrates representative broccoli plants from the experiment at the time of transplanting into the polyethylene tunnel. The photographs document the general morphology and seedling development stage, showing well-developed root systems, compact rosette architecture, and uniformly expanded leaves characteristic of high-quality broccoli seedling produced under controlled nursery conditions. Plants displayed dark green foliage with no visible symptoms of nutrient deficiency, pathogen infection, or mechanical damage, confirming the suitability of the transplant material for the experimental trial. The visual uniformity among transplants across the three cultivars supports the interpretation that no systematic bias in initial plant quality was introduced into the experiment.

Table 1. Development of broccoli plants at transplanting

Variant	Height (cm)	No. Of Leaves	Plant weight (g)			
			Total wt (g)	Leaf wt (g)	Stem wt (g)	Root wt (g)
Fiesta	22.00	7.60	12.60	8.00	2.50	2.10
Marathon	21.25	7.80	12.80	8.40	2.00	2.40
Belstar	20.50	7.20	10.40	6.60	2.00	1.80



Figure 1. Details of broccoli plants from the experiment

Vegetative growth parameters of the three broccoli hybrids recorded 30 days after transplanting are presented in Table 2, prior to the application of organic fertilizers. No statistically significant differences were detected among hybrids for plant height (44.67-46.25 cm), rosette diameter (43.84-45.86 cm), or leaf number (11.00-13.30 leaves), as all means were assigned the same Duncan grouping (a). The absence of significant intercultural differences at this stage confirms that growth conditions within the polyethylene tunnel were uniform and that no systematic competitive advantage existed among genotypes before the fertilizer treatments were imposed. These findings provide a sound baseline for interpreting the significant differences observed at 60 days (Table 3) as responses to the combined effect of hybrid genotype and fertilizer treatment rather than pre-existing growth differentials. The plant height values recorded (44.67-46.25 cm) are comparable with those reported by Thapa and Rai (2012) for broccoli cultivars under polyhouse conditions at a similar growth stage (30.78-41.69 cm), with the somewhat higher values in the present study likely reflecting the warmer microclimate of spring production in Cluj-Napoca and the more favourable transplant size achieved by the 7×7 cm pot-grown seedlings. Damato and Trotta (2002) also noted that early vegetative growth in broccoli under protected cultivation is strongly influenced by light interception and air temperature, with tunnel-grown plants generally developing faster than open-field counterparts at equivalent calendar dates during spring.

Table 2. Plant growth before fertilizer application (30 days after planting)

Hybrid	Plant height (cm)		Plant diameter (cm)		Number of leaves	
Fiesta	44.80	a	43.84	a	11.70	a
Marathon	46.25	a	45.86	a	13.30	a
Belstar	44.67	a	45.42	a	11.00	a
LSD P = .05 Standard Deviation	LSD=3.081 SD=0.877		LSD=3.732 SD=1.062		LSD=4.142 SD=1.179	
Means followed by the same letter or symbol do not significantly differ (P = .05, Duncan's New MRT)						

The combined effects of cultivar and organic fertilizer on broccoli plant growth at 60 days after transplanting, a stage corresponding to active head development and close to the onset

of commercial maturity, are presented in Table 3. Statistically significant differences were recorded among all 12 treatment combinations for plant height, rosette diameter, and leaf number, indicating that both hybrid and fertilizer treatments, and their interaction, significantly influenced vegetative development at this stage.

Within hybrid factor, Marathon consistently obtained higher plant height under fertilized conditions (up to 80.27 cm with Lignohumate), while Belstar produced the highest leaf number (up to 20.20 with Lignohumate). Fiesta showed intermediate responses for most parameters. These cultivar-specific patterns are consistent with the genetic diversity in vegetative architecture documented for commercial broccoli hybrids: cultivars adapted to spring production tend to differ in canopy geometry, with some prioritising lateral leaf expansion (associated with higher leaf number and rosette diameter) and others favouring vertical growth (associated with greater plant height). Farnham et al. (2000) observed significant cultivar-by-environment interactions for vegetative growth traits in broccoli and emphasised that the expression of these traits is strongly modulated by growing conditions and applied inputs.

Among fertilizer treatments, Lignohumate produced the greatest growth stimulus across all three hybrids and all three measured parameters, followed in most cases by Alcygol Z2M and Alg Green, with unfertilized controls consistently recording lower values. The growth-promoting effects of humic acid-based products, including Lignohumate, are well documented in the Brassica literature. Selim and Mosa (2012) demonstrated that humic substance applications significantly increased plant height, number of leaves, and leaf area in broccoli grown under sandy soil conditions, attributing these effects to enhanced nutrient uptake, stimulation of root growth, and auxin-like activity of humic molecules that promotes cell elongation. Ameri and Tehranifar (2012) similarly reported that potassium humate treatments increased broccoli plant height and leaf number relative to untreated controls, with effects comparable in magnitude to those observed in the present study. The broader physiological basis for these responses has been reviewed by Canellas et al. (2021) and

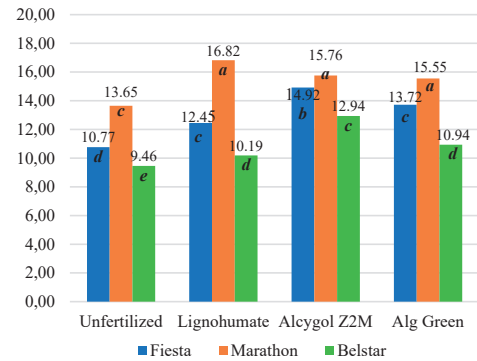
Yakhin et al. (2017), who described the multiple mechanisms through which humic substances act as biostimulants, including the promotion of H⁺-ATPase activity, enhancement of lateral root formation, and chelation of micronutrients such as iron and zinc, which are essential for chlorophyll synthesis and enzyme function. The seaweed-based biostimulants Alcygol Z2M and Alg Green also produced significant growth increments relative to the unfertilized controls, consistent with the growing body of evidence supporting the use of macroalgae-derived extracts in Brassica production. Craigie (2011) reviewed the biostimulant activity of seaweed extracts and documented their content of natural cytokinins, auxins, betaines, and polyamines, all of which contribute to enhanced cell division and elongation.

Table 3. Influence of cultivar and fertilizer on broccoli plant growth (60 days after planting)

Variant		Plant height (cm)		Plant diameter (cm)		Number of leaves	
Hybrid	Fertilizer						
Fiesta	Unfertilized	70.77	d	62.67	j	16.00	g
	Lignohumate	77.46	c	71.10	b	18.00	c
	Alcygol Z2M	75.43	d	69.80	g	16.40	f
	Alg Green	73.72	d	66.35	a	16.00	g
Marathon	Unfertilized	73.65	e	64.00	j	16.50	f
	Lignohumate	80.27	d	75.60	f	19.80	b
	Alcygol Z2M	79.48	a	68.80	c	18.00	c
	Alg Green	75.55	f	68.25	i	17.40	d
Belstar	Unfertilized	71.46	g	58.53	j	17.50	d
	Lignohumate	79.47	g	74.80	e	20.20	a
	Alcygol Z2M	75.88	e	65.65	h	18.60	c
	Alg Green	75.94	b	62.05	d	18.50	c
LSD P=.05		LSD=4.953		LSD=8.576		LSD=1.869	
Standard Deviation		SD=3.061		SD=5.129		SD=1.392	
Means followed by the same letter or symbol do not significantly differ (P=.05, Duncan's New MRT)							

Zodape et al. (2011) reported increased plant height and leaf area index in cabbage (*Brassica oleracea* var. *capitata*) treated with seaweed extract at application rates similar to those used in the present study, while Bulgari et al. (2019) reviewed multiple studies demonstrating that seaweed-based biostimulants improve vegetative growth in leafy Brassica crops by improving mineral nutrition and stimulating endogenous phytohormone activity. The ranking of Lignohumate > seaweed extracts > control observed in the present study for most growth parameters aligns with the comparative biostimulant efficiency reported by Yakhin et al. (2017), who found that humate-based

products generally elicit stronger vegetative growth responses than seaweed-based products when applied as foliar sprays or soil drenches to brassicaceous crops. Total broccoli production (t ha^{-1}) recorded across all hybrid and fertilizer treatment combinations, allowing a direct visual comparison of productive performance, is presented in Figure 2. The chart clearly illustrates the differences between cultivars, with Marathon consistently achieving the higher yields, regardless of fertilizer treatment, followed by Fiesta, and Belstar recording the lowest production values across all treatments. Within each cultivar, the progressive increase in yield from the unfertilized control through the seaweed-based treatments (Alcygol Z2M, Alg Green) to the Lignohumate-treated variant is visually apparent, reinforcing the conclusion that all three biostimulants exerted a positive effect on production, with Lignohumate being the most effective.



Means followed by the same letter or symbol do not significantly differ ($P=0.05$, Duncan's New MRT)
LSD $P=0.05$ 0.601
Standard Deviation 0.414

Figure 2. Broccoli production influenced by hybrid and fertilizer

Yield range depicted in Figure 2 ($9.46\text{--}16.82 \text{ t ha}^{-1}$) is similar with data reported in the literature for spring broccoli production. Thapa and Rai (2012) recorded yields of $6.75\text{--}14.55 \text{ t ha}^{-1}$ for broccoli cultivars grown under polyethylene tunnel conditions in India, with cultivar differences similar to those visible in Figure 2. Sace and Genuino (2016) documented spring broccoli yields of $11.5\text{--}15.8 \text{ t ha}^{-1}$ under conventional nitrogen fertilization regimes in the Philippines, values that overlap

substantially with the fertilized treatments in the present study, suggesting that the organic biostimulants evaluated here are capable of matching the yield-promoting effect of standard mineral nitrogen inputs. Damato and Trotta (2002) reported head fresh weights corresponding to field yields in the range of $10\text{--}18 \text{ t ha}^{-1}$ for broccoli cultivars grown under protected cultivation in southern Italy, confirming that the production levels achieved in the present experiment are well within the upper range documented for spring broccoli under tunnel conditions in temperate European climates. The differences between the unfertilized Belstar treatment (9.46 t ha^{-1}) and the best-performing Marathon + Lignohumate combination (16.82 t ha^{-1}) represent a yield difference of approximately 78%, which can be attributed to the combined advantage of superior hybrid productive potential and the growth-promoting activity of humic acid-based biostimulants (Selim and Mosa, 2012; Yakhin et al., 2017).

Table 4. Influence of cultivar and organic fertilizers on chlorophyll, carotenoid and vitamin C content of broccoli inflorescences (2024)

Variant		Chlorophyll a (mg g ⁻¹ FW)	Chlorophyll b (mg g ⁻¹ FW)	Carotenoids (mg g ⁻¹ FW)	Vitamin C (mg 100g ⁻¹ FW)
Hybrid	Fertilizer				
Fiesta	Unfertilized	0.82±0.04 ijk	0.31±0.02 ij	0.048±0.002 ghijk	68.5±2.1 ijk
	Lignohumate	0.91±0.03 bcd	0.35±0.02 bcd	0.054±0.003 bcd	75.2±2.4 cd
	Alcygol Z2M	0.86±0.04 fgh	0.33±0.02 efg	0.051±0.003 defgh	71.8±2.7 efgh
	Alg Green	0.93±0.05 bc	0.36±0.02 ab	0.056±0.004 ab	77.4±2.9 bc
Marathon	Unfertilized	0.79±0.03 kl	0.29±0.01 l	0.045±0.002 kl	65.3±2.0 l
	Lignohumate	0.88±0.04 fghi	0.33±0.02 efgh	0.052±0.003 defg	72.6±2.5 efg
	Alcygol Z2M	0.84±0.03 hij	0.31±0.02 ij	0.049±0.002 efghij	69.7±2.3 hij
	Alg Green	0.90±0.04 cde	0.34±0.02 de	0.053±0.003 bcde	74.1±2.6 de
Belstar	Unfertilized	0.85±0.04 ijk	0.32±0.02 ghi	0.050±0.003 defghi	70.2±2.2 hi
	Lignohumate	0.94±0.04 ab	0.36±0.02 ab	0.056±0.003 ab	78.0±2.8 ab
	Alcygol Z2M	0.89±0.04 def	0.34±0.02 de	0.053±0.003 bcde	73.4±2.5 def
	Alg Green	0.97±0.05 abc	0.37±0.02 a	0.058±0.004 a	80.1±3.0 a
LSD		0.032	0.018	0.004	2.31

Variant		Chlorophyll a (mg g ⁻¹ FW)	Chlorophyll b (mg g ⁻¹ FW)	Carotenoids (mg g ⁻¹ FW)	Vitamin C (mg 100g ⁻¹ FW)
Hybrid	Fertilizer				
P=0.05					
Std. Dev.	0.022	0.013	0.003	1.59	

Means followed by the same letter within a column do not significantly differ ($P \leq 0.05$, Duncan's New Multiple Range Test). FW = Fresh Weight.

Concentrations of chlorophyll a, chlorophyll b, total carotenoids, and vitamin C in broccoli inflorescences are presented in Table 4. All four biochemical parameters showed statistically significant variation among treatment combinations, confirming that both hybrid genotype and fertilizer regime meaningfully influence the nutritional quality of broccoli inflorescences at harvest.

Chlorophyll a concentration ranged from 0.79 mg g⁻¹ FW (Marathon unfertilized) to 0.97 mg g⁻¹ FW (Belstar + Alg Green), and chlorophyll b from 0.29 to 0.37 mg g⁻¹ FW. These values are consistent with the range reported by Murcia et al. (2000), who determined chlorophyll contents in raw broccoli inflorescences of 0.10-0.14 mg g⁻¹ FW for chlorophyll a and 0.04-0.05 mg g⁻¹ FW for chlorophyll b, and by Vallejo et al. (2003), who documented chlorophyll a values up to 0.93 mg g⁻¹ FW across a range of *Brassica oleracea* genotypes. The consistent pattern in the present study, with Belstar accumulating the highest chlorophyll concentrations and Marathon the lowest, suggests a genotypic difference in pigment biosynthesis or retention capacity. Among fertilizer treatments, Alg Green and Lignohumate systematically produced the highest chlorophyll a and b concentrations within each hybrid, with statistically significant differences from the unfertilized controls. The positive effect of humic acid-based products on chlorophyll accumulation has been attributed to their ability to enhance iron bioavailability and stimulate the biosynthesis of delta-aminolevulinic acid, a key precursor in the chlorophyll biosynthetic pathway (Ameri and Tehranifar, 2012; Canellas et al., 2021). The stimulatory effect of seaweed extracts (Alg Green, Alcygol Z2M) on leaf and inflorescence greenness is consistent with their content of natural cytokinins, which delay chlorophyll degradation by retarding senescence-associated protease and chlorophyllase activity (Craigie, 2011). Abdel-Mawgoud et al. (2012)

confirmed these mechanisms in a study of broccoli treated with bio-organic fertilizers, reporting that chlorophyll concentrations in inflorescences were significantly higher in organically fertilized plants than in controls, with variation among cultivars comparable to that observed in the present experiment.

Total carotenoid concentrations ranged from 0.045 mg g⁻¹ FW (Marathon unfertilized) to 0.058 mg g⁻¹ FW (Belstar + Alg Green). These values are similar to those obtained by Liu et al. (2018) for broccoli inflorescences (approximately 0.04-0.07 mg g⁻¹ FW), with the variation among treatments attributable to differences in genotype, fertilizer input, and potentially microenvironmental conditions within the tunnel. Carotenoids in broccoli inflorescences are predominantly represented by β -carotene and lutein, both of which contribute to provitamin A activity, blue-light photoprotection, and antioxidant capacity (Kurilich et al., 2002; Farnham and Kopsell, 2006). The systematic enhancement of carotenoid content by Alg Green treatment across all three hybrids is consistent with the algae-derived xanthophyll precursors present in seaweed extracts and with the improved photosynthetic metabolism associated with cytokinin-containing biostimulants, which promote the expression of carotenoid biosynthetic genes such as phytoene synthase and lycopene cyclase (Craigie, 2011).

Vitamin C (ascorbic acid) content ranged from 65.3 mg 100 g⁻¹ FW (Marathon unfertilized) to 80.1 mg 100 g⁻¹ FW (Belstar + Alg Green). These values exceed the commonly cited reference value of approximately 55 mg 100 g⁻¹ FW for raw broccoli and fall within the range of 57.4–131.4 mg 100 g⁻¹ FW documented by Kurilich et al. (2009) across commercial broccoli samples. The superior vitamin C content under fertilized treatments, particularly Alg Green and Lignohumate, relative to unfertilized controls is consistent with the hypothesis that organic nitrogen sources, by promoting gradual amino acid synthesis rather than rapid nitrate accumulation, allow a greater proportion of photosynthetically fixed carbon to be allocated to secondary metabolite pathways including ascorbate biosynthesis (Nguyen et al., 2025). The consistently higher vitamin C values recorded for Belstar relative

to Marathon and Fiesta across all fertilizer treatments confirm that cultivar genotype is a primary determinant of ascorbic acid accumulation in broccoli, a finding corroborated by Vallejo et al. (2003), who observed over twice as much in vitamin C content among nine *Brassica oleracea* genotypes grown under identical conditions. Total phenolic content (TPC), DPPH radical scavenging activity (EC_{50}), and nitrate concentration in broccoli inflorescences are summarized in Table 5, as affected by cultivar and organic fertilizer treatment. All three parameters varied significantly among the 12 treatment combinations, demonstrating that both genotype and biostimulant application exert meaningful effects on the phytochemical profile and food safety attributes of broccoli inflorescences. Total phenolic content ranged from 19.8 mg GAE 100 g⁻¹ FW (Marathon unfertilized) to 30.2 mg GAE 100 g⁻¹ FW (Belstar + Alg Green).

Table 5. Influence of cultivar and organic fertilizers on total phenolic content, antioxidant activity and nitrate concentration of broccoli inflorescences (2024)

Variant		Total Phenolic Content (mg GAE 100g ⁻¹ FW)	DPPH EC ₅₀ (mg mL ⁻¹)	Nitrate (mg kg ⁻¹ FW)
Hybrid	Fertilizer			
Fiesta	Unfertilized	21.4±0.8 k	0.85±0.04 ijk	342±18 bc
	Lignohumate	27.3±1.1 de	0.96±0.05 abcd	318±15 ghi
	Alcygol Z2M	24.6±0.9 gh	0.91±0.04 defgh	335±16 cdef
	Alg Green	29.1±1.2 ab	0.98±0.06 ab	310±14 hijk
Marathon	Unfertilized	19.8±0.7 l	0.80±0.03 kl	358±19 a
	Lignohumate	25.9±1.0 f	0.93±0.05 bcdef	325±16 efg
	Alcygol Z2M	23.2±0.9 i	0.88±0.04 fghi	341±17 bcd
	Alg Green	27.7±1.1 cd	0.95±0.05 bcde	319±15 gh
Belstar	Unfertilized	22.6±0.9 ij	0.87±0.04 ghij	350±18 ab
	Lignohumate	28.5±1.1 bc	0.97±0.05 abc	312±14 hij
	Alcygol Z2M	25.4±1.0 fg	0.92±0.05 cdefg	337±16 cde
	Alg Green	30.2±1.3 a	1.01±0.06 a	305±13 jkl
LSD P=0.05	1.12	0.052	12.4	
Std. Dev.	0.77	0.036	8.51	

Means followed by the same letter within a column do not significantly differ ($P \leq 0.05$, Duncan's New Multiple Range Test). FW = Fresh Weight. GAE = gallic acid equivalents; DPPH = 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity expressed as EC_{50} .

These values are consistent with the range of 17-42 mg GAE 100 g⁻¹ FW reported for fresh broccoli inflorescences by Naguib et al. (2012) using the Folin-Ciocalteu method under comparable extraction conditions, and they overlap with the values documented by Abdel-Mawgoud et al. (2012) for organically fertilized broccoli (20-32 mg GAE 100 g⁻¹ FW). The highest TPC values were consistently observed under Alg Green treatment across all three cultivars, suggesting that the phenolic catalyst activity of algae-derived compounds, including oligosaccharides and polysaccharide fragments released during extract preparation, may actively stimulate the phenylpropanoid pathway in broccoli inflorescences. Comparable seaweed-induced enhancement of phenolic content in *Brassica* crops has been reported by Zodape et al. (2011) and reviewed by Bulgari et al. (2019), who attributed this effect to a mild oxidative stress response triggered by algae-derived elicitors that up-regulates the expression of phenylalanine ammonia-lyase (PAL) and downstream biosynthetic enzymes. Lignohumate also significantly enhanced TPC relative to unfertilized controls in all hybrids, consistent with the findings of Selim and Mosa (2012) and with the proposed role of humic substances as indirect triggers of secondary metabolism through their effects on soil microbial activity and root exudate composition. DPPH EC_{50} values ranged from 0.80 mg mL⁻¹ (Marathon unfertilized) to 1.01 mg mL⁻¹ (Belstar + Alg Green). In the DPPH assay, higher EC_{50} values indicate that a greater extract concentration is required to achieve 50% radical inhibition; therefore, Marathon unfertilized exhibited the lowest antioxidant capacity per unit mass, while Belstar + Alg Green required the highest extract concentration. This apparent paradox - in which the treatment with the highest TPC also shows the highest EC_{50} (lowest efficiency per mg) - may reflect qualitative shifts in the phenolic profile rather than a simple quantitative increase. As noted by Gülçin et al. (2004), the DPPH assay is sensitive to the chemical structure of individual phenolics, with hydroxycinnamic acids and flavonols being more reactive than hydroxybenzoic acid derivatives; if biostimulant treatment promotes

the accumulation of less-reactive structural phenolics (e.g., cell wall-bound hydroxycinnamic acid esters), TPC may increase without a proportional improvement in DPPH scavenging efficiency. This interpretation is consistent with the observation by Slávińska et al. (2025) that the relationship between TPC and antioxidant capacity in broccoli is non-linear and highly dependent on the relative proportions of individual phenolic classes. The moderate range of EC₅₀ variation among treatments (0.80-1.01 mg mL⁻¹) indicates that all combinations maintained substantial antioxidant capacity, confirming broccoli as a valuable dietary source of antioxidants regardless of cultivar or fertilizer treatment.

Nitrate concentrations in inflorescences ranged from 305 mg kg⁻¹ FW (Belstar + Alg Green) to 358 mg kg⁻¹ FW (Marathon unfertilized). All recorded values fall well below the maximum limits established by European Union Regulation (EC) No 1258/2011 for high-accumulating vegetables (1000-3000 mg kg⁻¹ FW for leafy vegetables), confirming the food safety of the harvested inflorescences under all treatment conditions. The relatively low nitrate concentrations in broccoli inflorescences relative to leaf tissue are consistent with the species-level data reviewed by Santamaria (2006) and Bian et al. (2020), who classified Brassica inflorescences as moderate-to-low nitrate accumulators compared to spinach, lettuce, or celery. A reverse relationship between organic fertilizer application and nitrate concentration was observed in the present study: plants receiving Alg Green and Lignohumate accumulated significantly less nitrate than unfertilized controls in all three hybrids. This finding is consistent with the mechanism proposed by Colla et al. (2018), whereby organic nitrogen sources promote the gradual mineralisation of nitrogen and its rapid assimilation into amino acids and proteins via enhanced glutamine synthetase activity, reducing the pool of free nitrate remaining in inflorescence tissue. Nguyen et al. (2025) confirmed a similar nitrate-diluting effect of organic fertilizers in leafy Brassica crops, attributing it to both the slow-release nitrogen supply and the stimulation of nitrate reductase activity by humate and seaweed-derived

compounds. From a food quality perspective, the reduction in nitrate content under organic fertilization, combined with concurrent increases in vitamin C and phenolic content, represents a favourable nutritional outcome that adds value to organically produced broccoli.

CONCLUSIONS

The present study investigated the vegetative growth, yield, and biochemical quality of three broccoli hybrids (Fiesta, Marathon, and Belstar) cultivated under polyethylene tunnel conditions in Cluj-Napoca, Romania, in response to three organic fertilizer treatments - Lignohumate (1 kg ha⁻¹), Alcygol Z2M (5 L ha⁻¹), and Alg Green (1 L ha⁻¹) - applied 30 days after transplanting, alongside an unfertilized control. The results consistently demonstrated that both cultivar and fertilizer type independently and significantly influenced every parameter assessed.

Regarding vegetative development, all three hybrids showed homogeneous growth at 30 days after transplanting, prior to fertilizer application, confirming the validity of the experimental starting point. By 60 days after transplanting, statistically significant differences were found among all 12 hybrid-fertilizer combinations for plant height, rosette diameter, and number of leaves. Lignohumate consistently promoted the greatest vegetative growth across all cultivars, recording the highest plant heights in Marathon (80.27 cm) and Belstar (79.47 cm) and the greatest leaf number in Belstar (20.20 leaves). Seaweed-based biostimulants (Alcygol Z2M and Alg Green) produced intermediate but significant growth increments relative to the unfertilized controls.

Total inflorescence yield ranged from 9.46 t ha⁻¹ (Belstar unfertilized) to 16.82 t ha⁻¹ (Marathon + Lignohumate), with Marathon consistently outperforming Fiesta and Belstar across all fertilizer treatments. Lignohumate application increased yield by 7.7% in Belstar, 15.6% in Fiesta, and 23.2% in Marathon relative to their respective unfertilized controls, demonstrating that the humate-based biostimulant not only elevated absolute yield but also amplified genotype-specific productive potential. The yield levels recorded for

fertilized treatments compare favourably with spring broccoli production data reported in the international literature under both protected and open-field conditions, confirming the agro-climatic suitability of the Cluj-Napoca polyethylene tunnel environment for high-yielding spring broccoli cultivation.

Biochemical analyses of inflorescence tissue revealed that organic fertilization significantly enhanced nutritional quality across all measured parameters. Chlorophyll a content ranged from 0.79 to 0.97 mg g⁻¹ FW, chlorophyll b from 0.29 to 0.37 mg g⁻¹ FW, and total carotenoids from 0.045 to 0.058 mg g⁻¹ FW, with Belstar and Alg Green treatments consistently producing the highest pigment concentrations. Vitamin C content ranged from 65.3 mg 100 g⁻¹ FW (Marathon unfertilized) to 80.1 mg 100 g⁻¹ FW (Belstar + Alg Green), exceeding cited reference values for commercial broccoli and confirming the superior nutritional quality achievable through organic biostimulant application. Total phenolic content ranged from 19.8 to 30.2 mg GAE 100 g⁻¹ FW, with Alg Green treatment eliciting the highest accumulation of phenolics across all three cultivars, likely through the elicitor activity of algae-derived oligosaccharides on the phenylpropanoid biosynthetic pathway. DPPH radical scavenging capacity (EC₅₀: 0.80-1.01 mg mL⁻¹) did not show a direct linear relationship with total phenolic content, indicating that antioxidant capacity is influenced not only by the total amount of phenolics but also by their specific chemical composition and reactivity.

Nitrate concentrations in broccoli inflorescences were low across all treatments (305-358 mg kg⁻¹ FW), well below the European Union regulatory limits for vegetables. Plants treated with Alg Green and Lignohumate consistently accumulated less nitrate than unfertilized controls. This result is attributable to the slow-release organic nitrogen supply and the stimulation of nitrate reductase activity associated with humate- and seaweed-derived compounds.

From a practical standpoint, the results of this study support the following recommendations for spring broccoli production under protected cultivation in temperate climates: (i) Marathon hybrid should be preferred where maximising

total inflorescence yield is the primary objective; (ii) Belstar hybrid is recommended where superior biochemical quality - particularly vitamin C, chlorophyll, and phenolic content - is the priority; (iii) Lignohumate at 1 kg ha⁻¹, applied 30 days after transplanting, represents the most effective single biostimulant treatment for simultaneously improving both vegetative growth and yield; (iv) Alg Green at 1 L ha⁻¹ is the preferred option for maximising nutritional quality parameters, particularly total phenolic content and vitamin C, while simultaneously minimising nitrate accumulation.

In conclusion, this study demonstrates that the combined selection of an appropriate broccoli hybrid and a well-matched organic biostimulant can substantially improve both the agronomic and nutritional performance of spring broccoli grown under polyethylene tunnel conditions. The consistent superiority of Lignohumate for growth and yield promotion, and of Alg Green for biochemical quality enhancement, provides a sound evidence base for the development of integrated, organic-compatible production strategies for broccoli in the north-western Romania climate. Future research should investigate multi-season reproducibility of these results, the effects of combined biostimulant applications, and the potential interaction between organic fertilization and post-harvest storage quality of the inflorescences.

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MODULATION OF GROWTH AND STRESS-RELATED PHYSIOLOGY IN ROUND PEPPER (*CAPSICUM ANNUUM* L.) SEEDLINGS VIA OPTIMIZED FERTILIZATION

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Abstract

The present study investigates the effects of some fertilization treatments on the growth and physiological attributes of 'Creolica' round pepper (*Capsicum annuum* L.) seedlings cultivated under controlled conditions at the Vegetable Research and Development Station Bacău. Seven fertilization variants were assessed using distinct commercial products at different concentrations: V1 (Albit 0.1% + Turboroot 0.25%), V2 (Albit 0.01% + Turboroot 0.375%), V3 (Albit 0.01% + Turboroot 0.5%), V4 (Turboroot 0.25%), V5 (Turboroot 0.375%), V6 (Turboroot 0.5%), and V7 (Control - untreated). Key morphological and physiological parameters were evaluated, including stem height, stem weight and diameter, root length and weight, number of leaves per seedling, leaf length and width, leaf surface area, leaf shape (expressed as the ratio of leaf width to leaf length), chlorophyll content, anthocyanin concentration, dry matter content, and water content. The findings of this study provide valuable insights into the potential of Albit and Turboroot combinations to enhance seedling quality and vigor in round pepper cultivation.

Key words: seedling vigor, biostimulant-mediated regulation, controlled-environment, root morphogenesis, chlorophyll and anthocyanin dynamics.

INTRODUCTION

The efficient cultivation of horticultural crops in controlled environments is vital to addressing the global demand for high-quality produce while ensuring resource sustainability (Ahmed et al., 2024; Jain et al., 2023). *Capsicum annuum* L. var. Grossum, commonly known as round pepper, stands out among the diverse array of cultivated vegetable species due to its substantial economic importance, as well as its notable nutritional and medicinal attributes (da Veiga Jr. et al., 2022). The round pepper variety, characterized by its robust growth habit and high yield potential, holds particular promise in sustainable vegetable growing systems (Crosby, 2008). However, achieving optimal growth and physiological performance in greenhouse-grown seedlings requires precise management of nutritional and biostimulatory inputs (Zapata-García et al., 2024).

Biostimulants have emerged as innovative tools in modern horticulture, offering an environmentally friendly alternative to conventional fertilizers (Bell et al., 2022). These bioactive formulations often comprise plant

extracts, microbial agents, and signaling molecules designed to enhance plant vigor by improving nutrient assimilation, hormonal balance, and stress tolerance (Nephali et al., 2020; Aamir et al., 2020). Unlike traditional fertilizers, biostimulants operate through complex biochemical and physiological mechanisms, influencing processes such as root architecture development, photosynthetic efficiency, and stress resilience (Johnson et al., 2024). Given the high-value nature of pepper crops and the growing emphasis on sustainable agriculture, understanding the impact of biostimulant-based fertilization regimes on seedling development is of paramount importance (Farouk, 2023).

Seedlings represent a critical phase in the life cycle of plants, serving as the foundation for subsequent growth stages (Leck et al., 2008). Ensuring robust seedling development is particularly essential in controlled greenhouse systems, where environmental variables such as light, temperature, and humidity are carefully managed to maximize productivity (Paradiso & Proietti, 2022). The application of biostimulants during this stage has the potential to mitigate

environmental stressors and optimize morphological and physiological traits, including stem and root development, leaf expansion, photosynthetic pigment synthesis, and water-use efficiency (Melo-Sabogal & Contreras-Medina, 2024). Despite these advantages, the outcomes of biostimulant applications often vary depending on their composition, concentration, and frequency of use, requiring comprehensive evaluations tailored to specific crops and growing conditions (Parađiković et al., 2019).

Limited research has explored the synergistic effects of several biostimulants when applied concurrently or at varying concentrations, particularly in the context of round pepper seedlings. Such insights are crucial for determining optimal fertilization regimes that maximize the benefits of biostimulants while avoiding potential trade-offs, such as over-fertilization or imbalanced nutrient allocation.

A key objective of modern horticultural practices is the optimization of vegetative and physiological traits that underpin plant productivity (Langensiopen et al., 2020). Morphological characteristics, such as stem length, collar diameter, root architecture, and leaf area, are pivotal for resource acquisition and structural integrity (Zhang et al., 2024). Concurrently, physiological parameters, including chlorophyll and anthocyanin content, provide insights into the plant's photosynthetic capacity and stress response (Yan et al., 2022). Evaluating these parameters under varying fertilization regimes can elucidate the mechanisms by which biostimulants influence plant performance, offering a basis for tailored nutrient management strategies (Dalal et al., 2019).

The significance of this study extends beyond its immediate application to round pepper seedlings, contributing to the broader discourse on sustainable vegetable growing practices. As the global agricultural sector faces mounting challenges related to climate change, resource scarcity, and soil degradation (Hossain et al., 2020), the adoption of biostimulant-based fertilization regimes offers a viable pathway toward resilient and resource-efficient crop production systems (Pinto et al., 2023). Furthermore, the findings have potential implications for other high-value crops grown in

greenhouse settings, reinforcing the importance of evidence-based approaches to biostimulant application.

By leveraging controlled greenhouse conditions, this research provides a comprehensive evaluation of the interplay between biostimulant treatments and plant performance, thereby advancing our understanding of sustainable fertilization practices in vegetable growing.

MATERIALS AND METHODS

This research was conducted in 2024 and 2025 growing seasons at the Vegetable Research and Development Station situated in the northeastern region of Bacău city, under controlled conditions, in an Almeria-style heated greenhouse to ensure optimal environmental requirements for seedling development. Regarding the biological material, it encompassed 'Creolica' round pepper – an early semi-variety developed at the Vegetable Research and Development Station Bacău, characterized by a vegetation period of 125 days, round-flattened fruits with a smooth surface and slight ribbing, an average fruit weight of 180-220 g, and a yield capacity of 40-45 t/ha;

To produce the round pepper seedlings, seeds were sown in mid-February in trays specifically designed for this purpose, using Rekyva Remix 1 horticultural peat as a nutritive substrate. This substrate is characterized by small and/or medium fractions, with the following properties: pH 5.5-6.5, electrical conductivity of 0.5-1 mS/cm, and NPK (chlorine-free) ≤ 1 kg/m³. Subsequently, after full germination, the seedlings were transplanted into 70-cell alveolar trays (at the end of February), using Rekyva Remix 2 peat, which differs from the sowing variant by containing medium and/or large fractions, an electrical conductivity of 1-2 mS/cm, and NPK (chlorine-free) ≤ 2 kg/m³.

Regarding the two products used as fertilizers and/or biostimulants in the experiment, Albit is a poly-beta-hydroxybutyric acid (a natural biopolymer derived from beneficial soil bacteria: *Bacillus megaterium* and *Pseudomonas aureofaciens*), supplemented with a set of potentiating substances, including magnesium sulfate, potassium phosphate, potassium nitrate, and urea. On the other hand,

Turboroot is a comprehensive rooting and growth stimulator containing humic acids, L-alpha free amino acids, NPK, and micronutrients chelated with EDTA.

On the description of the experimental variants, the concentrations applied, as well as the number of treatments and the time intervals between them, the details are presented in Table 1.

Table 1. Fertilization Variants, Composition and Application Intervals

No.	Description of variants (commercial product and applied concentration)	Treatment application/interval between treatments
V1	Albit 0.01% + Turboroot 0.25%	I - immediately after transplanting; II - two weeks after the first treatment; III - two weeks after the second treatment.
V2	Albit 0.01% + Turboroot 0.375%	
V3	Albit 0.01% + Turboroot 0.5%	
V4	Turboroot 0.25%	
V5	Turboroot 0.375%	
V6	Turboroot 0.5%	
V7	Control (untreated)	-

Thus, the experiment included six fertilization treatments (Figure 1), along with a control variant; each consisted of 20 seedlings in three replications, resulting in a total of 60 plants per treatment.

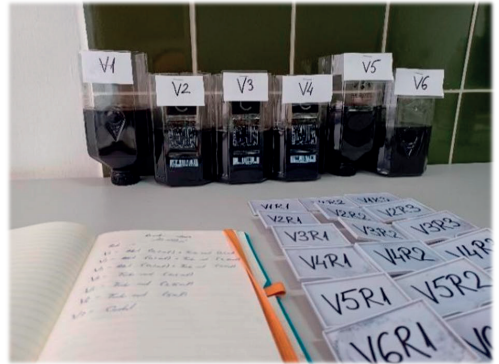


Figure 1. Methodological framework for the design and implementation of fertilization treatments

All three fertilization applications were carried out using a fine mist sprayer, ensuring uniform application across the entire leaf surface of the seedlings (Figure 2).

Furthermore, the experimental protocol adhered strictly to the prescribed conditions, including a controlled temperature range of 22-24°C, supplemental lighting using violet LED lights,

and irrigation in accordance with the applicable standards.



Figure 2. Different fertilization schemes applied using a fine mist sprayer

Quantitative and qualitative assessment of round pepper seedlings

Biometric assessments were performed using direct measurement and weighing techniques to evaluate the growth parameters of seedlings at their optimal age for transplantation (45-50 days). Measurements included seedling height, root length, collar stem diameter, and the number of leaves per seedling, while biomass assessments comprised stem and root weights (Figure 3).



Figure 3. Aspects concerning quantitative determinations in round pepper seedlings

The tools employed for these procedures consisted of a precision ruler for linear measurements, a caliper for diameter determinations, and a highly accurate electronic balance for mass quantification. This methodology ensured

precise and reliable data acquisition for the characterization of seedling morphological and physiological attributes.

Leaf width, leaf length, and leaf area were quantified using the Area Meter AM 350, a precision instrument manufactured by ADC BioScientific Ltd (Figure 4).



Figure 4. Determination of leaf area in round pepper seedlings

The measurements of leaf width and length were recorded in millimeters (mm), while the leaf area was calculated and expressed in square millimeters (mm²).

The quantification of both chlorophyll pigment and anthocyanin content was conducted using two comparable instruments, the CCM 200 Plus and the ACM 200 Plus, manufactured by Optisciences, by positioning the sensor on the seedling's leaf blade and recording the measured values (Figure 5).



Figure 5. The evaluation of chlorophyll pigment and anthocyanin content in round pepper seedlings

These readings were conducted under optimal conditions, with clear skies and a temperature of approximately 25°C. The data obtained from these measurements were expressed through specific indices, namely the Chlorophyll Concentration Index and the Anthocyanin Content Index, which provide a precise representation of the total concentration of chlorophyll pigments and anthocyanins, respectively. These indices ensure accurate and reliable assessment of pigment levels, facilitating a comprehensive analysis of the plant's biochemical composition.

Dry Matter Content (DM): Fresh, homogenized samples (Figure 6) were subjected to drying in a forced-air oven (Biobase) at a controlled temperature of $103 \pm 2^\circ\text{C}$ for 24 hours, until a stable weight was achieved, in accordance with the AOAC (2000) guidelines.



Figure 6. Sample preparation prior to forced-air oven placement for the determination of dry matter content

The dry matter content was then expressed as a percentage (%).

Water Content (W): The water content was determined by subtracting the dry matter percentage from 100%, using the formula:

$$W\% = 100\% - DM\%.$$

Statistical Analysis - The variations in the analyzed parameters were assessed using the ANOVA test, which revealed statistically significant differences among the various fertilization treatments. When significant differences were detected, multiple comparison tests based on Duncan's test ($p < 0.05$) were applied. All statistical analyses were conducted using SPSS version 21 (IBM® Corp., Armonk, NY, USA).

RESULTS AND DISCUSSIONS

This section evaluates the impact of different fertilization treatments (V1-V7) on various vegetative and physiological parameters of the Creolica round pepper seedlings under controlled greenhouse conditions. Treatments include combinations of Albit and Turboroot applications at varying concentrations and frequencies, with V7 serving as the untreated control. Parameters assessed include stem and root length, stem and root weight, collar diameter, leaf count, leaf dimensions, foliar surface area, dry and water content percentages, anthocyanin content index (ACI) and chlorophyll content index (CCI).

Vegetative Growth: Stem and Root Metrics

Stem length varied significantly across treatments, with V5 (Turboroot at 3.75 ml) yielding the highest stem length at 25.11 cm, indicating a 28.6% increase over the control (V7). This outcome suggests a robust positive effect of intermediate Turboroot concentrations on stem elongation, potentially enhancing light capture and photosynthetic efficiency. Similarly, root length was most pronounced in V5, recording an 8.79 cm length, which represented a 30.6% improvement over the untreated plants. This pronounced root growth under V5 may indicate enhanced nutrient and water uptake capabilities, essential for optimizing plant vigor in greenhouse environments (Ahmed et al., 2024).

In terms of biomass accumulation, V5 continued to show superior performance, with the highest stem weight at 6.32 g and root weight at 4.15 g, representing increases of approximately 17.5% and 29.7%, respectively, over V7. This enhancement in biomass suggests that Turboroot at 3.75 ml offers optimal conditions for root and stem tissue development, likely due to improved nutrient assimilation and cellular expansion facilitated by the bio-stimulant components in Turboroot.

The findings underscore the profound impact of biostimulant application on vegetative growth parameters (Johnson et al., 2024; Rajesaheb et al., 2024), emphasizing the efficacy of intermediate Turboroot concentrations (3.75 ml, V5). The significant elongation of stems and roots highlights the role of optimized biostimulant concentrations in augmenting

structural growth (Rani et al., 2024; Baltazar et al., 2021). These enhancements likely result from synergistic effects of nutrient mobilization and phytohormonal regulation (Savarese et al., 2022) induced by Turboroot.

Furthermore, the increases in biomass, sustain the biostimulant's capacity to enhance metabolic activity and cellular proliferation (Gatti et al., 2024; Lephatsi et al., 2022). Such improvements in structural integrity and resource acquisition suggest a potential for significant agronomic benefits, particularly in controlled environments where resource efficiency is paramount (Nephali et al., 2020; Sible et al., 2021). These results advocate for the strategic use of Turboroot at intermediate dosages to maximize vegetative growth and overall plant vigor, paving the way for further exploration into biostimulant-mediated growth optimization.

Collar Diameter and Leaf Count

Collar diameter, an indicator of stem robustness, showed the greatest values in V7 and V1, at 4.30 mm and 4.31 mm, respectively. This suggests that while fertilization treatments generally enhance vegetative growth, collar thickness may not directly correlate with the application of Albit or Turboroot, as the untreated control performed comparably well.

Maximum leaf count was observed in V1, with an average of 11 leaves per plant, indicating that the combined application of Albit and Turboroot effectively promotes leaf proliferation, thereby enhancing the photosynthetic surface and potentially increasing overall productivity. Conversely, leaf production was lowest in V5, with an average of 10.67 leaves per plant, highlighting potential constraints such as over-fertilization or nutrient allocation dynamics that may influence foliar development (Table 2).

Thus, collar diameter exhibited minimal variation across treatments. This suggests that collar thickness may not be significantly influenced by Albit or Turboroot, potentially indicating a limited role of these biostimulants in fortifying structural stability (Parađiković et al., 2019). compared to untreated control.

Similarly, leaf production exhibited minimal variation, indicating that while the combined application of Albit and Turboroot may support enhanced foliar proliferation (Şesan et al., 2020; Kisvarga et al., 2022) - potentially mediated by improved hormonal equilibrium and nutrient

assimilation mechanisms to maximize photosynthetic capacity (Rathor et al., 2023; Martínez-Lorente et al., 2024) -evidence of over-fertilization effects or resource allocation trade-offs was also apparent. These trade-offs suggest a potential shift in nutrient allocation dynamics, prioritizing root development at the

expense of foliage growth under specific treatment conditions (Monson et al., 2022). The outcomes emphasize the paramount significance of optimizing dosage in biostimulant application strategies to achieve balanced and targeted enhancement of plant growth parameters (Trejo-Téllez et al., 2023).

Table 2. Morphological and Biomass Parameters of Round Pepper Seedlings Under Different Fertilization Treatments

Variant	Seedling Height (cm)	Root length (cm)	Collar Stem Diameter (mm)	Stem Weight (g)	Root Weight (g)	Number of Leaves per Seedling
V1	23.38±1.55bc	7.43±1.08a	4.32±0.38a	6.13±0.77ab	6.02±1.88b	11.00±1.66a
V2	24.51±0.86ab	7.27±0.54a	4.18±0.23a	6.36±0.87a	7.73±1.61a	10.78±0.83a
V3	22.85±0.43c	7.08±1.03a	4.15±0.45a	6.11±0.90ab	4.47±1.75c	10.61±1.19a
V4	23.71±0.85abc	7.09±0.60a	4.01±0.26a	5.61±0.71ab	3.94±1.08c	10.00±0.82a
V5	25.11±2.03a	7.39±0.67a	4.13±0.38a	6.32±1.26a	4.15±1.11c	10.67±0.50a
V6	25.11±1.13a	7.10±0.57a	4.00±0.25a	6.07±0.77ab	3.70±0.70c	10.56±1.24a
V7	19.51±1.61d	6.73±1.13a	4.30±0.39a	5.38±0.92b	5.70±1.69b	10.56±0.88a

Within each column and within each experimental factor, different letters mean fertilization treatments differ significantly according to Duncan's test at $p \leq 0.05$

Leaf Morphology and Foliar Surface Area

Leaf morphology, assessed through leaf length and width, showed that V1 produced the largest leaves, with a length of 92.14 mm and a width of 8.51 mm, leading to the highest foliar area of 2258.56 mm². This result implies that the V1 treatment, combining Albit and Turboroot at lower concentrations, effectively promotes leaf expansion, which can enhance the plant's photosynthetic potential (Table 3).

This pronounced leaf expansion suggests an enhanced physiological response likely driven by improved hormonal modulation and nutrient efficiency (Paponov et al., 2021; Zhang et al., 2023). The resulting increase in photosynthetic surface area underscores the critical role of balanced biostimulant application in optimizing leaf growth dynamics (Namratha et al., 2024). to maximize photosynthetic capacity (Vitale et al., 2021) and overall plant productivity (Li et al., 2022). Furthermore, the formation of more elongated leaves highlights a characteristic that enhances light capture and facilitates gas exchange (Zhang et al., 2022; Xie et al., 2020).

Such morphological adaptations underline the potential of V1 fertilization scheme to improve resource acquisition efficiency in controlled greenhouse systems.

Ratio Analysis: Length-to-Width and Width-to-Length

The analysis of leaf length-to-width and width-to-length ratios reveals significant insights into the morphological responses of plants to different fertilization treatments. V1 and V5 exhibited the highest length-to-width ratios (2.24 and 2.20, respectively), indicating a shift towards more elongated leaves relative to the control, which maintained a more balanced ratio. This elongation likely confers an adaptive advantage in controlled environments (Steed et al., 2021), enhancing the plant's capacity for efficient light interception (Williams et al., 2021; Slattery & Ort, 2021). In contrast, width-to-length ratios remained relatively stable across all treatments, suggesting that while fertilization influences leaf size, it does not substantially alter the fundamental leaf shape.

Table 3. Leaf Morphological Parameters of Round Pepper Seedlings Under Different Fertilization Treatments

Variant	Leaf width (mm)	Leaf length (mm)	Length/Width Ratio	Width/Length Ratio	Leaf Area (mm ²)
V1	41.28±4.46a	92.14±8.51a	2.24±0.20a	0.45±0.04a	2258.56±316.86a
V2	41.19±4.00a	81.78±6.62a	1.99±0.14a	0.50±0.03a	2132.89±395.51a
V3	40.43±4.66a	83.15±4.83a	2.08±0.23a	0.49±0.05a	2094.28±307.83a
V4	40.44±2.50a	87.62±5.45a	2.17±0.12a	0.46±0.03a	2103.78±246.41a
V5	40.30±5.99a	86.89±8.90a	2.20±0.39a	0.47±0.06a	2156.89±344.39a
V6	42.89±3.60a	91.76±10.01a	2.14±0.23a	0.47±0.05a	2383.00±431.34a
V7	39.21±2.50a	81.57±6.34a	2.08±0.11a	0.48±0.03a	1987.67±196.21a

Within each column and within each experimental factor, different letters mean fertilization treatments differ significantly according to Duncan's test at $p \leq 0.05$.

Anthocyanin Content Index (ACI) and Chlorophyll Content Index (CCI)

The ACI values, reflecting pigment concentration associated with stress response and antioxidant activity, were highest in the untreated control (5.08) and lowest in V4 (4.12), suggesting that fertilization treatments might reduce the anthocyanin production by minimizing stress-related responses in the plants (Figure 7). Treatments V1 and V2 also exhibited elevated ACI values at 4.58 and 4.34, respectively, possibly due to mild stress induced by Albit and Turboroot at these concentrations, which may stimulate pigment synthesis without compromising growth.

CCI values, indicating chlorophyll concentration, were most pronounced in V1 (16.14) and V7 (15.31), suggesting that Albit and Turboroot can marginally improve chlorophyll synthesis, thereby enhancing photosynthetic capacity. CCI was lowest in V4 (12.86), possibly due to lower nutrient availability, which could impair chlorophyll synthesis pathways.

The observed variations in the Anthocyanin Content Index (ACI) and Chlorophyll Content Index (CCI) reflect complex physiological responses to different fertilization treatments. The high ACI in the untreated control suggests that plants may produce more anthocyanins in response to stress, likely as part of an antioxidant defense mechanism (Li & Ahammed, 2023; Kaur et al., 2023). In contrast, the lower ACI in fertilized treatments, particularly V4, indicates that nutrient supplementation may reduce stress-induced pigment production by optimizing plant health and minimizing stress responses (Kumari et al., 2022). This suggests that fertilization may

help mitigate stress-related pathways that typically stimulate anthocyanin synthesis (Mannino et al., 2021).

Regarding chlorophyll content, the highest CCI values in V1 and V7 highlight the potential of Albit and Turboroot, particularly at lower concentrations, to enhance chlorophyll synthesis. This enhancement in chlorophyll content may improve the plants' photosynthetic capacity and overall productivity (Simkin et al., 2022). The lowest CCI in V4 suggests that insufficient nutrient availability could impair chlorophyll biosynthesis, thereby limiting photosynthetic efficiency (Morales et al., 2020; Muhammad et al., 2021). These findings underline the importance of optimizing fertilization strategies to balance nutrient availability and metabolic responses (Barlóg et al., 2022), ultimately improving plant growth and stress resilience.

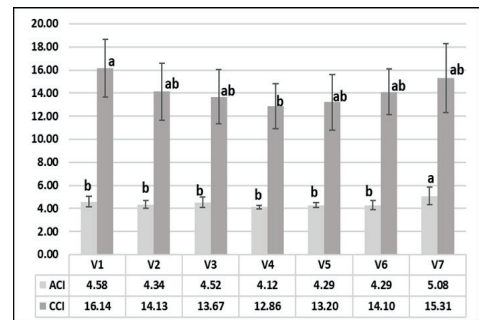


Figure 7. Chlorophyll Concentration Index (CCI) and Anthocyanin Content Index (ACI) in Round Pepper Seedlings Across Different Fertilization Treatments

Note: Same letters show no significant difference. Different letters between items indicates significant differences according to Duncan's multiple range test; $p \leq 0.05$

Water Content and Dry Matter Analysis

The analysis of water content and dry matter in seedlings provides valuable insights into their physiological responses to fertilization treatments. Higher dry matter percentages in V4 and V3 (10.12% and 9.77%, respectively) suggest enhanced seedling vigor, indicative of more efficient nutrient and biomass accumulation (Cui et al., 2024), which typically correlates with better plant quality and improved resilience (Penella & Calatayud, 2018). In contrast, V5 and V6 exhibited lower dry matter content (7.8% and 6.6%) and higher water retention, suggesting that these treatments promote greater hydration in seedling tissues (Figure 8). While this may support early growth, excessive water retention could compromise long-term stability and drought resistance (Shoaib et al., 2022). The inverse relationship between water content and dry matter, with V7 showing the highest water content (94.4%), emphasizes the role of bio-stimulants in regulating osmotic balance within seedlings. These findings highlight the potential of specific treatments to optimize water use efficiency and stress tolerance (Anli et al., 2020), ultimately enhancing seedling establishment and survival in controlled environments (He et al., 2020).

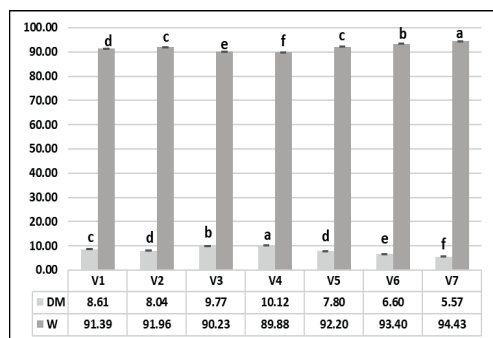


Figure 8. Dry Matter (DM) and Water Content (W) in Round Pepper Seedlings Across Different Fertilization Treatments

(Note: Same letters show no significant difference. Different letters between items indicates significant differences according to Duncan's multiple range test; $p \leq 0.05$)

CONCLUSIONS

The results demonstrate the pivotal influence of biostimulant treatments on the morphological and physiological performance of *Capsicum annuum* L. seedlings cultivated under controlled

conditions. Intermediate dosages - particularly V5 (Turboroot 3.75 ml) - significantly improved key growth parameters, including stem height, root development and biomass accumulation, confirming their effectiveness in promoting vigorous vegetative growth. Notable synergistic effects between Albit and Turboroot in V1 further enhanced leaf morphology, foliar area and chlorophyll content, thereby increasing photosynthetic capacity and overall seedling quality.

The observed variation in dry matter and water content reflects treatment-dependent adjustments in osmotic balance, while differences in chlorophyll and anthocyanin concentrations indicate a dynamic interplay between growth promotion and stress-related metabolic responses. Collectively, these findings emphasize the necessity of precise dosage calibration in biostimulant application to optimize seedling performance, providing essential guidance for optimizing resource-efficient and sustainable horticultural practices.

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INVASIVE AND POTENTIALLY INVASIVE SPECIES IN VEGETABLE CROPPING SYSTEMS OF NORTHEASTERN ROMANIA

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Abstract

Vegetable crop production is increasingly affected by invasive and potentially invasive species, including weeds, insect pests, and plant pathogens. The aim of this study was to inventory weed species and to assess their role as ecological drivers influencing both pest incidence and beneficial entomofauna within vegetable agroecosystems.

Field observations were conducted to identify and document the weed species present in vegetable crops. In parallel, the incidence of major pests and beneficial entomofauna was analysed in order to evaluate their relationship with spontaneous flora. Among the 76 species identified, belonging to 26 botanical families, a total of 19 taxa were classified as invasive or potentially invasive in Romania.

The results showed that the presence of certain weed species shapes the dynamics and overall quality of the ecosystem, as some weeds can serve as hosts for important vegetable crop pests, while others provide essential resources for beneficial entomofauna, thereby mitigating pest pressure.

Key words: biodiversity, beneficial arthropods, ecological services, weed-insect interactions.

INTRODUCTION

Weeds are an integral component of ecosystems. Despite the common perception of weeds as detrimental, their role in promoting ecological balance, safeguarding biodiversity, preserving soil resources, and sustaining natural cycles within both natural and anthropized ecosystems is widely acknowledged.

Recent studies have underscored the heterogeneity of weed species and the composition of allogenic flora at both the European (Lambdon et al., 2008) and national levels (Anastasiu et al., 2024; Răduțoiu & Ștefănescu, 2022; Culiță, 2016).

Research has demonstrated that anthropogenic stressors, including habitat disturbance and herbicide use, can interact synergistically with climate-change stressors to expedite the evolution of weeds.

Perceived as a direct competitor to crops, weeds are understood to hinder agricultural productivity by utilizing vital resources such as

water, light, heat, and nutrients, resulting in a decline in both the quality and quantity of crop yield (Kaur et al., 2018; Novac et al., 2021).

However, a nuanced understanding of agroecosystems reveals a much more complex interaction (Allen et al., 2022).

Vegetable production systems in Northeastern Romania are increasingly challenged by a diverse assemblage of invasive and emerging weed species, including highly competitive annual dicotyledons such as *Amaranthus retroflexus*, *Galinsoga parviflora*, and *Hibiscus trionum*, alongside perennial taxa like *Cirsium arvense* and *Convolvulus arvensis*, which collectively contribute to persistent weed pressure (Calara et al., 2023).

High constancy values observed for key species - such as *Senecio vulgaris* and *Echinochloa crus-galli*, reaching up to 100% occurrence before critical management interventions - highlight their strong establishment capacity and underline the need for targeted, stage-specific weed management strategies (Calara et al., 2024).

The objectives of this study are to identify invasive or potentially invasive weed species and to investigate the dual role of weeds, examining both their detrimental impact and their beneficial potential in maintaining ecological balance, with a particular focus on their relationships with invasive insect species.

MATERIALS AND METHODS

The study was conducted at the Vegetable Research and Development Station in Bacău, which is located in the northeastern region of Romania (46°35' north latitude, 26°55' east longitude, 165 m altitude). The area between the Bistrița and Siret rivers in Bacău County, particularly around their confluence (downstream from the city of Bacău), exhibits a geobotanical character typical of wide floodplains and low terraces. This character is strongly influenced by the hydrological regime and human activities.

The present study entailed the collection, identification, and inventorying of weed species present in vegetable crops. The collected botanical material was identified using specialized literature (Gurău, 2007).

In conducting this study, the binary nomenclature from Flora Europaea (Tutin et al., 1964-1980) and Flora R.P.R. (Săvulescu, 1952-1976) was utilized.

The plants were preserved and stored in the VRDS Bacău herbarium in accordance with standard practices described in the scientific literature.

For insects, the direct visual observation method was carried out by systematically examining the plants (leaves, stems, flowers and fruits). For each monitored plot, samples of 10-20 plants were randomly selected, and the presence of various species was recorded.

RESULTS AND DISCUSSIONS

A comprehensive taxonomic analysis of the collected biological material was conducted, resulting in the identification of 76 taxa belonging to 26 families, which were subsequently grouped into 16 orders. According to Figure 1, the best-represented order is Asterales with 16 taxa, followed by Caryophyllales (13), Poales (12), Lamiales (11),

Fabales (7), Solanales (4), Apiales (2), Malpighiales (2), Rosales (2), Boraginales (1), Brassicales (1), Equisetales (1), Malvales (1), Papaverales (1), Ranunculales (1), Sapindales (1).

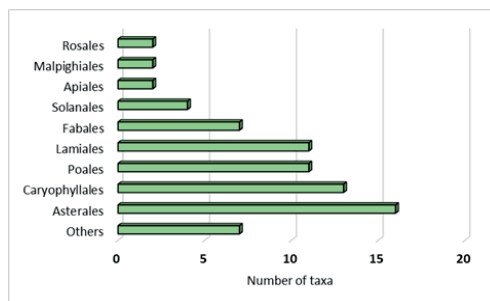


Figure 1. Graphical representation of orders

In the wake of observations made in vegetable crops within the VRDS Bacău, a concerted effort was made to collect, identify, and inventory weeds. This endeavour culminated in the formulation of the following list of weed species:

Fam. Amaranthaceae: *Amaranthus albus* L.; *Amaranthus blitoides* S. Wats; *Amaranthus blitum* L.; *Amaranthus hybridus* L.; *Atriplex patula* L.; *Chenopodium album* L.

Fam. Apiaceae: *Daucus carota* L.; *Torilis arvensis* (Huds.) Link.

Fam. Asteraceae: *Achillea setacea* L.; *Arctium lappa* L.; *Cichorium intybus* L.; *Cirsium arvense* (L.) Scop.; *Cirsium vulgare* (Savi) Ten.; *Erigeron annuus* (L.) Pers; *Erigeron canadensis* L.; *Galinsoga parviflora* Cav.; *Helianthus tuberosus* L.; *Lactuca serriola* L.; *Senecio vulgaris* L.; *Solidago gigantea* Alton.; *Sonchus asper* (L.) Hill; *Taraxacum officinalis* (L.) Weber ex F.H.Wigg; *Tripleurospermum inodorum* (L.) Sch.Bip.; *Xanthium strumarium* L.

Fam. Boraginaceae: *Symphytum officinale* L.

Fam. Brassicaceae: *Rorippa sylvestris* (L.) Besser.

Fam. Caryophyllaceae: *Saponaria officinalis* L.

Fam. Convolvulaceae: *Convolvulus arvensis* L.; *Ipomoea rubro-caerulea* Hook.

Fam. Equisetaceae: *Equisetum arvense* L.

Fam. Euphorbiaceae: *Euphorbia agraria* M. Bieb.

Fam. Fabaceae: *Coronilla varia* (L.) Lassen; *Gleditsia triacanthos* L.; *Medicago minima* L.; *Medicago sativa* L.; *Trifolium hybridum* L.; *Trifolium pratense* L.; *Trifolium repens* L.

Fam. Hypericaceae: *Hypericum perforatum* L.

Fam. Lamiaceae: *Ballota nigra* L.; *Glechoma hirsuta* L.; *Marrubium vulgare* L.; *Mentha longifolia* (L.) Huds; *Origanum vulgare* L.; *Prunella vulgaris* L.

Fam. Malvaceae: *Hibiscus trionum* L.

Fam. Papaveraceae: *Chelidonium majus* L.

Fam. Plantaginaceae: *Kickxia spuria* Dumort.; *Plantago lanceolata* L.; *Plantago major* L.

Fam. Poaceae: *Bromus* spp., *Bromus sterilis* L.; *Calamagrostis epigeios* (L.) Roth; *Dactylis glomerata* L.; *Digitaria sanguinalis* (L.) Scop; *Echinochloa crus-galli* (L.) P.Beauv.; *Elymus repens* Gould; *Festuca drymeja* Mert. & W.D.J. Koch; *Lolium perenne* L.; *Phragmites australis* (Cav.) Trin. ex Steud.; *Setaria verticillata* (L.) P.Beauv.; *Setaria viridis* (L.) P.Beauv.

Fam. Polygonaceae: *Polygonum convolvulus* L.; *Polygonum aviculare* L.; *Polygonum lapathifolium* (L.) Delarbre; *Rumex crispus* L.; *Rumex sanguineus* L.

Fam. Portulacaceae: *Portulaca oleracea* L.

Fam. Ranunculaceae: *Clematis vitalba* L.

Fam. Rosaceae: *Agrimonia eupatoria* L.

Fam. Rutaceae: *Ruta graveolens* L.

Fam. Sapindaceae: *Acer negundo* L.

Fam. Scrophulariaceae: *Verbascum* sp.

Fam. Solanaceae: *Solanum nigrum* L.

Fam. Urticaceae: *Urtica dioica* L.

Fam. Verbenaceae: *Verbena officinalis* L.

In the realm of family classification, the Asteraceae family exhibits a predominant presence, constituting 21.05% of the total taxa identified within the study. Next in order are the families Poaceae (15.79%), Fabaceae (9.21%), Amaranthaceae (7.89%), Lamiaceae (7.89%), Polygonaceae (6.58%), Plantaginaceae (3.95%), Apiaceae (2.63%), Convolvulaceae (2.63%). The remaining 17 families comprise 1.32% each (Figure 2).

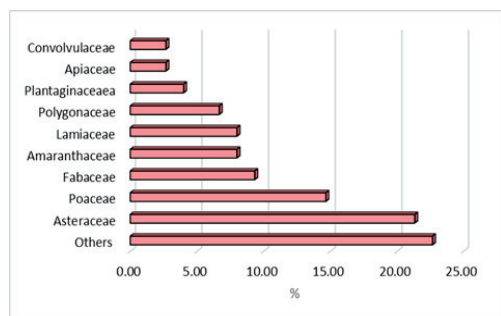


Figure 2. Graphical representation of botanical families

Of the total 76 species identified, dicotyledons predominated, accounting for 84%, with monocotyledons comprising a significantly smaller proportion (Figure 3).

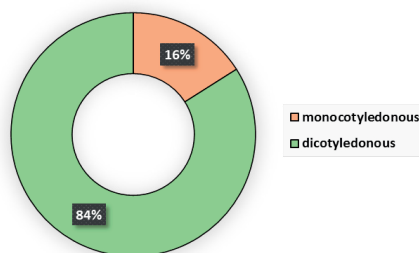


Figure 3. The relationship between monocotyledonous and dicotyledonous plants

Of the total number of species identified, the majority are classified as perennial weeds (67%) followed by annuals (29%) and then biennials (4%) (Figure 4).

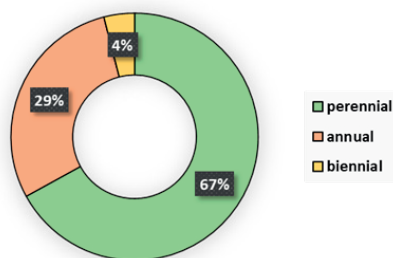


Figure 4. The ratio between annual, biennial, and perennial weeds

In vegetable crops within the VRDS Bacău, phytocenotic analysis showed that the most frequent, dominant, and abundant weed species were white amaranth (*Amaranthus albus* L.), cockspur grass (*Echinochloa crus-galli* (L.) P.Beauv.), green foxtail (*Setaria viridis* (L.) P.Beauv.), pale persicaria (*Polygonum lapathifolium* (L.) Delarbre), and gallant soldier (*Galinsoga parviflora* Cav.).

Identifying invasive or potentially invasive weed species

An important ecological consequence of global change is the invasion of alien species that can influence biodiversity.

Weeds, in general, have been shown to be deleterious to vegetable crops, affecting them from the germination stage through to harvest.

According to Reddy (2018), the presence of weeds and their density can cause significant losses during the early stages of plant growth. As the duration of interaction between weeds and crops increases, the intensity of competition between them escalates. This heightened competition gives rise to quantitative and qualitative changes in the crops, ultimately leading to a decline in yield. Invasive or potentially invasive weed species have the potential to cause significant losses to agricultural production. These losses can be classified as either direct or indirect. Direct losses manifest as a reduction in crop yield per hectare. Indirect losses are associated with the costs of controlling these pests, whether through mechanical, chemical, or biological means (Sirbu, 2011).

A total of 19 taxa have been identified as invasive or potentially invasive species for our country, according to the invasive species inventory guide within the POIM 2014+ 120008 project. This classification is in accordance with EU Regulation 1143/2014 on the prevention and management of the introduction and spread of invasive alien species (Anastasiu et al., 2019; Anastasiu et al., 2022; Anastasiu et al., 2024). The 19 taxa are grouped into eight families. The Asteraceae family has the highest number of invasive plant species (6), followed by the Amaranthaceae (4), Poaceae (3), Fabaceae (2) and the remaining four families (Convolvulaceae, Euphorbiaceae, Portulacaceae, and Sapindaceae) were represented by one species each. (Table 1).

Table 1. List of invasive or potentially invasive alien plants

Nr. Crt.	Taxon	Family	Native range	Initial reporting	Bioforma	Plant mono/di	Life span	Invasive potential
1	<i>Acer negundo</i>	Sapindaceae	NAm	1946	PhM	di	pe	High
2	<i>Amaranthus albus</i>	Amaranthaceae	NAm	1905	T	di	an	Moderate
3	<i>Amaranthus blitoides</i>	Amaranthaceae	NAm	1931	T	di	an	Moderate
4	<i>Amaranthus blitum</i>	Amaranthaceae	NAm	1816	T	di	an	Moderate
5	<i>Amaranthus hybridus</i>	Amaranthaceae	NAm	1898	T	di	an	High
6	<i>Bromus sterilis</i>	Poaceae	Eur	1816	T	mono	an/bi	Moderate
7	<i>Bromus spp.</i>	Poaceae	Eur	1816	T	mono	an/bi	Moderate
8	<i>Echinochloa crus-galii</i>	Poaceae	Eur	1866	T	mono	an	High
9	<i>Erigeron annuus</i>	Asteraceae	NAm	1936	TH	di	an/bi	High
10	<i>Erigeron canadense</i>	Asteraceae	NAm	1814	T	di	an/bi	High
11	<i>Euphorbia agaria</i>	Euphorbiaceae	Eur	1879	H	di	pe	Low
12	<i>Galinsoga parviflora</i>	Asteraceae	Sam	1866	T	di	an	High
13	<i>Gleditsia triacanthos</i>	Fabaceae	NAm	1898	PhM	di	pe	Moderate
14	<i>Helianthus tuberosus</i>	Asteraceae	NAm	1858	G	di	pe	High
15	<i>Ipomoea rubro-caerulea</i>	Convolvulaceae	SAm	1906	T	di	an	Low
16	<i>Medicago sativa</i>	Fabaceae	As	1879	H	di	an	Low
17	<i>Portulaca oleracea</i>	Portulacaceae	Eur	1879	T	di	an	Moderate
18	<i>Solidago gigantea</i>	Asteraceae	Nam	1900	H	di	pe	High
19	<i>Xanthium strumarium</i>	Asteraceae	Eur	1879	T	di	an	Moderate

As - Asia; Eur - Europa; Nam - North America; Sam - South America; G - geophytes; H - hemicryptophytes; PhM - megaphanerophytes; T - terophytes; TH - hemiterophytes; mono - monotiledonata; di - dicotiledonata; an - annual; bi - biennial; pe - perennial.

Table 1 presents a comprehensive list of the 19 identified invasive species, along with their respective family classifications, geographical origins, dates of first documentation in Romania, life forms (classified as either monocotyledonous or dicotyledonous), estimated lifespans, and levels of invasiveness. In terms of geographic origin, the flora analyzed in Table 1 is dominated by species native to North America, in line with the trend observed

in European adventive flora (Danci & Drăgulescu, 2023). Two families, Asteraceae and Amaranthaceae, are particularly prominent in terms of species richness. This finding has also been reported in other regions of the country (Răduțoiu & Băloniu, 2021). Many taxa were introduced either accidentally or intentionally (for agricultural, ornamental, or forestry purposes) and have since become naturalized.

The analysis of plant forms (Figure 5) reveals a high percentage of therophytes (63.16%), followed by hemicryptophytes (15.79%), megaphanerophytes (10.53%), hemitherophytes (5.26%), and geophytes (5.26%). The predominance of therophytes is congruent with the study's execution within an agricultural ecosystem under temperate seasonal climate conditions. The low abundance of perennial forms (hemicryptophytes, geophytes, and phanerophytes) suggests that many of the analyzed species are adventive or ruderal species, adapted to short life cycles and rapid dispersal.

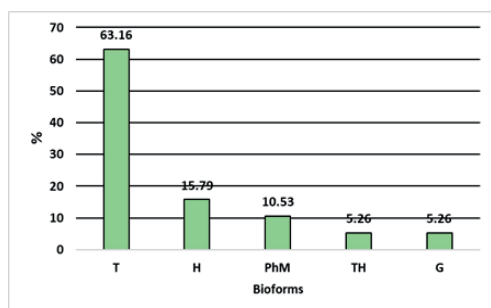


Figure 5. Percentage distribution of bioforms (G – geophytes; H – hemicryptophytes; PhM – megaphanerophytes; T – therophytes; TH – hemitherophytes)

Most of the invasive species identified are neophytes (introduced after 1492), and only a few are archaeophytes, having been introduced before 1492: *Amaranthus blitum* L.; *Portulaca oleracea* L. (Culiță, 2007, 2011)

According to the scientific literature, only three species have a low potential for invasiveness. The others have a moderate potential, which is on par with those with a high potential. Among the highly invasive species, *Echinochloa crus-galii* (L.) P. Beauv stands out as the worst weed (Holm et al., 1977).

Invasive pest species of economic interest for vegetable crops in the Bacău region

The spread of invasive insects of tropical origin in Romania's agricultural ecosystems is exacerbated by global warming. Some species are increasingly being recognized as quarantine pests, posing a significant threat to national plant health. Among the most important species at the national and regional levels for vegetable crops are:

- *Bemisia tabaci* Gennadius (silverleaf whitefly), a quarantine pest (non-European populations), listed in Annex 1, Section 1 of Government Decision No. 563/2007. It is an extremely polyphagous species, capable of feeding on over 600 host plant species (Martin et al., 2000). Its main danger lies not only in feeding on plant sap, but also in its ability to transmit over 100 types of viruses (Soumia et al., 2020; Ghosh et al., 2022).

Regarding the presence of whiteflies in horticultural areas in Bacău County, different trends were observed among species. For example, *Trialeurodes vaporariorum* (Westwood) showed an extremely low infestation level, while *Aleyrodes proletella* (L.) had a higher incidence in field cabbage crops. However, the infestation pressure of the latter remained below the critical threshold, with a maximum infestation rate of 2.2% during 2024.

- *Leptinotarsa decemlineata* Say (Colorado potato beetle) is one of the most well-known and destructive pests of solanaceous crops, including potatoes, tomatoes, and eggplants. It is notable for its extraordinary ability to develop resistance to over 50 different insecticides, often within just a few years of their introduction (Chen et al., 2023).

- *Tuta absoluta* Meyrick (tomato leaf miner) has become a major pest of tomato crops in Romania, causing extensive damage. It mainly attacks plants of the Solanaceae family, feeding on all the above-ground parts of the plant, with a clear preference for tomatoes (Savranschii et al., 2000). In the Bacău area, the species has been reported but does not have a level of infestation that requires control measures.

- *Nezara viridula* L. (green stink bug): A polyphagous species native to Africa that has spread globally and become a permanent presence in temperate regions. It prefers legumes and other plants with fruits or pods, injecting toxic saliva into plant tissues and facilitating the penetration of pathogens (Mitchell et al., 2018; Jones, 1988). In the Bacău area, the species has been reported but does not have a degree of attack that requires control measures.

The role of beneficial invasive insect species

In an attempt to control agricultural pests, certain insect species considered “beneficial” have been intentionally introduced. A notable

example is *Harmonia axyridis* (Asian ladybird) (also present at VRDS Bacău), brought to Europe to control pest populations (Roy et al., 2016). However, this seemingly “noble” intervention has generated a new set of ecological challenges. The Asian ladybird has become an invasive species itself, competing with native ladybirds for resources and, moreover, consuming their larvae, thus destabilizing the natural balance of the local entomofauna. This paradox highlights the complexity of ecological management (Roy et al., 2016; Farrow, 2023). An external biological solution can, over time, become a new threat to biodiversity, demonstrating that ecological balance is not static and that any intervention, even one with positive intentions, requires careful monitoring and long-term evaluation (Roy et al., 2016).

Weeds as host plants and reservoirs for pests and diseases

Weeds play a pivotal role in the epidemiology of agricultural crops, functioning as ecological "bridges" that facilitate the transmission of pest

and pathogen populations from one growing season to the next.

This mechanism of pest survival and proliferation is a direct causal relationship, essential to understanding how infestations are maintained and spread (Ansar & Ghatak, 2019; Gupta & Thind, 2018). A salient example can be found in the relationship between weeds of the Solanaceae family and specialized pests. Notable examples of such species include *Solanum nigrum* (black nightshade), which is listed among the identified weeds and which has been found to play a significant role in serving as an alternative host for pests such as the Colorado potato beetle (*L. decemlineata*) and the tomato leaf miner (*T. absoluta*) (Ben-Abdallah et al., 2019; Kroschel et al., 2020). The presence of these weeds facilitates the completion of the life cycle of pests, thereby enabling their survival between vegetable crops. This, in turn, creates the conditions for a subsequent massive invasion once new crops are planted (Reddy, 2018).

Table 2. Correlation between weed families and invasive pests and assimilated diseases

Weeds family	Weeds species	Invasive pests	Transmitted viral diseases
Solanaceae	<i>Solanum nigrum</i>	<i>Leptinotarsa decemlineata</i>	
Solanaceae	<i>Datura stramonium</i>	<i>Tuta absoluta</i>	
Amaranthaceae	<i>Amaranthus</i> sp.	<i>Aphids, Bemisia tabaci</i>	Tomato Mosaiv Virus (ToMV)
Polygonaceae	<i>Festuca drimeja</i>	<i>Aphids, Bemisia tabaci</i>	Cucumber Mosaic Virus (CMV)

Furthermore, weeds act as reservoirs for viruses, with major implications for plant health (Wisler & Norris, 2005). A number of viruses, including tomato mosaic virus (ToMV) and cucumber mosaic virus (CMV), have been observed to infect and persist in various weeds, such as chickweed and lamb's quarters (Naik & Chaudhary, 2025). These viruses are then transmitted to crops by mobile vectors, such as aphids and whiteflies. A single generation of aphids has been shown to transmit over 50 viruses to crops, thereby demonstrating the rapid and extensive dissemination of pathogens. Conversely, the whitefly (*B. tabaci*) has been identified as the exclusive vector for the tomato yellow leaf curl virus (TYLCV), which has the capacity to infect weeds, beans, and peppers (Brown & Czosnek, 2002; Sarwar et al., 2020). This intricate interplay constitutes a causal chain:

- *Presence of weeds:* Species from families such as Solanaceae and Amaranthaceae are ubiquitous in agricultural ecosystems.
- *Reservoir effect:* These weeds act as alternative hosts for polyphagous pests (aphids, whiteflies) and specialized pests (Colorado potato beetle).
- *Virus vectors:* Pests feed on infected weeds, becoming virus carriers themselves.
- *Disease transfer:* Due to their high mobility, vectors rapidly transfer viruses from weeds at the edge of crops to vegetable plants, triggering widespread epidemics.

The beneficial role of weeds in maintaining beneficial insect fauna

In the context of an agroecological approach, it has been acknowledged that certain weeds and plants in the spontaneous flora can exert a beneficial role, thereby supporting biodiversity and ecosystem services. These organisms can

function as a source of sustenance (i.e., nectar and pollen) and a habitat for beneficial insects, thereby contributing to natural biological control and reducing reliance on chemical interventions (Jordan & Vatovec, 2004; Gaba et al., 2014, 2016).

A notable example is represented by nectar-producing plants, such as those in the Lamiaceae family (sage, rosemary, lavender), which attract natural predators of pests, such as ladybugs (Coccinellidae), lacewings (Chrysopa), and hoverflies (Syrphidae). These predators consume aphids, thereby helping to regulate their population levels and prevent them from reaching levels that would cause economic damage. It has been demonstrated that other families of weeds, such as Apiaceae, which are renowned for their ability to attract beneficial insects, can be employed in a strategic manner to enhance the agricultural landscape (Smith & Hagen, 1956; Elekcioglu, 2020; Chellappan & Ranjith, 2023).

The positive impact of weeds on soil fertility has been well documented (Parks & Gallandt, 2023). Weeds belonging to the Fabaceae family (legumes) possess the capacity to fix atmospheric nitrogen in the soil, thereby enhancing its structure and fertility. This process is analogous to that of cover crops (Xu & Deng, 2017). These practices, which are part of a broader framework of conservation agriculture, demonstrate that not all weeds are necessarily detrimental and that intelligent management can leverage their ecological functions (Monteiro & Santos, 2022). The following is a description of this beneficial chain:

- *Strategic presence of functional plants:* Weeds are maintained or plants from the spontaneous flora with insect-attracting properties are introduced.
- *Attracting beneficial entomofauna:* These plants provide food sources and shelter for a variety of beneficial insects, predators, and pollinators.
- *Strengthening biological control:* Beneficial insect populations thrive, exerting natural and effective control over pests, which reduces the need for pesticides.

CONCLUSIONS

An analysis of phyto-entomological interactions at the Vegetable Research and Development Station Bacău reveals a complex and dynamic picture of the agricultural ecosystem. Weeds, it should be noted, play a dual role in this ecosystem. They serve as both hosts and reservoirs for invasive pests and the viruses they transmit. At the same time, weeds can act as a potentially beneficial element that supports natural biological control. The relationship between weeds and invasive insects is deeply interconnected, and their impact is dynamic, accelerated by climate change, which allows the geographical expansion of pests such as the green tomato bug. In order to develop sustainable and region-specific management strategies, further research is needed. It is recommended that future studies at the Bacău Resort focus on:

- *Identification and Quantification:* Conduct in-depth local studies to identify specific species of beneficial entomofauna and pests, quantify their distribution in relation to the prevalence of weeds within the spontaneous flora.

- A *long-term evaluation* is necessary to ascertain the effectiveness of the intervention. It is imperative to monitor the long-term impact of invasive species, such as *H. axyridis*, in order to comprehensively understand their effects on native biodiversity. This monitoring is necessary to develop a stricter regulatory framework for the introduction of new organisms for biological control purposes.

- *Predictive models:* The development of predictive models is imperative, with these models being based on local climatic conditions and weed and insect population dynamics. The anticipation and prevention of major infestations are best achieved through the implementation of such predictive models.

The effective management of weeds and invasive insects at the Vegetable Research and Development Station Bacău should not aim to eradicate them; rather, it should focus on an ecological approach that capitalizes on the beneficial role of certain elements of spontaneous flora in maintaining a dynamic balance in the agroecosystem. This approach is imperative to ensure long-term sustainability.

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PRODUCTIVITY ELEMENTS, YIELD, AND QUALITY INDICES IN TOMATOES AS A RESULT OF 'GENOTYPE×FERTILIZER' INTERACTION

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Abstract

The study evaluated some productivity parameters and quality indices in tomatoes in relation to fertilization. The tomato hybrid 'Polfast F1' was cultivated in relation to Naturamin WSP fertilizer (V1) and Crop Max (V2), and the tomato variety 'San Marzano' in relation to Naturamin WSP (V3) and Crop Max (V4). The 'San Marzano' variety showed an advantage for the number of clusters (PCN) and the number of fruits per plant (PFN). The 'Polfast F1' hybrid showed an advantage for average fruit weight (AFW). In terms of yield (Y/plt) the V4 variant showed an advantage, with statistically significant differences ($p < 0.05$). In terms of quality indices, variant V1 showed an advantage for vitamin C (VitC), $p < 0.05$, and variant V4 showed an advantage for the calcium (Ca) content, $p < 0.01$, and the lycopene (LYCO) content, $p < 0.05$. Multivariate analysis (PCA) explained the positioning of the productivity parameters and the fruit quality indices in relation to the principal components, with positive effects in PC1 (PCN, PFN, LYCO) and in PC2 (Ca) and negative effects in PC1 (VitC, AFW).

Key words: fertilizers, PCA, productivity elements, quality indices, tomatoes, yield.

INTRODUCTION

Tomatoes are important in human nutrition due to their complex nutritional profile, given by the content of nutrients and specific phytochemical compounds with an antioxidant role (e.g. vitamins, minerals, essential amino acids, monounsaturated fatty acids, phytosterols, and lycopene), with a specific role in human health (Ali et al., 2020; Sattar et al., 2024).

In order to show the importance of tomatoes, various formulations have been made, which highlighted the important position of tomatoes in the structure of vegetable crops (Schwarz et al., 2014), fruit quality and benefits for human health (Bozo et al., 2019), ecological plasticity (Diouf et al., 2020), suitability for different cropping systems (Sattar et al., 2024), importance for different industries (Sattar et al., 2024), versatility and dominance in consumer preferences (Li et al., 2025).

Tomato cultivation faces several challenges related to the cropping system, soil conditions, nutrient imbalances, microbial activity,

allelopathic interactions, and crop management practices, all of which may influence plant growth, productivity, and fruit quality (Li et al., 2025; Singh et al., 2025).

Pretreatments of tomato seeds (e.g. shikimic acid) generated positive effects on the agronomic parameters of tomato plants and fruits, and on the content of phytochemical compounds in fruits (Al-Amri, 2013). The advantages of growing tomatoes on nutrient solutions were communicated through higher yields and superior quality indices, compared with the soil cultivation system (Bozo et al., 2019).

Soilless tomato cultivation systems are considered a promising and intensive, sustainable approach with a number of advantages, especially in relation to water and nutrient management (Aydi et al., 2023).

Plant morphological parameters, productivity elements, and quality indices in cherry tomatoes were studied under greenhouse cultivation conditions, and favorable effects were recorded in relation to longer day length (Arshad et al., 2023). Under greenhouse conditions, proper

water and fertilizer management is necessary to optimize tomato yield and quality (Yu et al., 2023). Tomatoes can be cultivated, for productive purposes or for research, in soil, in different substrates (established recipes or accessible mixtures from local resources), or on nutrient solutions. They have the ability to capitalize on growth environments with specific responses (Schwarz et al., 2014).

Growing substrates that contained/integrated shredded corn plant residues favored fruiting in tomatoes and positively influenced dimensional parameters of the fruits, firmness, and total soluble solids content in tomato fruits (Tzortzakis & Economakis, 2008). Alternative growing media were studied compared with commercial media, with the aim of reducing the costs of growing cherry tomatoes (Silva et al., 2017). The study authors identified different variants of alternative substrates made from local components (e.g., compost, soil) with comparable results in plant vigor and yield, or better results, compared with the commercial substrates tested (Silva et al., 2017). Comparative analysis of different growing substrates showed that including a mixture of fermented tomato waste, along with peat and vermiculite, positively influenced vegetable seedlings and seedlings (e.g. chili peppers, greenhouse cucumbers, greenhouse zucchini, and tomatoes) used in the test (Abdel-Razzak et al., 2019).

The water and nutrient retention properties of some vegetable growing media have been improved by components such as vermiculite, perlite, or biochar (Vaughn et al., 2021). Biocarbon-infused growing media (infused with inorganic and organic fertilizers) differentially influenced germination, seedling growth, and transplanting of tomato seedlings (Vaughn et al., 2021).

Certain substrates established for such "soilless" systems (e.g. coconut fiber) raise economic and logistical issues, and various substrates of local origin have been analyzed (Aydi et al., 2023), which, moreover, integrate well into the concept of circular economy.

The use of renewable substrates, or substrates based on renewable components, is a current concern for replacing dedicated substrates in vegetable cultivation (Wang et al., 2024). Seedling growth parameters and water regime in growing media showed that alternative ingredients (e.g.

fermented corn residues) to commercial media led to positive results in greenhouse tomato cultivation (Wang et al., 2024).

Tomato plants performance and yield were evaluated in relation to different conventional growing media, and with new ingredients (e.g. white champignon mushroom substrate - as a peat substitute), in different proportions (da Silva Alves et al., 2024). Based on the recorded results, the authors formed the proportion in which the newly tested component, substrate from champignon mushrooms, should be included in the mixture for tomato substrates (da Silva Alves et al., 2024).

In studies on the utilization of fermented organic residues, growing substrates that included biochar improved tomato yields in urban agriculture experiments (de Celis et al., 2025). Fermented organic materials (e.g. food scraps, wood chips) have been used alongside peat, in different proportions, to create growing media for tomatoes, with positive results in plant emergence rate, morphological and physiological parameters, and nutrient content (Hamilton et al., 2025).

Concerns regarding yield, production quality and potential post-harvest losses in tomato cultivation have been identified within communities of "small farmers" under open and protected cultivation conditions (Molelekoa et al., 2025). The development index of tomato plants was positively influenced in relation to organic fertilizers (Mineva, 2025).

Yield variation in tomatoes was recorded in relation to different distances between rows and between plants per row (considered factors of crop geometry) under "poly-house structures" cultivation conditions (Singh et al., 2025). According to the authors, a spatial geometry of the type "60 × 40 cm" led to significantly larger polar and equatorial dimensions of fruits ($p < 0.05$), compared to other geometric arrangements of plants, as well as to improved energy efficiency in tomato cultivation (Singh et al., 2025).

In the context of interest in capitalizing on local resources, the circular economy, the sustainability of vegetable cultivation in protected areas, and accessible solutions for "small farmers", the present study comparatively evaluated two tomato genotypes in relation to fertilizers in a solar growing system in nutrient bags, on a growing substrate predominantly made of local components.

MATERIALS AND METHODS

The study was conducted in the area of Ticvaniu Mic, Caras-Severin County, Romania, during the 2023-2024 (Figure 1). The experiment was

conducted in a protected, tunnel-type solar space.

The biological material was represented by two tomato genotypes: the hybrid 'Polfast F1' and the variety 'San Marzano' (Figure 2).



Figure 1. Study location, Ticvaniu Mic ATU, Caras-Severin County, Romania (Google Maps)



Figure 2. Cultivated tomato genotypes; 'Polfast F1' - left; 'San Marzano' - right

The plants were obtained by sowing in alveolar trays (OZ-KA VAKUM), in a professional peat substrate (Zelota Zeme; 80% blond peat, 20% black peat), with a fine granulation, up to 7 mm. Sowing was done on April 10, 2024. During the seedling production period, the temperature was maintained at 22-25°C during the day and at 17-20°C at night. On May 12, the seedlings were transplanted into EPE 28 trays. The substrate was represented by peat for transplanting (Thomaflor; 60% blond peat, 40% black peat) and perlite, in a volumetric proportion of 85% peat and 10% perlite. For plant protection, treatments with Bioprotekt and Previcur Energy (0.2%) were applied. On June 6, the seedlings were planted in vegetation bags (volume 45 L). The growing substrate in the vegetation bags

was a homogeneous mixture of alluvial soil (60%), fermented manure (30%), and peat (10%).

Two fertilizer products were applied: Naturamin WSP (0.1%) and Crop Max (0.2%). The experimental variants resulted: V1 ('Polfast F1' + Naturamin WSP), V2 ('Polfast F1' + Crop Max), V3 ('San Marzano' + Naturamin WSP), V4 ('San Marzano' + Crop Max).

Productivity elements were evaluated, Plant clusters number (PCN), average fruit weight (AFW, g), plant fruits number (PFN), yield per plant (Y/plt), yield per hectare (Y/ha), vitamin C content (VitC, mg/100 g), calcium content (Ca, ppm), lycopene content (LYCO, mg/100 g).

Fruit samples were taken randomly from each experimental variant and replication. Productivity elements (PCN, PFN) were determined by counting, on plants, experimental variants and replications. Fruit weight (AFW, g) was determined by weighing with a technical balance. The content of vitamin C (VitC, mg/100 g), calcium (Ca, ppm) and Lycopene (LYCO, mg/100 g) was determined within the Interdisciplinary Research Platform, ULS "King Mihai I" from Timisoara, by accredited methods (Iodometric - Vitamin C, mg/100 g; AAS spectrometry - Ca, ppm; UV-VIS spectrophotometry - Lycopene, mg/100 g).

To assess the reliability of the data and the presence of variance, the ANOVA test was applied with Alpha = 0.05. Correlation analysis (Pearson's R) was applied to assess the interdependence of the elements of productivity, yield, and quality indices. Comparative analysis (t Test) was applied to evaluate the level of confidence of the differences between the values of the variants and the experimental mean for each determined parameter. The PAST software (Hammer et al., 2001), and the calculation module in EXCEL were used for the statistical

analysis of the data and the generation of graphs. Established statistical parameters were used to determine the confidence of the results.

RESULTS AND DISCUSSIONS

Under experimental conditions, the interaction of the two tomato genotypes ('Polfast F1', 'San Marzano'), with the applied fertilization (Naturamin WSP, Crop Max), resulted in specific values of productivity, yield, and quality index parameters (Table 1).

Table 1. Productivity elements and quality indices in tomatoes, average values across experimental variants

Trial	PCN	AFW	PFN	Y _(plt)	Y _(ha)	Vit C	Ca	LYCO
	(No)	(g)	(No)	(kg/plt)	(t/ha)	(mg/100 g)	(ppm)	(mg/100 g)
V1	3.50	105.00	14.50	1.53	22.95	17.800	88.149	18.870
V2	3.83	117.00	14.80	1.73	25.95	13.580	95.146	19.100
V3	5.00	93.00	22.80	2.13	31.80	9.850	77.357	21.700
V4	5.00	103.00	24.60	2.55	38.25	11.730	125.897	22.430
SE	±0.25	±1.72	±2.99	±0.14	±2.14	±1.05	±5.67	±0.59

Plant cluster number (PCN) ranged from 3.50±0.25 (V1) to 5.00±0.25 (V3, and V4). AFW ranged from 93.00±1.72 g (V3) to 117.00±1.72 g (V2). The PFN parameter recorded values between 14.50±2.99 (V1) and 24.60±2.99 (V4). The yield per plant (Y/plt) recorded values between 1.53±0.14 kg/plt (V1) and 2.55±0.14 kg/plt (V4), and the yield per hectare (Y/ha) recorded values between 22.95±2.14 t/ha (V1) and 38.25±2.14 t/ha (V4). In the case of quality indices, the vitamin C (Vit C) content recorded values between 9,850±1.05 mg/100 g (V3) and 17,800±1.05 mg/100 g (V1). The calcium (Ca) content varied between 77,357±5.67 ppm (V3) and 125,897±5.67 ppm (V4). The lycopene content (LYCO) recorded values ranging from 18,870±0.59 mg/100 g (V1) to 22,430±0.59 mg/100 g (V4). The ANOVA test (Alpha = 0.05) confirmed the statistical reliability of the experimental data, and the presence of variance in the data set ($F > F_{crit}$; $p < 0.001$).

According to the coefficient of variation values, moderate variability was recorded for the PFN parameter ($CV_{PFN} = 23.76$) and for the Vit C parameter ($CV_{VitC} = 21.07$). Low variability was recorded for the other parameters, namely $CV_{PCN} = 15.54$, $CV_{AFW} = 7.57$, $CV_{Y/plt} = 19.11$, $CV_{Y/ha} = 19.08$, $CV_{Ca} = 15.53$, $CV_{LYCO} = 7.58$. High variability has been reported in tomato

fruits as a result of polymorphism (Schwarz et al., 2014). Tomato has been used as a “model plant” for biochemical, cellular, molecular, genetic, physiological, and fruit development studies (Schwarz et al., 2014).

The interdependence between morphological parameters, yield, and quality indices was evaluated through correlation analysis (Table 2). The PCN parameter showed a positive correlation of very strong intensity with PFN ($r = 0.986^{***}$), and with the lycopene content, LYCO ($r = 0.986^{***}$). The PCN parameter showed a positive correlation of strong intensity with the yield per plant and per unit area, Y/plt ($r = 0.947^{**}$), Y/ha ($r = 0.944^{**}$). The PCN parameter showed a strong negative correlation with vitamin C, VitC ($r = -0.931^{**}$). The PFN parameter showed a very strong positive correlation with yield ($r = 0.968^{***}$, in the case of Y/plt; $r = 0.966^{***}$, in the case of Y/ha) and with lycopene ($r = 0.999$). The PFN parameter showed a strong negative correlation with Vit C ($r = -0.859$), and the PFN parameter showed a moderate negative correlation with AFW ($r = -0.766$). Yield per plant, Y/plt showed a positive correlation of very strong intensity with Y/ha ($r = 0.999^{***}$) and with LYCO ($r = 0.976$). It showed a negative correlation of strong intensity with Vit C ($r = -0.826$). Yield per unit area, Y/ha showed a positive, very strong correlation

with LYCO ($r = 0.974^{***}$) and a negative, strong correlation with VitC ($r = -0.922$). Vitamin C content, Vit C, showed a negative correlation with LYCO ($r = -0.862$).

Table 2. Correlation table

Variable	Statistical parameters	PCN	PFN	AFW	$Y_{(plt)}$	$Y_{(ha)}$	VitC	Ca	LYCO
PCN	Pearson's r	—							
	p-value	—							
PFN	Pearson's r	0.986***	—						
	p-value	< .001	—						
AFW	Pearson's r	-0.751	-0.766*	—					
	p-value	0.051	0.045	—					
$Y_{(plt)}$	Pearson's r	0.947**	0.968***	-0.585	—				
	p-value	0.001	< .001	0.167	—				
$Y_{(ha)}$	Pearson's r	0.944**	0.966***	-0.579	0.999***	—			
	p-value	0.001	< .001	0.173	< .001	—			
VitC	Pearson's r	-0.931**	-0.859*	0.601	-0.826*	-0.822*	—		
	p-value	0.002	0.013	0.153	0.022	0.023	—		
Ca	Pearson's r	0.348	0.439	0.126	0.630	0.637	-0.169	—	
	p-value	0.444	0.324	0.787	0.130	0.124	0.717	—	
LYCO	Pearson's r	0.986***	0.999***	-0.745	0.976***	0.974***	-0.862*	0.461	—
	p-value	< .001	< .001	0.055	< .001	< .001	0.013	0.298	—

Note: “*” symbol of significance: *significance for $p < 0.05$; **significance for $p < 0.01$; ***significance for $p < 0.001$

A positive correlation of high undertensity was recorded between total yield and the the height of tomato plants under greenhouse conditions ($r = 0.91$ for the Grando variety, and $r = 0.89$ for the Vermilanza variety) under organic fertilization conditions (Mineva, 2025).

The comparative analysis was performed between the experimental variants for the parameters considered.

In the case of the number of clusters per plant (PCN), the average value calculated at the experiment level was $\bar{PCN} = 4.33$. Compared to the average value, variant V1 recorded a value lower than the average, within statistical safety conditions ($p < 0.05$). In the case of the other variants, the differences did not present statistical safety. The average value of the PCN parameter at the level of the hybrid 'Polfast F1' (variants V1, V2) was 3.67, and the average value of the PCN parameter at the level of the variety 'San Marzano' (variants V3, V4) was 5.00.

In the case of the number of fruits per plant (PFN), the average value calculated at the experiment level was $\bar{PFN} = 19.18$. Compared

to the average value, variant V4 recorded a value higher than the average, in conditions of statistical safety ($p < 0.05$). In the case of the other variants, the differences did not present statistical safety. The average value of the PFN parameter at the level of the hybrid 'Polfast F1' (variants V1, V2) was 14.65, and the average value of the PFN parameter at the level of the variety 'San Marzano' (variants V3, V4) was 23.70.

In the case of the average fruit weight (AFW), the average value calculated at the experiment level was $\bar{AFW} = 104.50$ g. Compared to the average value, variant V2 recorded a value higher than the average under conditions of statistical safety ($p < 0.05$), and variant V3 recorded a value lower than the average under conditions of statistical safety ($p < 0.05$). In the case of the other variants, the differences did not present statistical certainty (Table 3). The average value of fruit weight at the level of the hybrid 'Polfast F1' (variants V1, V2) was 111.00 g, and the average value of yield at the level of the variety 'San Marzano' (variants V3, V4) was 98.00 g (Figure 3).

Table 3. Results of comparative analysis for tomato fruit weight

Statistical parameters	V1	V2	V3	V4
Given mean	105.00	117.00	93.00	103.00
Sample mean	104.50			
95% conf. interval	(95.406 113.59)	(95.406 113.59)	(95.406 113.59)	(95.406 113.59)
Difference	0.50	12.50	11.50	1.50
95% conf. interval	(-8.5944 9.5944)	(3.4056 21.594)	(2.4056 20.594)	(-7.5944 10.594)
t	-0.1413	-3.5332	3.2505	0.4240
p (same mean)	0.8931	0.0167	0.0227	0.6892
Significance	ns	*	o	ns

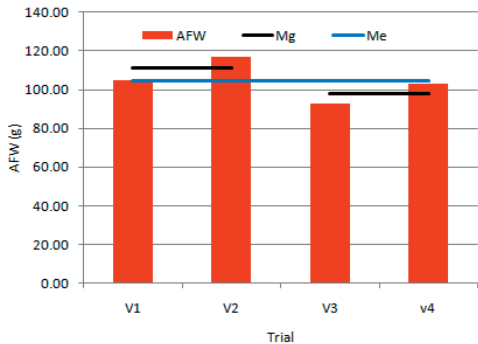


Figure 3. Average weight of tomato fruits in relation to the experimental variants; Mg – mean of genotype; V1, V2 – 'Polfast F1'; V3, V4 – 'San Marzano'; Me – mean of experiment

Positive variations, in quantitative and qualitative terms, were recorded in tomato fruits under the influence of treatments with microorganisms (Dragomir et al., 2025). In the case of yield per plant (Y/plt), the average value calculated at the experiment level was =1.985 kg (Table 4). Compared to the mean value, variant V4 recorded a value higher than the mean under statistical safety conditions ($p < 0.05$), and variant V1 recorded a value lower than the mean under statistical safety conditions ($p < 0.05$). In the case of the other variants, the differences did not show statistical safety (Table 4). The average yield value for the 'Polfast F1' hybrid (variants V1, V2) was 1.630 kg/plt, and the average yield value for the 'San Marzano' variety (variants V3, V4) was 2.340 kg/plt (Figure 4).

Table 4. Results of comparative analysis for yield per plant

Statistical parameters	V1	V2	V3	V4
Given mean	1.530	1.730	2.130	2.550
Sample mean	1.985			
95% conf. interval	(1.5487 2.4213)	(1.5487 2.4213)	(1.5487 2.4213)	(1.5487 2.4213)
Difference	0.455	0.255	0.145	0.565
95% conf. interval	(0.018669 0.89133)	(-0.18133 0.69133)	(-0.29133 0.58133)	(0.12867 1.0013)
t	2.6806	1.5023	-0.8543	-3.3286
p (same mean)	0.0438	0.1933	0.4320	0.0208
Significance	o	ns	ns	*

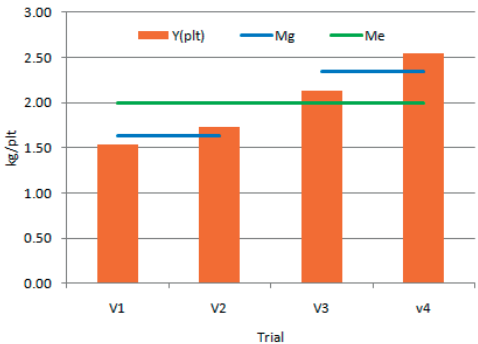


Figure 4. Tomato yield per plant, in relation to experimental variants; Mg – mean of genotype; V1, V2 – 'Polfast F1'; V3, V4 – 'San Marzano'; Me – mean of experiment

In the case of vitamin C (Vit C) content, the average value calculated at the experiment level was $\overline{\text{VitC}} = 13.24 \text{ mg}/100 \text{ g}$ (Table 5).

Table 5. Results of comparative analysis for vitamin C content

Statistical parameters	V1	V2	V3	V4
Given mean	17.80	13.58	9.85	11.73
Sample mean	13.24			
95% conf. interval	(10.033 16.447)	(10.033 16.447)	(10.033 16.447)	(10.033 16.447)
Difference	4.56	0.34	3.39	1.51
95% conf. interval	(1.3532 7.7668)	(-2.8668 3.5468)	(0.18324 6.5968)	(-1.6968 4.7168)
t	-3.6554	-0.2726	2.7175	1.2104
p (same mean)	0.0147	0.7961	0.0419	0.2802
Significance	*	Ns	o	ns

Compared to the average value, variant V1 recorded a value higher than the average under conditions of statistical safety ($p<0.05$), and in the case of variant V3, a value lower than the average was recorded under conditions of statistical safety ($p<0.05$). In the case of the other variants, the differences did not show statistical significance (Table 5). The average value of the vitamin C content in the hybrid 'Polfast F1' (variants V1, V2) was 15.69 mg/100g, and the average value of the vitamin C content in the variety 'San Marzano' (variants V3, V4) was 10.79 mg/100 g (Figure 5).

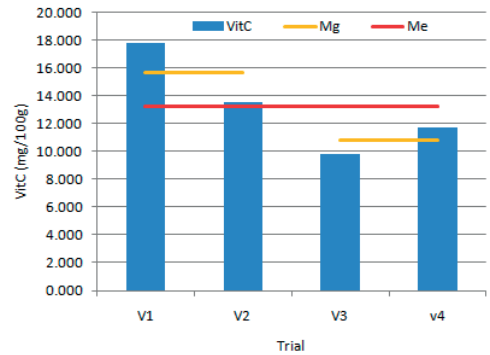


Figure 5. Vitamin C content in tomatoes, in relation to experimental variants; Mg – mean of genotype; V1, V2 – 'Polfast F1'; V3, V4 – 'San Marzano'; Me – mean of experiment

In the case of calcium (Ca) content, the average value calculated at the experiment level was $\overline{\text{Ca}} = 96.638$ ppm (Table 6).

Table 6. Results of the comparative analysis for Ca content in tomato fruits

Statistical parameters	V1	V2	V3	V4
Given mean	88.149	95.146	77.357	125.897
Sample mean	96.638			
95% conf. Interval	(79.382 113.89)	(79.382 113.89)	(79.382 113.89)	(79.382 113.89)
Difference	8.489	1.492	19.281	29.259
95% conf. Interval	(-8.7672 25.746)	(-15.764 18.749)	(2.0248 36.538)	(12.002 46.515)
t	1.2646	0.2223	2.8722	-4.3585
p (same mean)	0.2618	0.8329	0.0349	0.0073
Significance	ns	ns	o	**

Compared to the calculated average value, variant V3 recorded a value below the average under conditions of statistical safety ($p<0.05$),

and variant V4 recorded a value above the average, with the difference under conditions of statistical safety ($p<0.01$). In the case of variants V1 and V2, the differences did not show statistical significance (Table 6). The average value of Ca content in the hybrid 'Polfast F1' (variants V1, V2) was 91.65 ppm, and the average value of Ca content in the variety 'San Marzano' (variants V3, V4) was 101.63 ppm (Figure 6).

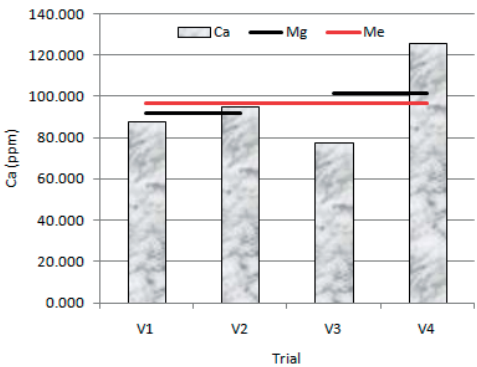


Figure 6. The Ca content of tomatoes, in relation to the experimental variants; Mg – mean of genotype; V1, V2 – 'Polfast F1'; V3, V4 – 'San Marzano'; Me – mean of experiment

In the case of lycopene content (LYCO), the average value calculated at the experiment level was $\overline{\text{LYCO}} = 20.527$ mg/100 g (Table 7).

Table 7. Results of the comparative analysis for lycopene content in tomato fruits

Statistical parameters	V1	V2	V3	V4
Given mean	18.870	19.100	21.700	22.430
Sample mean	20.527			
95% conf. interval	(18.738 22.315)	(18.738 22.315)	(18.738 22.315)	(18.738 22.315)
Difference	1.657	1.427	1.173	1.903
95% conf. interval	(-0.13185 3.4452)	(-0.36185 3.2152)	(-0.61518 2.9618)	(0.11482 3.6918)
t	2.3811	2.0505	-1.6864	-2.7356
p (same mean)	0.0631	0.0956	0.1525	0.0410
Significance	ns	ns	ns	*

Compared with the calculated average value, variant V4 had a lycopene content above the average, with a statistically significant difference ($p<0.05$). In the case of the other variants, the differences did not show statistical

significance (Table 7). The average value of lycopene content in the 'Polfast F1' hybrid (variants V1, V2) was 18,985 mg/100 g, and the average value of lycopene content in the 'San Marzano' variety (variants V3, V4) was 22,065 mg/100 g (Figure 7).

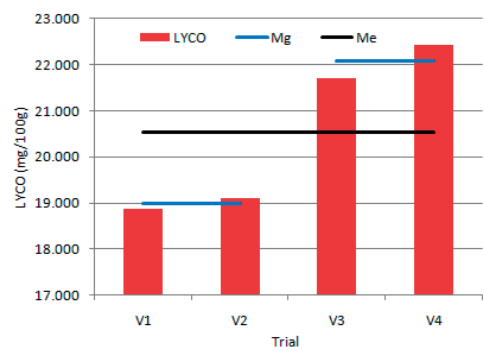


Figure 7. Lycopene content in tomatoes, in relation to experimental variants; Mg – mean of genotype; V1, V2 – 'Polfast F1'; V3, V4 – 'San Marzano'; Me – mean of experiment

Lycopene is a dietary carotenoid present in tomatoes, with an important role in human health (Ali et al., 2020). The increase in lycopene content, with values of up to 26.64% in tomato fruits, was recorded under the influence of treatments with biostimulating substances (Bălan et al., 2024). The variation in the content of phytochemical compounds in tomatoes was analyzed in relation to environmental factors, and the role of temperature in the formation of lycopene and beta-carotene content was recorded (ElShamey et al., 2025). The impact of the experimental variants on the values of productivity parameters, yield, and quality indices in tomatoes was evaluated (Figure 8). Variant V1 showed a negative effect (with statistical certainty) on the PCN parameter, yield (Y/plt, Y/ha) and a positive impact on vitamin C content. Variant V2 showed a positive effect (with statistical certainty) on fruit weight (AFW), and effects without statistical significance on the other elements evaluated. The Crop Max product improved the performance of the 'Polfast F1' hybrid compared to the Naturamin WSP product. Variant V3 showed a negative effect (with statistical certainty) on the AFW parameter, vitamin C (VitC) content, and calcium (Ca) content in

tomato fruits. On the other parameters evaluated, the effect was without statistical certainty. Variant V4 generated the most positive effects (with statistical certainty) in tomatoes, as an expression of the interaction of the 'San Marzano' variety with the Crop Max fertilizer (Figure 8).

	Polifast F1		San Marzano	
	Naturamin WSP	Crop Max	Naturamin WSP	Crop Max
	V1	V2	V3	V4
PCN	o	ns	ns	ns
PFN	ns	ns	ns	*
AFW	ns	*	o	ns
Y/plt	o	ns	ns	*
Y/ha	o	ns	ns	*
VitC	*	ns	o	ns
Ca	ns	ns	o	**
LYCO	ns	ns	ns	*

Figure 8. Synthetic diagram of the effect generated by the experimental variants on tomatoes

Advantages of some types of local substrates for tomatoes have been recorded, with positive effects on physiological indices, morphological parameters of plants, productivity, and fruit quality elements (Aydi et al., 2023). The variation in tomato fruit production and quality was analyzed in relation to climatic parameters under greenhouse cultivation conditions, on a “coconut-containing growbag (Jiffy growbag)” (Arshad et al., 2023). Mathematical models have described quality indicators in tomatoes under greenhouse conditions in relation to the water regime and variable fertilization rates (Yu et al., 2023). In the present study, the growing substrate made mainly from local resources, alluvial soil (60%), mranita (30%), and improved with peat (10%), created good conditions for the growth of tomato plants ('Polfast F1', 'San Marzano') in a tunnel-type greenhouse. From the interaction with the fertilizer products, the tomato genotypes generated differentiated values of the evaluated

parameters, in relation to their specific biological potential.

CONCLUSIONS

The cultivation conditions in a tunnel-type greenhouse, in container bags (volume of 45 L), with a homogeneous growth substrate consisting of alluvial soil (60%), fermented manure (30%) and peat (10%), ensured uniform growing conditions for the tomato plants.

The two tomato genotypes, the 'Polfac F1' hybrid and the 'San Marzano' variety, benefited differentially from treatments with Naturamin WSP and Crop Max.

The 'San Marzano' showed advantages for the number of clusters per plant (PCN) and for the number of fruits per plant (PFN). The interaction between the 'San Marzano' variety and the product Crop Max (V4) led to positive, statistically significant differences for the parameter PFN ($p < 0.05$).

For average fruit weight (AFW), the 'Polfac F1' hybrid presented advantages, and the interaction between 'Polfac F1' and the Crop Max fertilizer (V2) generated positive differences compared to the experimental average, with statistical certainty ($p < 0.05$).

For yield per plant (Y/plt) and associated yield per hectare (Y/ha), the 'San Marzano' variety presented advantages, and the interaction between 'San Marzano' and Crop Max generated positive differences compared to the experiment average ($p < 0.05$).

For vitamin C content (Vit C, mg/100 g), the 'Polfac F1' genotype presented advantages, and the interaction between 'Polfac F1' and Naturamin WSP generated values above the average of the experiment ($p < 0.05$).

For calcium content (Ca, ppm) and lycopene content (LYCO, mg/100 g) the 'San Marzano' genotype presented advantages, and the interaction between 'San Marzano' and Crop Max generated values above the average of the experiment ($p < 0.01$ for Ca; $p < 0.05$ for LYCO). The Crop Max fertilizer product, in combination with both genotypes, generated the most advantages for fruit parameters, yield, and quality indices, except for average fruit weight (AFW) and vitamin C content (Vit C) where the Naturamin WSP product showed better performance.

Overall, the 'San Marzano' variety, in combination with Crop Max, generated the most advantages for the fruit parameters, yield, and quality indices evaluated in tomatoes under the study conditions.

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GENETIC RESOURCES AND BREEDING STRATEGIES FOR SUSTAINABLE IMPROVEMENT OF EGGPLANT (*SOLANUM MELONGENA* L.) A REVIEW

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Abstract

*The broad yet underutilized genetic diversity of eggplant (*Solanum melongena* L.) provides a crucial foundation for breeding strategies aimed at enhancing yield, resilience, and adaptability. In light of accelerating climate change and mounting biotic and abiotic stresses, the development of improved cultivars necessitates a strategic and effective deployment of these genetic resources. This review synthesizes current knowledge on eggplant genetic diversity and its practical integration into breeding programs, with emphasis on germplasm evaluation, trait-based selection, stress tolerance, and yield and quality improvement. Particular attention is given to conventional and hybrid breeding approaches, the role of phenotypic diversity in cultivar adaptation, and the linkage between genetic variation and nutritional or functional traits. By consolidating recent advances and breeding outcomes, this review highlights pathways for strengthening eggplant improvement programs and supports the development of resilient cultivars suited to sustainable agricultural systems.*

Key words: eggplant, germplasm evaluation, genetic resources, quality improvement, breeding strategies, sustainable improvement.

INTRODUCTION

Eggplant (*Solanum melongena* L.) production is expected to be highly vulnerable to the accelerating impacts of climate change, which increasingly threaten global agricultural sustainability and food security (del Pozo et al., 2019; Habib-ur-Rahman et al., 2022). Rising temperatures, altered precipitation regimes, and the growing frequency and intensity of extreme climatic events are reshaping agroecosystem stability and crop performance. In particular, recurrent and prolonged drought episodes represent a critical constraint for water-stress-sensitive crops such as eggplant, disrupting physiological processes, impairing biomass accumulation, and ultimately compromising yield stability and fruit quality (Plazas et al., 2022). Eggplant ranks among the most economically and nutritionally important solanaceous vegetable crops cultivated worldwide (Yu et al., 2026). It constitutes a dietary staple for numerous populations, particularly across Africa, the Middle East, Southeast Asia, and subtropical regions including India, Bangladesh, and Central America (Chapman, 2019). In

addition, its cultivation extends to warm temperate zones such as the Mediterranean basin and the southern United States (Mat et al., 2020). As a high-value horticultural crop, eggplant harbors extensive and largely underexploited genetic diversity, representing a substantial reservoir for crop improvement. Addressing contemporary production constraints and enhancing resilience to climate change will require a paradigm shift toward advanced, strategically integrated breeding approaches capable of delivering qualitative genetic gains (Gramazio, 2023).

This study was designed as a comprehensive literature-based analysis aimed at systematically evaluating the global body of scientific evidence related to the research topic. Relevant publications were identified through structured searches of major academic databases, including Google Scholar, Web of Science, PubMed, ScienceDirect, MDPI, Frontiers, and ResearchGate, among others.

Only peer-reviewed articles published in reputable scientific journals within recent years were considered to ensure methodological rigor and relevance. An initial pool of more than 300

publications was screened according to predefined inclusion criteria, including scientific quality, topical relevance, and data transparency. In addition, secondary statistical data were retrieved from FAOSTAT (Food and Agriculture Organization of the United Nations). These datasets were systematically processed, statistically analyzed, and interpreted to support and contextualize the findings derived from the literature review.

This paper intends to highlight the importance of genetic diversity in eggplants (*Solanum melongena*) and how it can be effectively used in breeding programs to obtain varieties with high productivity, increased tolerance to biotic and abiotic stresses, and greater adaptability to conditions generated by climate change, in order to develop high-performing and sustainable cultivars for modern agricultural systems.

RESULTS AND DISCUSSIONS

Solanum melongena (eggplant), *Solanum aethiopicum* (scarlet eggplant) and *Solanum macrocarpon* (gboma eggplant) represent the three principal cultivated eggplant species distributed across Europe, Asia, and Africa. These taxa exhibit substantial phenotypic diversity, primarily distinguished by fruit skin color, shape, size, and weight. However, environmental influences, onset of fruiting, and biochemical composition further contribute to the development of morphological variability and diversification among cultivated forms (Mishra et al., 2024).

Eggplant breeding programs have historically focused on elucidating the genetic architecture of adaptive traits, particularly through the exploitation of wild relatives as reservoirs of beneficial alleles (Oladosu et al., 2021). Despite their recognized value, the utilization of wild germplasm has been constrained by limited genomic resources, including incomplete genome sequence information, which hampers efficient introgression strategies (Gramazio et al., 2018). This limitation is particularly significant given that resistance to biotic and abiotic stresses is frequently derived from wild species (Dempewolf et al., 2017).

In recent years, substantial progress has been achieved through the development of multiple mapping populations derived from interspecific

crosses between cultivated eggplant and its wild relatives (Kaushik et al., 2016). These efforts have enabled the successful introgression of resistance traits, including Fusarium wilt resistance from *Solanum aethiopicum* (Toppino et al., 2008) and Verticillium wilt resistance from *Solanum linnaeanum* into cultivated eggplant backgrounds (Liu et al., 2015).

Current Insights into the Origin and Domestication of Eggplant

The genus *Solanum*, comprising 13 major clades, represents one of the most taxonomically and morphologically diverse lineages among angiosperms. Eggplant is assigned to the *Leptostemonum* clade (Knapp et al., 2013), commonly referred to as the “*Solanum spinosum*” group due to the presence of prominent epidermal spines on stems and leaves (Vorontsova et al., 2013).

Although eggplant is widely regarded as a vegetable crop of Asian origin, most of its wild relatives are distributed across Africa (Weese & Bohs, 2010). Archaeobotanical evidence indicates a long history of eggplant cultivation in both India and China, suggesting that the exploitation of wild progenitors may have begun in India prior to its introduction into China. In addition, an independent center of domestication has been proposed in the Philippines (Meyer et al., 2012). It has been hypothesized that certain local landraces may represent some of the earliest domesticated forms.

Subsequent molecular analyses have provided further insights into the domestication history of eggplant. Meyer et al. (2012) demonstrated that eggplant underwent multiple domestication events. More recent evidence indicates that domestication occurred independently on several occasions from *Solanum insanum*, a wild species naturally distributed throughout tropical Asia, from Madagascar to the Philippines (Knapp et al., 2013). Nevertheless, despite advances in phylogenetic and genomic research, important questions concerning the domestication process remain unresolved. (Taher et al., 2017).

The identification and taxonomic delimitation of wild relatives of eggplant remain challenging due to the extensive diversity within the genus. However, the integration of recent genetic and phenotypic datasets, along with the

classification of primary, secondary, and tertiary gene pools and improved knowledge of domestication pathways and wild progenitors, has substantially enhanced the potential for utilizing these resources in breeding programs (Taher et al., 2017).

Archaeological findings further corroborate the historical significance of eggplant. Fuks et al. (2020) reported the presence of mineralized plant remains, including seeds of *Solanum melongena*, recovered from excavations of an 8th–9th century CE Abbasid market in Jerusalem, providing material evidence of its cultivation and trade during the early medieval period.

Eggplant in the Global Agri-Food Economy

Eggplant (*Solanum melongena* L.) is a horticultural crop of considerable global economic relevance. Approximately 94% of worldwide production is concentrated in the subtropical regions of Asia, where its widespread cultivation and culinary prominence have earned it the designation “queen of vegetables” (Caruso et al., 2017).

According to preliminary statistics and recent reports derived from the FAOSTAT database of the Food and Agriculture Organization of the United Nations (FAO), global eggplant production reached record levels in recent years. Production totaled 58.70 million metric tons in 2021, followed by a 1.0% increase in 2022 compared with the previous year, further consolidating eggplant’s status as a major crop, particularly within Asian agricultural systems (FAO, 2023).

As illustrated in Figure 1, China remains the leading producer, with 39.244 million tons, followed by India with 12.792 million tons and Egypt with 1.859 million tons.

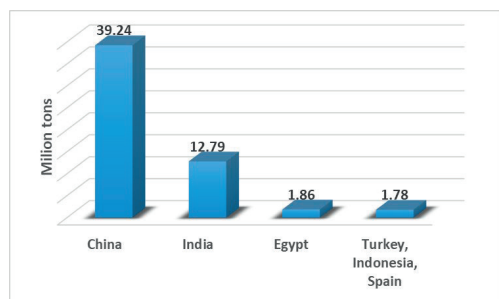


Figure 1. Top producers of eggplant

Among the subsequent major producers, Turkey (817.59 thousand tons), Indonesia (699.89 thousand tons), and Spain (260.41 thousand tons) rank prominently in global production statistics (Al-Salmi et al., 2025).

Nutritional and Functional Traits as Targets for Genetic Improvement

Eggplant (*Solanum melongena* L.) represents not only a crop of agronomic importance but also a valuable source of nutritional and bioactive compounds, making its health-related attributes a key consideration in breeding programs aimed at sustainable improvement. The fruit is characterized by low caloric content and appreciable levels of vitamins, minerals, and diverse phytochemicals, including phenolic compounds and flavonoids (Taher et al., 2017; Al-Salmi et al., 2025). These nutritious qualities make eggplant a crucial part of diets focused on health and justify its inclusion among priority crops for quality-oriented genetic enhancement. Wild relatives within the *Solanum* genus constitute an important reservoir of bioactive metabolites, particularly saponins and steroidal alkaloids, which have demonstrated significant pharmaceutical potential (Oladosu et al., 2021). The exploitation of these genetic resources provides opportunities to broaden the phytochemical profile of cultivated eggplant through introgression breeding and pre-breeding strategies. Comprehensive phytochemical analyses have identified numerous functional compounds in eggplant fruits and leaves, including aspartic acid, tropane alkaloids, flavonoids, lanosterol, gramisterol, steroidal alkaloids, glycoalkaloids, histidine, nasunin, oxalic acid, solasodine, ascorbic acid, and tryptophan (Naeem et al., 2019). The enhancement and stabilization of such traits through molecular breeding and selection approaches may contribute to the development of nutritionally superior cultivars.

In addition to their nutritional value, eggplant-derived compounds exhibit documented bioactivities relevant to human health. Extracts have been shown to exert stronger cytotoxic effects on cancer cells than on normal cells (Afshari et al., 2016), while chlorogenic acid demonstrates anticarcinogenic activity by inducing apoptosis in leukemia and lung cancer cell lines (Tajik et al., 2017). Antimicrobial

activity against pathogens such as *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Vibrio cholerae*, *Pseudomonas* spp., and *Bacillus cereus* has also been reported (Ahmed et al., 2016). These functional properties highlight the potential of breeding strategies aimed at increasing specific phenolic compounds and secondary metabolites without compromising agronomic performance. Furthermore, the contribution of eggplant to cardiovascular health, together with its content of vitamins, carbohydrates, and proteins, reinforces its value as a functional food (Eyid Abbas et al., 2021). The integration of nutritional quality traits into breeding objectives aligns with contemporary approaches to sustainable crop improvement, where yield stability, stress tolerance, and enhanced health-promoting properties are addressed simultaneously. Collectively, the nutritional and pharmacological attributes of eggplant emphasize the strategic importance of conserving and utilizing genetic resources to develop cultivars that meet both agronomic and public health goals.

Functional Metabolites in Eggplant: Implications for Breeding Strategies

Eggplant synthesizes a diverse array of primary and secondary metabolites, as summarized in Table 1. These phytochemicals represent a significant source of bioactive molecules with applications in both conventional and modern medicine, contributing to the prevention and management of various human diseases. Among these compounds, chlorogenic acid (5-*O*-caffeoylquinic acid; CGA), predominantly localized in the fruit peel, is one of the major phenolic constituents of eggplant. CGA has been reported to exhibit anti-obesity, anti-inflammatory, anti-diabetic, and cardioprotective activities, underscoring its relevance for functional food development and nutraceutical applications (Plazas et al., 2013). In this context, the integration of metabolomic approaches into plant breeding programs has gained increasing importance, as it enables the identification, quantification, and selection of desirable phytochemical traits in cultivated germplasm (Oladosu et al., 2021).

Table 1. Metabolites produced by eggplant

Type	Description	Role	Literature
Primary metabolites	vitamins, lipids, proteins, and carbohydrates	play a direct role in regulating plant growth and essential physiological functions	Mistry et al., 2016
Secondary metabolites	phenols, glycoalkaloids (solasodine), carotenoids, anthocyanins, and antioxidants	are essential for maintaining physiological homeostasis and conferring protection against biotic and abiotic stressors.	Naeem et al., 2019

Growing consumer demand for health-promoting foods has increased interest in specific secondary metabolites. Mansurat et al. (2023) report that health-conscious consumers prioritize phenolic content, glycoalkaloids,

carotenoids, and antioxidant capacity (Table 2). Therefore, the characterization and enhancement of these bioactive compounds have become key objectives in modern eggplant breeding strategies.

Table 2. Secondary metabolites from eggplant

Type	Description	Literature
Phenols	Eggplant (<i>Solanum melongena</i>) possesses the highest phenolic content within the Solanaceae family, establishing it as a primary dietary source.	Helmja et al., 2007
	Intravarietal variation in phenolic profiles among local cultivars provides critical insights for the development of targeted breeding programs.	Oladosu et al., 2021
	Polyphenolic composition in eggplant is strongly influenced by cultivar genotype, environmental conditions, agronomic practices, developmental stage, and specific fruit tissues.	Mansurat et al., 2023
	Seasonal fluctuations markedly affect phenolic levels, reporting a pronounced decline from spring to summer, suggesting that elevated temperatures inhibit phenolic biosynthesis.	
	Seasonal fluctuations markedly affect phenolic levels, reporting a pronounced decline from spring to summer, suggesting that elevated temperatures inhibit phenolic biosynthesis.	García-Salas, et al., 2014
	Eggplants are enriched with a diverse spectrum of phenolic compounds, including anthocyanins, rutin, naringin, naringenin, syringic acid, caffeic acid, chlorogenic acid, p-coumaric acid, and sinapic acid.	Mansurat et al., 2023
Carotenoids	Dark purple cultivars exhibit superior antioxidant capacity compared with white and green variants, despite the latter occasionally presenting higher total phenolic content.	Mansurat et al., 2023
	Carotenoid accumulation in eggplant is modulated by multiple factors, including post-harvest handling, environmental stress, developmental stage, and culinary processing, as these compounds are dietary essentials that cannot be synthesized endogenously in humans.	Sánchez-Maldonado et al., 2016; Linnewiel-Hermoni et al., 2015
	Storage at 0 °C effectively preserves carotenoid content, with the highest levels observed at early stages of fruit maturity, followed by a gradual decline throughout ripening.	Zaro et al., 2014
	Dietary intake of carotenoid-rich foods is strongly correlated with a reduced risk of certain malignancies.	Linnewiel-Hermoni et al., 2015
	Consuming carotenoid-rich diets, including eggplant, contributes to the prevention of vitamin A deficiency, particularly in pediatric populations.	Kamga et al., 2013; Mishiba et al., 2020
Glycoalkaloids	Glycoalkaloids play a critical role in enhancing plant defense mechanisms against pests and pathogens.	Zaro et al., 2014
	Eggplant-derived glycoalkaloids exhibit notable anticarcinogenic properties.	Shen et al., 2017
	The principal steroidal glycoalkaloids in eggplant, α -solanine and α -solanoside, exert anticancer activity by reducing cancer cell proliferation across multiple malignancies, including basal cell carcinoma, osteosarcoma, lung, liver, leukemia, and gastric cancers.	Gürbüz et al., 2018 & Shen et al., 2017
Antioxidant compounds	Eggplant ranks among the top ten antioxidant-rich vegetables out of 120 evaluated species.	Nisha et al., 2009
	Total antioxidant content ranges from 2664 to 8247 mmol Trolox/kg, influenced by cultivar, fruit size and shape, skin pigmentation, and post-harvest storage conditions.	Gürbüz et al., 2018

Morpho-Physiological Assessment of Eggplant Germplasm for Breeding and Conservation

Eggplant (*Solanum melongena* L.) exhibits considerable diversity in fruit shape, color, and productivity (Figure 2) (Yurlisa et al., 2019).



Figure 2. Representative variety of eggplant fruit

Due to its greater economic importance compared with the native African species *Solanum aethiopicum* L. and *Solanum macrocarpon* L., *S. melongena* has been the primary focus of genetic and breeding research on diversity and interspecific crossability (Daunay et al., 2019).

At the germplasm access level, gene banks provide detailed information on phenotypic traits, including plant growth habits, branching patterns, leaf, flower, and fruit morphology, plant height, as well as flowering and fruiting periods (Barchi et al., 2019). Morpho-physiological evaluation and characterization of

available germplasm for target traits represent a fundamental component of eggplant breeding programs (Musa et al., 2020). These morpho-physiological traits facilitate rapid and accurate discrimination of phenotypic variation, which is typically highly heritable and moderately influenced by environmental conditions (Kumar et al., 2008).

In response, organizations such as the European Cooperative Program for Plant Genetic Resources (ECPGR), Bioversity International, and the International Union for the Protection of New Varieties of Plants (UPOV) have developed standardized descriptors and methodologies for eggplant characterization (Prohens et al., 2012; Hurtado et al., 2012).

Morpho-physiological assessments, which are essential for the sustainable management of genetic resources, are classified into two categories:

- Primary characterization: involves the quantification of readily observable traits at multiple growth stages, including the vegetative phase, inflorescence descriptors, maturity stage, and germination/seedling phase.

- Secondary morphological characterization: encompasses additional complex agronomic traits, such as biochemical composition, yield potential, fruit set, and resistance to pests and diseases (Musa et al., 2020).

These assessments provide the foundation for effective utilization and conservation of eggplant genetic resources in breeding programs.

Breeding Approaches for Sustainable Eggplant Improvement

With advances in breeding technologies and artificial selection, eggplants are increasingly cultivated for enhanced yield and fruit quality. However, as varietal uniformity improves, the genetic diversity of cultivated germplasm is progressively narrowing (Mohanty et al., 2023). Wild relatives, representing native populations, remain a critical reservoir of genetic variation, harboring genes conferring resilience to biotic and abiotic stresses that have been naturally selected over millennia, making them valuable for improving cultivated varieties (Yu et al., 2026).

Eggplant breeding has historically focused on enhancing productivity and improving resistance to diseases and pests while optimizing

fruit quality (Daunay et al., 2007). Over time, domestication and selection have undergone multiple stages of refinement, resulting in modern cultivars that significantly outperform local landraces in terms of yield and overall agronomic performance (Mishra et al., 2024). Despite the crop's global cultivation, relatively limited effort has been directed toward exploiting local germplasm for hybrid development compared with other solanaceous crops such as tomato and potato (Mistry et al., 2016).

Current eggplant cultivars have largely been developed using the genetic resources of cultivated *S. melongena*, which have facilitated the creation of improved selections, breeding lines, and F1 hybrids (Solberg et al., 2022; Gramazio et al., 2023). Intensive breeding programs in regions of extensive cultivation, including China, India, Bangladesh, Western Europe, Turkey, and Japan, have yielded numerous open-pollinated and F1 hybrids exhibiting distinct phenotypic traits (Şekara et al., 2007). However, the reliance on cultivated germplasm has narrowed the genetic base of elite breeding lines; for instance, Muñoz-Falcón et al. (2009) reported that contemporary F1 hybrids of black eggplant share a common genetic origin and display reduced diversity.

The potential of wild *Solanum* species in breeding programs has been largely underutilized, primarily due to interspecific incompatibilities that often manifest as hybrid sterility (Laura et al., 2025; Bletsos et al., 2004). Nonetheless, biotechnological approaches, including genetic transformation, protoplast fusion, somatic hybridization, and haploidization, have enabled the transfer of desirable agronomic traits from wild relatives into cultivated backgrounds (Oladosu et al., 2021). Interspecific hybridization remains a critical strategy for broadening the genetic base of farmed eggplants and developing novel cultivars with enhanced stress tolerance, with some hybrids also exhibiting heterosis (Laura et al., 2025).

Somatic hybridization has been investigated as a tool to overcome cross-incompatibility barriers and integrate agronomically valuable traits from wild species into *S. melongena*. While successful in producing hybrids with desired characteristics, sterility and the tetraploid nature

of somatic hybrids often limit their practical application in breeding programs (Iwamoto et al., 2007; Rizza et al., 2002; Rotino et al., 2005). Marker-assisted selection (MAS) has emerged as a powerful tool to accelerate breeding cycles and enhance selection efficiency, thereby reducing the time required for crop improvement (Chen et al., 2024; Yu et al., 2026).

Some wild species exhibit superior tolerance to abiotic stress compared with cultivated forms. For example, García-Forte et al. (2019) demonstrated that *Solanum elaeagnifolium* possesses a more extensive root system than *S. melongena*, conferring improved drought tolerance. Despite such promising traits, introgression from wild species remains challenging (Adithya et al., 2025). Conventional breeding approaches are time-consuming and labor-intensive, often limiting their effectiveness in generating cultivars with enhanced pest and abiotic stress resistance (Alam et al., 2021). Consequently, most breeding initiatives continue to rely heavily on cultivated *S. melongena* germplasm, despite significant advances in breeding strategies (Gramazio et al., 2023).

Compared with other major vegetable crops such as tomato, eggplant lags in both genetic improvement and the effective utilization of allied species' genetic resources, highlighting the need for innovative strategies to expand the genetic base and accelerate sustainable improvement (Schouten et al., 2019).

Worldwide Collection and Preservation of Eggplant Genetic Resources.

According to the latest Global Strategy for the Conservation and Utilization of Eggplants, a total of 110 germplasm banks and collections worldwide maintain 19,020 accessions of cultivated eggplants and related species (Solberg et al., 2022). The most extensive collections are held by several key institutions, including the National Plant Genetic Resources Office in India, the World Vegetable Center in Taiwan, which conserves 3,036 accessions, the National Gene Bank for Plant Germplasm Resources in China (1,601 accessions), the INRAE Gene Bank in France (2,388 accessions), and the NARO Gene Bank in Japan (1,501 accessions) (Salinier et al., 2022; Solberg et al., 2022).

Pest and Disease Constraints in Eggplant Breeding

A major challenge in contemporary eggplant breeding is the development of cultivars exhibiting enhanced resistance or tolerance to prevalent pests and diseases, which can result in yield losses of up to 100% (Kole et al., 2020; Vezzulli et al., 2022). The high genetic complexity among *Solanum* species complicates the identification of beneficial local variants and the improvement of cultivated lines with enhanced productivity and biotic stress resistance. Advances in biotechnological tools and methods have facilitated the genetic enhancement and ideotype development of eggplant (Mishra et al., 2024).

Although cultivated eggplants are susceptible to a range of bacterial and fungal pathogens, many wild relatives possess natural resistance, underscoring the importance of elucidating the genetic basis of disease resistance for breeding programs (Oladosu et al., 2021). Among diseases, bacterial wilt caused by *Ralstonia solanacearum* is particularly detrimental, especially in tropical regions, often necessitating the use of resistant rootstocks to sustain production (Lebeau et al., 2013; Barik et al., 2020; Namisy et al., 2019). Other economically significant diseases affecting eggplant include *Verticillium* and *Fusarium* wilts, as well as damage caused by root-knot nematodes (*Meloidogyne* spp.) (Vezzulli et al., 2022).

Pests represent another primary biotic constraint in commercial eggplant production. Major insect pests include mites, whiteflies, aphids, and the hadda beetle (*Henosepilachna vigintioctopunctata* Fabricius), while nematodes - particularly root-knot nematodes - pose serious challenges in both greenhouse and open-field cultivation (Nayak et al., 2021; Caliskan et al., 2023). The genetic basis of nematode resistance in eggplant remains poorly understood, highlighting an important gap for future breeding efforts aimed at sustainable pest management (Caliskan et al., 2023).

CONCLUSIONS

Eggplants contain essential primary metabolites for growth and secondary metabolites with significant nutritional, pharmaceutical, and stress-resilience roles. Evidence of their activity

against cancer cells and pathogenic bacteria highlights their health-promoting potential, emphasizing the need to integrate metabolomic approaches in breeding programs.

The conservation and strategic use of genetic resources, particularly wild species, remain critical for developing high-yielding, stress-tolerant, and locally adapted varieties. Modern breeding tools, including interspecific hybridization, somatic hybridization, marker-assisted selection, and genetic transformation, can overcome cross-compatibility barriers and expand the genetic base of cultivated eggplants. Enhancing resistance to pests and diseases is essential for sustainable production, reducing reliance on chemical controls and securing the global food supply. International initiatives such as the Global Crop Diversity Trust's climate adaptation programs illustrate the value of global collaboration in improving conservation and utilization of eggplant germplasm (Dempewolf et al., 2014; Eastwood et al., 2022; Gramazio, 2023). Continued coordinated efforts are required to fully realize the potential of these genetic resources in sustainable eggplant agriculture.

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LEG(O)NEST - OPTIMIZING THE VEGETABLE AGRI-FOOD SYSTEM THROUGH THE BROAD APPLICATION OF RESEARCH AND DEVELOPMENT RESULTS TO STRENGTHEN FARMERS' COMPETENCIES

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Abstract

The diversity of life on Earth is the foundation of agriculture - from the food we eat to the ecosystem 'services' that sustain our existence. The loss of biodiversity can have a catastrophic impact on economic prosperity, agriculture, and other aspects of life. Biological resources are an essential contribution to the country's economy, enhancing the sustainability of agri-food products. The genetic base of today's crops is alarmingly narrow, posing a threat to food security for a growing population. Current aggressive agricultural practices and the excessive use of synthetic inputs severely endanger biodiversity, leading to genetic uniformity. Modern, imported varieties have replaced local varieties and populations in traditional agro-ecosystems, resulting in increased genetic vulnerability to diseases and pests. Leg(O)Nest proposes a model to promote biodiversity development through: identifying new genes/traits that enhance adaptation to specific cultivation conditions; identifying traits that ensure suitability for organic farming systems; developing new high-yielding varieties with a broad genetic base; enhancing the value of a wide range of newly created varieties in the agri-food chain.

Key words: vegetables, plant genetic resources, varieties, resilience, use.

INTRODUCTION

It is well known that Romania, including the North-East region, is increasingly becoming merely a market for vegetable imports; that farmers struggle to access supermarket chains; and that protected cultivation spaces (greenhouses, tunnels) are severely insufficient, even though implementing such systems and specific technologies would significantly increase the capacity to supply raw materials throughout the year. Moreover, the availability of high-quality seed material, characterised by tolerance/ resistance to different stressors, capable of performing under specific climatic conditions and meeting the nutritional and organoleptic expectations of consumers as well as the productivity needs of growers, is a recurrent necessity expressed by both farmers and consumers.

Leg(O)Nest represents a Regional and National Initiative to Support Romanian Research Competitiveness, materialized in a project cofunded by the European Union, Romanian Government, implemented through the Regio

Nord Est Programm 2021-2027, ADR NORD EST. The initiative entitles the Research Centre for the Smart, Integrated and Multi-Purpose Use of Plant Genetic Resources Across the Agri-Food Chain, for the Benefit and with the Involvement of the Community.

This work represents a positioning paper, and it has as an objective the communication related to the project potential at the regional and national level. The Leg(O)Nest consists of two major components: research and **investment** in infrastructure, the second one being major in terms of allocated funds. It is considered to have potential beyond regional borders, being designed to reinforce research for vegetable production in different systems, as organic and conventional, open-field and protected systems, aiming for a solid impact on the national economy. Even though the focus is on organic agriculture, the developed solutions and materials are not restricted to it, contrary, they are designed for wide application also in conventional systems.

The objectives are clearly harmonized and linked to national and European legislation,

especially when approaching the organic sector, which is more restrictive and resources demanding. The European Commission has established several instruments, including the European Action Plan for Organic Production Development (2021), which is structured around three pillars: consumption-production- sustainability. All these pillars are addressed in the research framework proposed by the Leg(O)Nest center. The project's implementation aims to achieve the targets of the Farm to Fork and Biodiversity strategies, which include reducing the use of chemical fertilizers, pesticides, nutrient losses, and greenhouse gas (GHG) emissions, while also forecasting an increase in agricultural and organic farming areas.

Leg(O)Nest will work to strengthen a scientific framework in which farmers have access to biological materials and know-how to facilitate and motivate adherence to organic agriculture and performance in conventional systems by exploiting agroecological practices. The main challenges in implementing organic agriculture are related to insufficient financial incentives for conversion, confusion with other quality schemes, and low awareness of the benefits of organic production for health, climate, and the environment.

THE INTERNATIONAL AND NATIONAL CONTEXT

Strategies and legislation

Leg(O)Nest proposes a robust intervention in the productive sector through scientific initiatives, while also engaging in awareness-raising, information, and education activities for consumers across different age groups and educational levels.

The project will draw on key references, including: The European Green Deal (2019), The European Commission's Communication to the European Parliament on the Action Plan for Organic Production Development (2021), translated into Romania's National Action Plan for Organic Production Development (2021), The Farm to Fork Strategy (2020), alongside the upcoming Zero Pollution Action Plan for Water, Air, and Soil (2021), The Circular Economy Action Plan (2020), The EU Biodiversity Strategy for 2030 (2021), Regulation 848/2018

on Organic Production and Labeling of Organic Products (2018). From these international documents, transposed into the national legislation of member states, we highlight the following European objectives and approaches to which Leg(O)Nest actively contributes:

The Commission's goal is to achieve 25% of organic agricultural land by 2030 and 100% use of organically certified seeds, which also involves supplying farmers with seeds produced under organic conditions. Consequently, there must be more land and dedicated solutions for organic seed production.

The New Action Plan for Organic Agriculture (2021-2027) forecasts remarkable growth in the organic sector over this decade, encouraging farmers to transition to organic farming, thereby increasing the accessibility of organic food and closing the gap between the status quo scenario and the "additional effort" needed to reach the 25% target by 2030.

The Commission aims to increase the share of research and innovation (R & I) and to dedicate at least 30% of the R & I budget in agriculture, forestry, and rural areas to actions with specific or significant relevance to the organic sector. Research will focus on, among other things, changing farmer and consumer behavior, higher crop yields, genetic biodiversity, and alternatives to controversial products.

Biodiversity loss can have a catastrophic impact on our economic prosperity, agriculture, and other aspects of our lives. Biological resources are essential for the EU economy, contributing to the sustainability of agri-food products, according to the EU Biodiversity Strategy for 2030.

Since 2021, greater emphasis has been placed on organic production, among the themes targeted by the annual calls for proposals for information measures regarding the CAP (EU (COM(2021), 141 final).

Since 2022, data on the environmental, economic, and social benefits of organic agriculture have been continuously collected, and citizens and farmers are informed about these benefits through more intensive use of social media platforms. Since 2022, the Commission has measured consumer awareness of the EU organic logo to monitor progress since the 2020 Eurobarometer survey (EU (COM(2021), 141 final).

Reorienting the current EU food system toward a sustainable model, ensuring food security and safety, providing sufficient, affordable, and nutritious food, and promoting healthier and more sustainable food consumption and diets, according to the Circular Economy Action Plan: For a cleaner and more competitive Europe (2020).

Concrete actions are needed across the entire food chain, from production to consumption. The aim is to align food production with environmental protection while stimulating investment and sustainable production.

In its resolution of January 15, 2020, on the European Green Deal, the European Parliament emphasized that agriculture has the potential to contribute to reducing EU emissions through sustainable practices such as organic farming.

In its conclusions on the Farm to Fork Strategy, the Council highlighted the role of organic products in a sustainable food system. The strategy comprehensively addresses the challenges of sustainable food systems, recognizing the links between healthy people, healthy societies, and a healthy planet. It challenges the development of a sustainable food system capable of improving primary producers' incomes, strengthening sector competitiveness, and ensuring sufficient, healthy, and financially accessible food for all consumer categories.

Agriculture is both the primary source of food and a significant polluter through soil cultivation, pesticide residues in soil, water, and products. The use of chemical pesticides in agriculture contributes to soil, water, and air pollution, biodiversity decline, and can harm non-target plants, insects, birds, mammals, and amphibians. Excess nutrients (especially nitrogen and phosphorus) in the environment, resulting from overuse, are another major source of pollution with climate impacts. Organic agriculture has a positive impact on biodiversity (biodiversity in organic fields is 30% higher than in conventional fields), creates jobs, and attracts young farmers. From a niche sector, organic agriculture has become a priority for decision-makers and consumers who recognize its value as an opportunity to improve lifestyles, health, and the environment. Sustainable food systems depend on seed security and diversity. The registration of seed varieties, including for organic agriculture, and easier market access for

traditional and locally adapted varieties are essential conditions for creating a functional, competent vegetable production system, as highlighted by the EU. Research must provide viable solutions to ensure the success of national action plans targeting the organic sector, and member states must build the necessary capacity for their implementation.

The current size of the sector in which the project's specific activity is carried out

The organic farming sector in the European Union has expanded rapidly in recent years in terms of agricultural areas under organic management, the number of organic farms, and the total number of registered organic operators in the Union. The area used for organic farming has increased by almost 66% over the last decade, rising from 8.3 million hectares in 2009 to 13.8 million hectares in 2019. At present, this area represents 8.5% of the EU's total utilized agricultural area. This growth in organic farmland has been accompanied by a substantial increase in retail sales, which have doubled in value over the last decade, from approximately EUR 18 billion in 2010 to over EUR 41 billion in 2019.

Romania has enormous potential for the development of this sector. Although it ranks among the top 10 European countries in terms of certified organic areas (Figure 1) - including grasslands, arable land, and perennial crops - a significant gap is still noticeable.

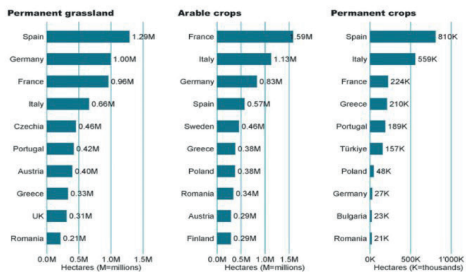


Figure 1. Top 10 countries - certified surfaces

In Romania, in 2022, the total area under organic farming amounted to 644,519.69 ha, of which 221,743.15 ha (34%) was in conversion and 422,776.55 ha (66%) was fully certified organic (Figure 2). According to FiBL 2024 data, in 2022, only 177 ha were dedicated to organic vegetable cultivation.

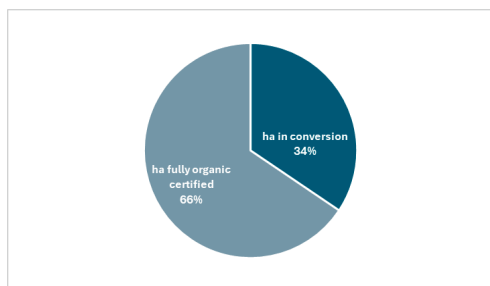


Figure 2. Distribution of areas: organic and conversion cultivation

The total number of registered organic operators in 2022 (Figure 3) included 13,260, as follows: 12,598 agricultural producers (including beekeepers), 203 processors, 502 traders, 25 importers, 25 exporters, and 6 aquaculture production units (MADR, 2024).

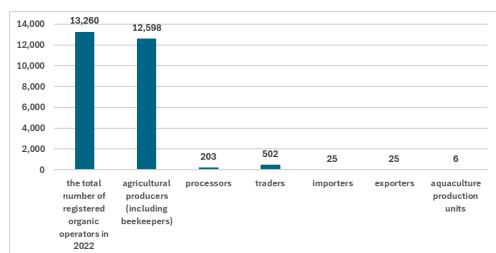


Figure 3. The total number of registered organic operators in 2022

Trends in national and international developments in the field targeted by the project

For the 2023-2027 programming period, the financial allocation for interventions targeting organic farming within the CAP Strategic Plan (SP) 2023-2027 amounts to EUR 389.12 million, of which EUR 162.6 million are dedicated to the intervention supporting commitments during the conversion period, and EUR 226.52 million to the intervention supporting commitments during the maintenance period of organic farming practices.

The *National Action Plan for the Development of Organic Production* of the Ministry of Agriculture and Rural Development (MADR) sets out the following national objectives for the development of organic agriculture:

- Increasing organically cultivated areas and the production of high-value organic products.
- Increasing and diversifying organic production, especially in sectors well

developed in conventional farming but poorly represented in organic farming (vegetables, fruits, milk, eggs, processed foods, etc.), including the creation of product chains.

- Achieving organic production aligned with consumption needs, at prices accessible to Romanian consumers.
- Integrating organic agri-food production into market supply chains.
- Advising and training farmers for the organic farming system.
- Developing local and regional networks to support the growth of organic agriculture and encouraging farmer associations.
- Encouraging and motivating farmers to obtain organic certification.
- Educating and informing consumers about certified organic products.
- Aligning research activities with the needs of the organic farming sector.
- Increasing consumer confidence by adapting control, prevention, and anti-fraud measures.

Statistical data on organic farming shows a general upward trend in the area under organic management. Considering the unpredictability of factors that may influence organic farming, as well as fluctuations in the number of operators, it can be estimated that the area under organic farming in 2030 could reach at least 800,000 ha, representing approximately 6% of the total agricultural area. Of this target, 488,325 ha represent the objective set by the CAP Strategic Plan 2023-2027 for supporting organic farming commitments.

Factors having a direct influence on the dynamics of the sector targeted by the project

- Economic factors have a determinant role in the adoption of the organic farming system (OF)
- Fiscal policy - fiscal incentives such as subsidies or tax reductions can encourage farmers to shift to organic practices. Clear fiscal policies supporting organic farming can make this system more financially attractive.
 - Investment in R & D - essential for improving agricultural techniques and practices. Research can lead to new pest-control methods, improved soil fertility, and increased productivity in organic farming.
 - Market access and international trade - a key factor; growing demand for organic products can motivate farmers to adopt organic practices to align with consumer preferences.

- Regulatory policy - government regulations on quality standards, labeling, and certification of organic products are crucial and must align with EU legislation. Clear and coherent policies build consumer trust and encourage farmers to comply with organic standards.
 - Education and awareness — investments in agricultural education and consumer awareness about the benefits of organic farming can be essential. Promoting knowledge about organic practices increases demand and encourages conversion.
 - Access to finance - policies that facilitate access to funding are key to supporting the adoption of organic farming.
 - Economic stability - a stable and predictable economy encourages investment in organic farming; economic instability discourages farmer engagement.
- b. Political factors - a favorable framework for organic farming can positively influence decisions by farmers and consumers.
- Subsidy and financial support policies - government subsidies and financial support for organic farmers are key factors. These incentives make conversion to organic farming more economically attractive.
 - Environmental and sustainability policies - policies that promote sustainable agricultural practices and natural resource protection influence decisions to adopt organic farming. These may include regulations on pesticide use, waste management, and soil and water conservation.
 - Public health policy - policies that encourage healthy diets and promote organic products can influence consumer demand, stimulating farmers to adopt organic practices.
 - Trade policy and market access - trade policies and free-trade agreements influence market access for organic products. Access to national and international markets demanding organic goods is a key driver.
 - R & D policy - government investment in research and development for organic agriculture can significantly impact the sector. Research improves techniques and practices, making organic farming more attractive and profitable.
 - Education and awareness policies - policies supporting agricultural education and consumer awareness about organic farming can increase demand and encourage conversion.
- c. Legislative factors - provide a solid legal framework for organic farming, sustainable practices, environmental protection, and consumer welfare.
- Regulations on pesticide use - restrict or prohibit chemical pesticides in organic farming, ensuring compliance with sustainable practices and protecting consumer and environmental health.
 - Legislation on labeling and certification - essential for guaranteeing that products labeled “organic” meet the required standards.
 - Waste management legislation - regulations encouraging recycling and composting reduce negative environmental impacts; proper management of organic and agricultural waste influences organic practices.
 - Soil and water protection regulations - important for organic farming; include measures to prevent soil erosion, ensure proper nutrient management, and protect water quality.
 - Market access regulations - include quality standards, labeling requirements, and certification procedures ensuring fair and transparent commercialization of organic products.
 - Consumer protection legislation - ensures the safety and quality of organic products through testing, monitoring, and traceability requirements.
 - Legislation on R & D - can support organic farming by allocating funds for research, improving techniques, and developing new sustainable technologies.
- d. Technological factors
- Must be adapted to organic farming conditions and require continuous R & D activities.
- Environmental monitoring and control – support farmers to make informed decisions on irrigation, fertilization, and pest control, improving resource efficiency; quantifying the benefits increases acceptance.
 - Irrigation and water-management systems - reduce water consumption and improve efficiency, contributing to resource

conservation and reduced environmental impact.

- Pest-control technologies - smart traps, monitoring systems, and biological control methods ensure efficient and sustainable pest management, reducing dependence on chemical pesticides.
- Soil-management systems - low-tillage practices, crop rotation, and intercropping enhance soil biodiversity and support informed decision-making.
- Waste-management technologies - organic waste can become a valuable resource through composting and anaerobic digestion, reducing waste and supplying nutrients to the soil.
- Data-management systems - help farmers collect, analyze, and interpret data on crops, soil, and environmental conditions to improve organic farming efficiency.
- Communication technologies and connectivity: internet access and communication tools facilitate information exchange, market access, and networking among farmers and experts.
- e. Social factors - play a decisive role in the adoption of organic farming.
- Consumer education and agricultural training.
- Informed consumers are more likely to choose organic products, stimulating demand and encouraging farmers to convert. Agricultural education helps farmers understand the advantages of organic farming and transition successfully.
- Consumer attitudes - positive attitudes toward organic farming and understanding its benefits motivate consumers to support and purchase organic products.
- Local culture and traditions - in some regions, strong traditions of sustainable agriculture facilitate the adoption and expansion of organic farming.
- Social movements and organizations - advocacy for sustainable agriculture can create strong social pressure on policymakers to support organic farming.
- Government policies - public health campaigns, subsidies for organic products in schools or hospitals, and food-safety regulations influence adoption.
- Cooperation and social networks - collaboration among farmers, researchers, and organizations supports the implementation of

organic farming through knowledge and resource sharing.

- Role of media and influencers - media coverage and influencers can significantly shape public perception; positive and educational messaging increases awareness and acceptance of organic farming.

LEG(O)NEST STRUCTURE AND VISION

Leg(O)Nest is a solid research infrastructure with immense potential for advancing the field of vegetable cultivation, high-value valorization of vegetables, and certified organic and conventional planting material, with an impact on food security, agriculture, as a response to climate change pressures, exploring judiciously the resources, and ensuring biodiversity conservation. The project responds to clearly identified market needs, extensively aligned with the regional challenges defined in the RIS3 strategy. The Leg(O)Nest infrastructure proposes an ambitious approach, starting from the discrepancy between the current status and the significant agricultural potential measurable in the performance that the agri-food sector in the North-East region could achieve through a real, efficient, and feasible alignment of research with the private sector (farmers, processors, retailers). The project addresses field/ market-identified needs:

- The imperative need to increase and improve vegetable production areas and yields through the development of productive, high-quality, resilient varieties suitable for cultivation in the region;
- Adapted and competitive practices and techniques for cultivation and for producing certified seed material;
- The necessity of consultancy and advisory services for vegetable farms;
- The need to increase added value through the development of valorization matrices/models; Specialized studies show that improving the performance of vegetable species in the context of combating the negative effects of climate change requires significant investments in plant breeding and in improving cultivation systems. There is a vast amount of genetic resources (GenRes) as old varieties, local landraces, crop wild relatives (CWR), cultivars, or materials with completely unknown biological status,

representing enormous variability that requires collection, characterization, evaluation, and conservation in order to be effectively valorized in various directions: the food industry, breeding, cultivation technology, organic agriculture, etc.

The Leg(O)Nest thematic framework adds value through activities carried out in experimental fields (TRL 2-4), progressing to the demonstration and validation of technologies under operational conditions (TRL 5-7), and culminating in (TRL 8-9) patent applications/variety registration and the full demonstration, implementation, and sustainability of the integrated, multi-purpose management of plant genetic resources.

To valorize and exploit previous breeding results (TRL 4-5), promising material - cultivars, varieties, and lines obtained through prebreeding and breeding, open-pollinated cultivars, will be investigated under specific organic and conventional farming conditions, where practices, methodologies, and specific inputs will be tested (TRL 6). Genetic resources will be phenotyped and genotyped to identify and demonstrate specific traits relevant for productive and qualitative performance (TRL 6). To exploit plant resistance (tolerance/resistance to biotic and abiotic stress), the genes responsible for these traits will be identified and investigated, enabling targeted and competitive use of the developed resources. High-value valorization models will be created, such as sprouts, food products, mulches, green manures, compost, and dedicated links. Protocols, tools, and guidelines will be developed to improve and standardize cultivation and harvesting processes, reducing losses and enhancing the quantitative and qualitative potential of vegetable species (TRL 6-7).

The economic impact will be strengthened by dedicated technological pathways for: exploiting the potential for multi-purpose food use, increasing yield and biomass, and reducing seed loss during maturation and harvesting.

Traits selected for the agri-food chain, based on market needs and preferences, will be identified (TRL 3-5), and the germplasm collection will be valorized and exploited thanks to its visibility and availability (TRL > 6).

Interference and potential impact in different segments of the agri food chain:

Contribution to strengthen food biosecurity and food safety, through:

- providing healthy, locally produced food resources, obtained through modern technologies adapted to regional specificities, by: (a) developing new varieties adapted to the agro-climatic conditions of the region, with superior nutritional profiles, high productivity, and a positive impact on balanced consumer development, with the potential to counteract the negative effects of low-quality food and unbalanced diets that lead to obesity and other nutrition-related diseases; (b) expanding the vegetable portfolio with neglected species and acclimatized species newly introduced into cultivation, with multiple potential uses.
- diversifying processed products based on natural principles to increase national nutritional quality and food security, ensuring independence from external markets. The COVID-19 pandemic and the conflict in Ukraine have highlighted the importance of robust and resilient EU food systems within a sustainable circular bioeconomy, capable of responding to global shocks and supply chain disruptions and of mitigating the socio-economic impact of crises, particularly food poverty.
- developing models for high-value valorization of secondary products resulting from seed production (pepper pulp, pumpkin, melon, physalis, tomato juice, root vegetables, etc.).
- creating good-practice models for vegetable producers and processors - safe processed products with high nutritional value.
- improving population nutrition by supplying high-quality indigenous raw materials, including organic products, as well as planting material for large-scale production.
- Expanding the product range by developing new types of products.
- Promoting and valorizing local varieties through the development of a pilot vegetable processing station (preserves: sauces, stews, pickled cucumbers, beans, long peppers, bell peppers, kapia peppers, cabbage, red beet, etc.), based on traditional recipes and customized methods within SCDL Bacău.
- Creating intelligent exploitation models aligned with the high expectations of

consumers and diverse dietary needs: minimally processed products (freeze-dried, dehydrated, sprouted), organic products.

- Using aromatic and spice species both for their intended culinary purpose and for their natural preservative potential.

The entire set of practices and measures developed - from biological material collection, use in breeding, creation of new cultivation technologies, and specific pathogen-control steps, to high-value food valorisation - has as its main goal biodiversity conservation, eliminating the negative effects of agriculture on the environment and human health.

Contribution to Smart Farming - Leg(O)Nest creates modern, alternative solutions to classical cultivation systems through the integration of advanced, automated technologies specific to protected cultivation, improving hydroponic, vertical, and container-based cultivation systems for high-value use of new varieties. One new greenhouse and two plastic tunnels (part of the research infrastructure) will ensure the implementation of research components.

Non-food uses of agricultural by-products

These uses bring added value to the vegetable sector and ensure efficient utilization of agricultural waste (plant residues). The aim is to identify new potential applications through models for high-value valorisation of secondary products from seed production, unsuitable for human consumption, redirected towards high-quality compost production;

As previously mentioned, the project consists of two convergent components: (1) Research and Innovation (2) Investments in infrastructure, which have as a common goal the creation of new capacities for the vegetable research domain in view to supporting and stimulating farmers' competitiveness. This will be feasible through the development of new solutions and cultivation schemes: intercropping, successive, vertical, container-based, new genetic resources: patented/ homologated and specific matrices for valorization in the agri food chain. Distinct RDI services that will be available through the new infrastructure include:

Variety and hybrid testing - evaluation of agronomic performance, including genetic resistance to specific pathogens. Testing in different systems (organic vs. conventional, protected vs. open field, fully controlled

environments). The novelty consists in the infrastructure's capacity to simulate different cultivation conditions, thus providing recommendations for growing varieties in specific areas, systems, and conditions.

Testing the quality of vegetable products - this service includes complex analyses aimed at validating the quality of the batches investigated. The categories of analyses will be diverse: physiological, morphological, organoleptic, biochemical, and genetic. Beyond quality validation, genetic analyses will contribute to ensuring traceability of materials and strengthening the organic certification process.

Assessment of capacity-potential and consultancy for implementing conversion towards organic certification - this service supports vegetable farms in evaluating their readiness and implementing the steps required for organic certification.

Training and professional specialization: professional training for vegetable growers and other economic operators in agriculture and rural development.

Educational networking and support for entrepreneurial initiatives - Activities aimed at fostering knowledge exchange, collaboration, and the development of new business ideas in the agri-food sector.

Seed multiplication services for higher biological categories - The newly created varieties developed in the project will be multiplied within the Leg(o)Nest infrastructure and transferred to beneficiaries for further multiplication and valorization.

Testing of certified inputs for the organic system - This service is offered both to farmers and to input producers, with the purpose of evaluating the effectiveness of these products in organic vegetable production.

Related Research and Innovation agenda, the project aims to create a functional and complete approach in terms of exploring diversity for the benefits of breeding and along agri-food chains. Four convergent themes with specific tasks and objectives will be implemented. The starting point of our initiative, materialized in Theme 1 aims to capture, investigate, and catalogue functional diversity to ensure an effective agroecological transition. This involves creating a broad and functional structure of vegetable

species by increasing the number of accessions/genetic resources (collected, inventoried, or obtained through exchanges) and by developing tools that enable the beneficial exploitation of this material.

Theme 2. Developing modern ecological practices to ensure the sustainability and resilience of the vegetable production system in line with circular economy principles, includes extensive activities aimed at developing new cultivation practices and modernizing certain technological steps for growing vegetables in protected spaces and in open fields, under ecological systems (without excluding conventional systems). These actions will ensure increased competitiveness of ecological vegetable production, reduced constraining effects of climate change on vegetable cultivation, **improved** technologically underdeveloped production capacities, more efficient use of resources, and reduced negative environmental impacts of vegetable production practices.

Theme 3. Strengthening the national vegetable germplasm heritage through innovation in breeding and seed production - has tremendous importance. This theme will support the development of new varieties with high cultivation potential and diverse valorization along the agri-food chain, the introduction of new varieties into cultivation, and the creation of specific technological steps for producing certified seed and planting material.

Theme 4. Developing value-added pathways for ecological plant resources. This theme has a strong economic impact and creates opportunities for valorizing research and development results in health and nutrition, contributing to well-being, generating ideas, and enabling partnerships and new activities in the agri-food sector. The results may also support the creation of regional eco-districts that strengthen local traditions and regional identity, with potential implications in related fields such as tourism revitalization and promoting a healthy lifestyle.

CONCLUSIONS

Leg(O)Nest is a new research infrastructure that proposes a model to promote agrobiodiversity and to ensure the development of the vegetable sector.

Leg(O)Nest plans to develop an innovative model of sustainable agriculture, grounded in agroecological principles and based on crop diversification, expanded use of plant genetic resources, and adaptable cultivation systems and solutions. The model is designed for broad applicability, including within conventional agriculture, and relies on participatory engagement of stakeholders across the agri-food sector.

This project ensures environmental benefits through: breeding for climate-adapted crops; developing environmentally friendly tools for exploiting new vegetable resources; conserving and exploring biodiversity; developing environmentally friendly methods, practices, and technologies for both conventional and organic cultivation, aimed at reducing the negative impact of agriculture; increasing soil fertility through the use of leguminous species; reducing synthetic inputs by developing intelligent cultivation schemes. Creation of adapted cultivation schemes and new varieties, identifying new genes/traits that enhance adaptation to specific cultivation conditions, are key to a successful system able to respond to the continuous pressure of climate change. Identifying traits that ensure suitability for organic farming systems, developing new high-yielding varieties with a broad genetic base, enhancing the value of a wide range of newly created varieties in the agri-food chain, and addressing key factors of a competitive sector.

Through its structure and research themes, the project assumes direct intervention in solving key problems in the vegetable agri-food sector and in creating a model-concept to serve as a reference for agricultural research, centered on issues identified or signaled by farmers, processors, and consumers.

The proposed initiative aims to enhance the competitiveness and sustainability of the vegetable sector by implementing an intelligent, integrated system for the multipurpose use of plant genetic resources. The approach responds to the current environmental and economic challenges by accelerating the transition toward regenerative growth while leveraging significant economic opportunities. The project is designed for close collaboration with economic actors, consumers, citizens, and civil-society organizations.

The expected impacts are broad and cross-sectoral:

Farmers will gain access to improved technologies, adapted varieties, efficient resource-use solutions, and training opportunities, enabling increased productivity and diversified valorization pathways.

Processors and producers will benefit from enhanced processing models, revitalized market opportunities, and know-how for developing new products.

Consumers will experience greater diversity of local products, improved awareness, and opportunities for direct engagement with research activities.

Environment and society will benefit from reduced agricultural impact, safer local food sources, and improved public health outcomes.

Research and education will see strengthened partnerships, new research directions, increased attractiveness for young researchers, and enhanced entrepreneurial skills.

At regional level, the project aims to contribute to economic growth, higher specialization, increased visibility, and improved societal well-being.

Anticipated benefits are numerous, but the needs associated with project implementation are equally significant and require specific measures, dictated by financial support mechanisms, farmers' reluctance, the need for specific methods and practices, the use of planting material capable of withstanding additional pressures, and the need for superior valorization pathways.

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WEED CONTROL IN TOMATO CROP USING ALLELOPATHIC SPECIES UNDER CONVENTIONAL AND ECOLOGICAL SYSTEMS

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Abstract

The aim of this study was to evaluate the allelopathic influence of white clover (*Trifolium repens*), barley (*Hordeum vulgare*), and oat (*Avena sativa*) on weed control in tomato crops. Two experimental factors were analyzed: (1) the impact of plant species with allelopathic potential on weed infestation and (2) the effects of organic versus conventional fertilization on plant development. Allelopathic species were sown simultaneously with tomato planting, using the determinate tomato (*Solanum lycopersicum*) cultivar 'Bacuni'. The main weed species identified were redroot pigweed (*Amaranthus retroflexus*), lamb's quarters (*Chenopodium album*), gallant soldier (*Galinsoga parviflora*), pale persicaria (*Persicaria lapathifolia*), purslane (*Portulaca oleracea*), field bindweed (*Convolvulus arvensis*), cockspur (*Echinochloa crus-galli*), and green foxtail (*Setaria viridis*). The allelopathic plants significantly reduced both the number and total biomass of weeds before the first mowing, regardless of whether the cultivation system was organic or conventional. Weed biomass increased substantially before the second tillage/mowing in all treatments, highlighting the need for continuous weed management to prevent competition with the crop. Regarding tomato production, superior results were obtained under the conventionally fertilized treatment compared to the organically fertilized one.

Key words: sustainable agriculture, weed management, tomato production, organic fertilization.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetable crops in the Solanaceae family worldwide (Picanço et al., 2007) and holds significant economic importance globally (Dewapriya et al., 2024; Avasiloaiei et al., 2025). Conventional tomato cultivation practices involving excessive nitrogen fertilization degrade soil and water quality by promoting soil erosion, accelerating organic matter mineralization, and increasing NO₃⁻ concentrations in both surface and groundwater. Consequently, the adoption of sustainable practices is essential to maintain tomato yield while improving soil and water quality. There are some challenges in implementing reduced-tillage technologies in organic farming, particularly due to ineffective weed control and suboptimal nutrient management (Antichi et al., 2019; Abou

Chehade et al., 2023). Therefore, it is crucial to identify cover crop species that can address these two issues while sustaining crop yield (Vincent-Caboud et al., 2017). Cover crops can modify soil properties, thereby influencing fruit yield and the growth of both above- and belowground tomato biomass, which are closely associated with root development (Sainju et al., 2001).

An international meta-analysis revealed that intercropping strategies, including living mulch, relay cropping, or synchronous planting of cover crops, can suppress weed populations by up to 82% compared with non-weeded control plots (Verret et al., 2017). This evidence underlines why, in the context of increasing concerns over herbicide-resistant weeds and the limited availability of new modes of action, many producers have adopted annual cover crops as a management strategy (Basinger & Hill, 2021). For instance, Grant

and Sallans (1964) demonstrated that white clover (*Trifolium repens*), extracts can inhibit the germination of various legumes and grasses. Clover plants, as reported by Taiz and Zeiger (2013), contain flavonoids and other bioactive compounds that exhibit significant biological and allelopathic effects. Once incorporated into the soil, white clover contributes to the suppression of phytoparasitic nematode populations (Carlsen & Fomsgaard, 2008) and simultaneously enhances soil fertility by providing organic matter and biologically fixed nitrogen (Hill et al., 2021). Baghestani et al. (1999) reported that barley germplasm contains a broader spectrum of allelochemicals, including phenolic acids, compared with wheat, emphasizing its value as a model crop for allelopathy research. Plant residues (Gubbels and Kenaschuk, 1989), seeds (Liu and Lovett, 1993), and root exudates (Bertholdsson, 2004; Bouhaouel et al., 2015) of barley (*Hordeum vulgare*) have also been investigated for their allelopathic potential against various crop and weed species, further supporting the species' well-recognized production of bioactive allelochemicals. According to Kato-Noguchi et al. (1994), in oats (*Avena sativa*), L-tryptophan may function as an allelochemical, affecting the germination and growth of a range of plant species.

The aim of the present study was to evaluate the effects of white clover, barley, and oat on tomato crop growth and weed control, and to assess the impact of organic versus conventional fertilization on tomato development.

MATERIALS AND METHODS

The study was conducted at the Vegetable Research and Development Station Bacău (VRDS), during the 2025 growing season and focused on the cultivation of determinate tomato varieties under both organic and conventional farming systems. The tomato cultivar 'Bacuni', patented by the VRDS Bacău, was used in the experiment. The allelopathic species investigated were white clover (*Trifolium repens*), barley (*Hordeum vulgare*), and oat (*Avena sativa*). The allelopathic species were sown simultaneously with the tomato planting. Barley and oat served as support crops to facilitate white clover

establishment during germination. The spacing between rows was 140 cm, while the spacing between plants within the row was 15 cm.

In this study, two experimental factors were analyzed: (1) the influence of allelopathic potential species on tomato crops and weeds, and (2) the effects of organic and conventional fertilization on tomato crop development (Table 1).

Table 1. Variants used in the study

Variant	Allelopathic species	Main crop	Fertilization
V1F1	White clover (12 kg/ha), barley (30 kg/ha), oat (30 kg/ha)	Tomato	Organic (1 & 2: Orgevit; 3: Crop Max; 4: Etamin; 5: Crop Max + Etamin)
V1 F2	White clover (12 kg/ha), barley (30 kg/ha), oat (30 kg/ha)	Tomato	Conventional (1 & 2: NPK 15-15-15; 3: Albit; 4: Albit + Vegenergy; 5: Albit + Final K)
V2 F1	Without allelopathic species	Tomato	Organic (1 & 2: Orgevit; 3: Crop Max; 4: Etamin; 5: Crop Max + Etamin)
V2 F2	Without allelopathic species	Tomato	Conventional (1 & 2: NPK 15-15-15; 3: Albit; 4: Albit + Vegenergy; 5: Albit + Final K)

Mowing was performed twice using a garden strimmer, while the controls were tilled manually twice and once mechanically.

The fertilization treatments included the following products and application rates: Orgevit, an organic fertilizer applied at 1-1.5 t/ha; Crop Max, a natural foliar growth stimulator applied by dissolving 10 mL of product in 10 L of water; Etamin, an ecological biostimulator containing microelements and L-amino acids, applied at 0.3 L per 100 L of water; Albit, an ecological biostimulator used to enhance plant immunity and growth; Vegenergy, an organic fertilizer for advanced nutrition and physiological protection, applied at 20-25 mL per 10 L of water; Final K, applied at 3-5 L/ha; and NPK, applied at 300-400 kg/ha.

A metric frame was used to evaluate weed presence. The fresh biomass and weed density per m² were recorded. Identification of weed species was performed using a weed descriptor (Gurău, 2007). Weed infestation was assessed at two intervals: A - before the first tillage/mowing, B - before the second tillage/mowing.

Chlorophyll and anthocyanin levels were measured using an OPTI-SCIENCES Chlorophyll Content Meter and an OPTI-SCIENCES Anthocyanin Content Meter. For each replicate, leaves for all measurements were randomly collected from three plants. Total soluble solids concentration was determined using a high-precision handheld portable refractometer. The analysis was performed on homogenized juice obtained by pressing fresh fruits, and the results are expressed in Brix, according to method 932.12 (AOAC, 2005; Brezeanu et al., 2022). Fresh fruits were randomly sampled from three plants within each replicate. At harvest, total seed yield (t/ha) was also recorded. Statistical analyses were performed using ANOVA, and mean comparisons were conducted with Tukey's HSD test at a significance level of $P < 0.05$ and the Bonferroni test, using IBM SPSS Statistics 20.

RESULTS AND DISCUSSIONS

In the tomato crop, several weed species were identified. The annual dicotyledonous weeds included redroot pigweed (*Amaranthus retroflexus*), lamb's quarters (*Chenopodium album*), gallant soldier (*Galinsoga parviflora*), pale persicaria (*Persicaria lapathifolia*), and purslane (*Portulaca oleracea*). The perennial dicotyledonous weeds were represented by field bindweed (*Convolvulus arvensis*). Among the annual monocotyledonous weeds, cockspur (*Echinochloa crus-galli*) and green foxtail (*Setaria viridis*) were identified. Allelopathic plants significantly reduced both weed density and total weed biomass prior to the first mowing, regardless of whether the cropping system was organic or conventional (Table 2). After mowing, in the absence of barley and oats, which provided rapid soil cover and facilitated the establishment of white clover, a pronounced increase in weed abundance was observed. This response was attributed to the poor germination and slow growth rate of white clover, which limited its ability to compete effectively with weeds. Meteorological conditions during the period immediately following sowing play a decisive role in the successful establishment of white clover, as they may lead to uneven germination and slow

early growth, thereby reducing the plant's capacity to develop adequately and to compete effectively with weeds.

Table 2. Total number of weeds per m² in the tomato crop: A - before the first tillage/mowing, B - before the second tillage/mowing

Variant	Number of weeds m ²	
	A	B
V1F1	86.67±14.43 b	38.67±4.16
V1F2	80.67±21.03 b	40.67±9.24
V2F1	312.67±6.43 a	49.3±4.16
V2F2	292±27.87 a	50.33±4.62
Sig.	*	ns

V1F1 – tomatoes grown under an organic farming system with white clover, barley, and oats; V1F2 – tomatoes grown under a conventional farming system with white clover, barley, and oats; V2F1 – tomatoes grown under an organic farming system; V2F2 – tomatoes grown under a conventional farming system. Results are expressed as means ± standard deviation (SD). Different letters indicate significant differences among treatments, as determined by Tukey's post hoc test ($P < 0.05$): a – highest value recorded for the test performed; * – significant differences; ns – non-significant differences.

Weed biomass increased substantially at time B (before the second tillage/mowing) across all experimental treatments, highlighting the need for continuous weed management to prevent competition with the main crop (Table 3).

Table 3. Total fresh weed biomass g/m² in the tomato crop: A - before the first tillage/mowing, B - before the second tillage/mowing

Variant	Fresh weed biomass g/m ²	
	A	B
V1F1	97±12.17 b	495.67±29.57
V1F2	101±23.30 b	488.67±22.14
V2F1	268.67±5.51 a	423±58.21
V2F2	259.67±5.03 a	444.67±26.95
Sig.	*	ns

V1F1 – tomatoes grown under an organic farming system with white clover, barley, and oats; V1F2 – tomatoes grown under a conventional farming system with white clover, barley, and oats; V2F1 – tomatoes grown under an organic farming system; V2F2 – tomatoes grown under a conventional farming system. Results are expressed as means ± standard deviation (SD). Different letters indicate significant differences among treatments, as determined by Tukey's post hoc test ($P < 0.05$): a – highest value recorded for the test performed; * – significant differences; ns – non-significant differences.

The main effect of the cropping (allelopathic species vs. tillage) system was not statistically significant ($F = 4.704$, $p = 0.062$), although the effect size was moderate ($\eta^2p = 0.370$). This trend suggests that, in the absence of

conventional fertilization, the differences between cropping systems are not sufficiently large to produce a statistically consistent yield response. The fertilization treatment had a significant effect on tomato yield ($F = 27.783$, $p = 0.001$), with a very large effect size ($\eta^2p = 0.776$). The mean yield obtained under the conventional fertilization regime ($F2$: 107.59) was significantly higher than that recorded under the organic fertilization regime ($F1$: 64.54), with a difference of 43.05 units ($p = 0.001$, Bonferroni test). This result confirms the decisive role of fertilization in determining yield levels (Table 4).

Table 4. Tomato yield and fruit morphological characteristics

Variant	Tomato yield (t/ha)	Fruit diameter (mm)	Fruit height (mm)
V1F1	60.88±9.66b	69.06±1.12	41.66±1.19
V1F2	93.53±5.93ab	68.63±1.08	42.10±1.58
V2F1	68.20±6.98b	66.11±2.64	43.523±1.10
V2F2	121.65±9.46a	76.98±4.68	46.78±0.71
Sig.	*	ns	ns
F1	64.54±5.58	67.58±1.44	42.59±0.83
F2	107.59±8.03	72.80±2.85	44.44±1.30
Sig.	*	ns	ns
V1	77.21±8.89	68.84±0.70	41.88±0.89
V2	94.92±13.06	71.55±3.42	45.15±0.94
Sig.	ns	ns	ns

V1F1 – tomatoes grown under an organic farming system with white clover, barley, and oats; V1F2 – tomatoes grown under a conventional farming system with white clover, barley, and oats; V2F1 – tomatoes grown under an organic farming system; V2F2 – tomatoes grown under a conventional farming system. Results are expressed as means ± Std. Error. Different letters indicate significant differences among treatments, as determined by Tukey’s post hoc test ($P < 0.05$): a – highest value recorded for the test performed; * – significant differences; ns – non-significant differences.

The interaction between cropping system and fertilization was not statistically significant ($F = 1.621$, $p = 0.239$), indicating that the effect of fertilization is consistent across both cropping systems. In other words, conventional fertilization increases yield regardless of whether tomatoes are grown under organic or conventional cropping systems. Regarding chlorophyll content in tomato leaves, the highest value before the first tillage/mowing (A) was recorded in V2F2

(53.23±1.9 a), which was significantly higher than V1F1 (34.73±10.42 b), V1F2 and V2F1 presented intermediate values without significant differences between them. At the second measurement (B), no significant differences were observed among the variants, indicating that the initial differences in chlorophyll content diminished over time (Figure 1).

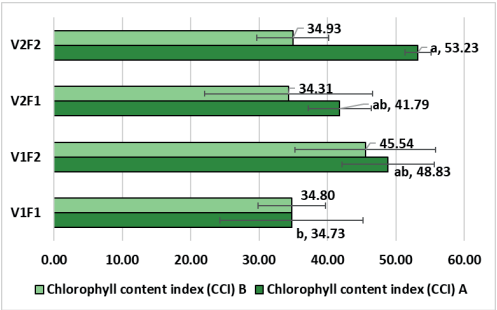


Figure 1. Chlorophyll content index in tomato leaves: A - before the first tillage/mowing, B - before the second tillage/mowing

Regarding anthocyanin content, no statistically significant differences were detected among the treatments at any of the evaluated time points (Figure 2).

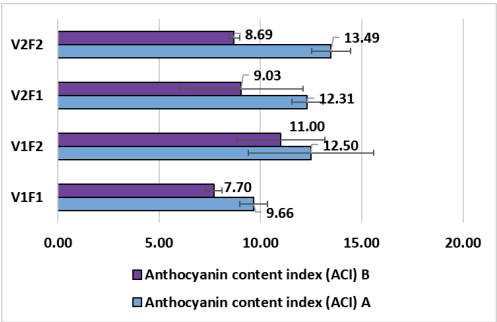


Figure 2. Anthocyanin content index in tomato leaves: A - before the first tillage/mowing, B - before the second tillage/mowing

Plant height before the first tillage/mowing (A) was greatest in V1F2 and V2F2, reaching approximately 31 cm, and was significantly higher than in V1F1 (27.22 cm) and V2F1 (23.56 cm) (Figures 3, 4).



Figure 3. Tomato plants cultivated with white clover, barley, and oats

At the second measurement (B), the tallest plants were observed in V2F2 and V2F1 (around 59 cm), followed by V1F2 (55.89 cm) and V1F1 (44.55 cm), with significant differences especially between V1F1 and the other variants.

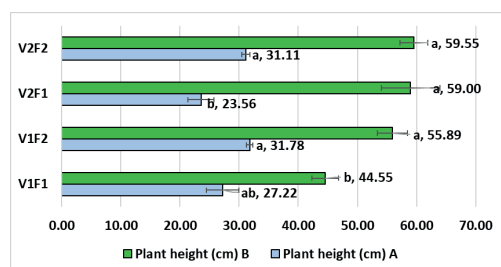


Figure 4. Plant height of tomato plants:
A - before the first tillage/mowing,
B - before the second tillage/mowing

A moderate positive correlation was observed between plant height and yield. However, the correlation was not strong, indicating that plant height is not the sole determinant of yield. Taller plants tended to produce higher yields, but yield is also influenced by other agronomic and physiological factors, such as nutrition, cropping system, competition, and abiotic stresses (Figure 5).

Linear regression analysis between tomato yield (t/ha) and weed biomass (g/m^2) revealed a very weak negative relationship, with a coefficient of determination of $R^2 = 0.008$. Weed biomass accounted for only 0.8% of the total variation in yield, indicating the absence of a statistically meaningful correlation. Under the given experimental conditions, yield performance was predominantly influenced by

the fertilization system, whereas weed competition did not exert a relevant effect on tomato productivity (Figure 6).

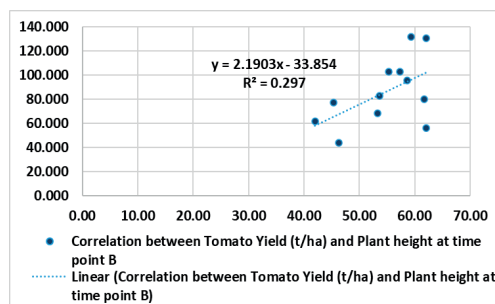


Figure 5. Correlation between tomato yield (t/ha) and plant height at time point B (before the second tillage/mowing)

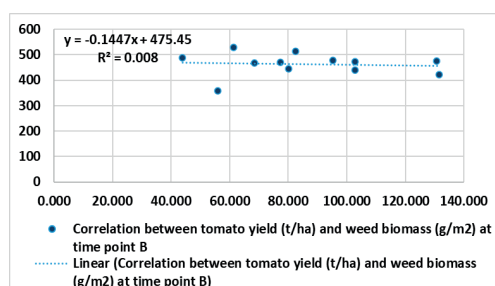


Figure 6. Correlation between tomato yield (t/ha) and weed biomass (g/m^2) at time point B (before the second tillage/mowing)

Statistical analysis revealed significant differences among treatments regarding soluble solids content ($P < 0.05$). The highest TSS value was recorded in treatment V1F2 (5.48 ± 0.19 °Brix), which was significantly higher than those observed in treatments V1F1, V2F1, and V2F2, among which no significant differences were detected (Figure 7).

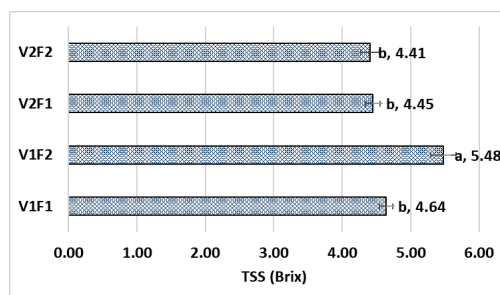


Figure 7. Total Soluble Solids (TSS, °Brix) in tomato fruits

Overall, the results demonstrate that fertilization optimization has a much more pronounced impact on tomato yield than the choice of cropping system, emphasizing the importance of nutrient management in achieving high and stable production levels.

CONCLUSIONS

Allelopathic plants (white clover, barley, and oats) significantly reduced weed density and total weed biomass prior to the first mowing; however, weed biomass increased substantially before the second management operation, highlighting the need for continuous weed management to prevent competition with the main crop.

The cropping system did not exert a statistically significant effect on most physiological traits, such as chlorophyll and anthocyanin content; however, it significantly influenced plant height, with the highest values recorded under conventional variants, particularly at sampling time B, indicating more intensive vegetative growth during the second part of the growing season.

Conventional fertilization resulted in a substantial increase in tomato yield compared to organic fertilization, confirming the essential role of nutrient supply in determining the productive performance of the tomato crop.

The cropping system, analyzed as a main effect, did not significantly influence tomato yield, although the relatively high effect size suggests a moderate biological trend. This observation indicates that differences between organic and conventional systems, in the absence of intensive fertilization, are not sufficient to generate statistically consistent yield variations.

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PRODUCTION PRACTICES AND USES AT SAFFRON. A MINI REVIEW

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Abstract

Saffron (Crocus sativus L.) is a bulbous perennial species of the Iridaceae family, genus Crocus, and is cultivated for the production of stigmas. These stigmas are used as a culinary spice, food colouring, in traditional medicine, or in the cosmetic industry. Saffron is often considered the "red gold" and the most expensive spice in the world, and for this reason it has gained great popularity worldwide as a "superfood" and as a profitable crop. The species is cultivated in diverse pedoclimatic regions and requires warm and dry conditions during the vegetative dormancy phase and sunny conditions for flower appearance. The productivity of saffron is influenced by several factors, namely: climatic conditions during the vegetation period, soil type, and production practices. The paper highlights cultivation methods and post-harvest management through the study of scientific works published in Google Scholar, Web of Science, Scopus and PubMed, for the implementation of appropriate agronomic practices. Therefore, scientific evidence supports the fact that adapting the best cultivation practices influences saffron productivity and quality.

Key words: *Crocus sativus L., agronomic practices, corms.*

INTRODUCTION

Saffron (*Crocus sativus* L.) is a geophyte plant belonging to the Iridaceae family and is valued for its dried red stigmas, used as a culinary spice and flavoring agent or in the pharmaceutical or cosmetic industry. This perennial species of the bulbous group is cultivated for its flowers. It is considered the most expensive spice in the world and is nicknamed "red gold" (Acharya et al., 2025). The most valuable part is the stigmas, which are used in the food, medicinal, and cosmetic sectors. It is valued as a spice for its taste, aroma, and pigments present.

The species is cultivated in Iran, parts of Turkey, and Greece, as well as in Spain, Italy, France, Switzerland, Israel, Balochistan (Pakistan), Azerbaijan, China, Egypt, the United Arab Emirates, Japan, Afghanistan, Morocco, Iraq, Australia, Mexico, New Zealand, and Argentina. The countries with the largest share of saffron production are Iran, Spain, Turkey, Morocco, Greece, India (Kashmir Valley), Switzerland, and Australia (Ahmed et al., 2023). This species shows considerable adaptability to a wide range of environments, both mild and arid, and can tolerate temperatures between -18°C and 45°C (Bharat et al., 2025). According to Koocheki et al. (2020) vegetative

development in saffron is maximum at a temperature of 27°C, and flowering is best at temperatures of 17°C.

Since ancient times, saffron has been widely used as a spice and food coloring, in the preparation of perfumes, or in the preparation of tea (Yousefi and Shafagh, 2019; Vlahova, 2022). In general, spices act as preservatives or colorants used in food or in painting (Yildirim et al., 2020; Lachguer et al., 2025).

Compared to other foods, saffron is particularly rich in vitamins such as riboflavin and thiamine (56-138 and 0.7-4 µg) and secondary metabolites: terpenes, flavonoids, anthocyanins, and carotenoids such as lycopene, α - and β -carotene, zeaxanthin, and crocetin, important in human nutrition (Cardone et al., 2020; Zorovski et al., 2025). The chemical composition of the stigmas is given by the presence of the following components: 14-16% water, 11-13% nitrogenous substances, 12-15% sugars, 41-44% soluble extracts, 0.6-0.9% essential oil, 4-5% fiber and 4-6% ash. Bioactive compounds include crocin, picrocrocin, and safranal (Cardone et al., 2020).

To enhance the value of saffron crops and increase profitability, saffron by-products have also been exploited, especially the tepals, for their use in traditional medicine because they

possess a series of properties: natural dye or antibacterial, antitussive, hepatoprotective, immunomodulatory, antidiabetic, or antioxidant properties (Lachguer et al., 2025).

A circular economy describes an economic system based on the principles of the 3Rs, i.e., reduce, reuse, and recycle materials to ensure environmental quality, economic prosperity, and social equity, for the benefit of current and future generations (Ghisellini et al., 2016). This approach is also gaining increasing attention in saffron cultivation through the use of its by-products, such as tepals, stamens, styles, leaves, and bulbs in various forms (Lahmass et al., 2018).

Recently, there has been interest in saffron cultivation in Europe with a particular focus on quality, but the technology used for saffron production needs to be improved to ensure profitability and quality (Renau-Morat et al., 2012). Increased collaboration between research institutions, producers, and policy makers, supported by specific government subsidies, will be essential to advance strategies that ensure the resilience of saffron production systems (Bharat et al., 2025).

Saffron areas are limited compared to other crops due to high human resource costs and difficulties in establishing or harvesting flowers, non-mechanized operations (Mariani et al., 2025). Numerous researchers have proposed several techniques to optimize saffron cultivation technology. In this regard, the present research includes a synthesis of recent studies on the botanical characteristics and cultivation methods of saffron.

MATERIALS AND METHODS

The research paper is a synthesis of scientific studies prepared by the authors who reported important information on saffron cultivation. This review included bibliographic information on saffron cultivation techniques from different areas, focusing mainly on planting date management, the effects, bulb size and weight at establishment, irrigation methods, and types of fertilization or other factors for successful cultivation. The specialized literature was consulted using electronic databases, including Google Scholar, Web of Science, Scopus and PubMed.

RESULTS AND DISCUSSIONS

Botanical traits

Saffron is a perennial geophyte plant, from the family *Iridaceae*, genus *Crocus*, and species *stativus*. The genus *Crocus* includes approximately 80 species in the world, of which 32 are found in the flora of Turkey, of which 18 are endemic to the country (Zorovski, 2025). It is a monocotyledonous plant, characterized by an embryo with a single cotyledon and leaves with parallel veins. The is a sterile triploid and bears 8 chromosomal bodies per set, making 24 in total (Hussain et al., 2019). This species reproduces vegetatively, exclusively through bulbs, and its size directly influences flower production and the ability to multiply new bulbs. Bulbs, underground morphological organs of the plant, have flat to ovoid shapes, with variable dimensions regarding horizontal diameter, ranging from 0.5 to 6.5 cm, and weight from 0.5 to 15 g. Each mother bulb produces 1 to 3 daughter bulbs of medium to large size from the apical buds, depending on the development of the mother bulb (Bakhtiari et al., 2013; Cardone et al., 2020; Acharya et al., 2025).

Crocus plants can reach a height of 15-20 cm, forming a rosette of lanceolate leaves from September to May, after which they dry up. During the fall, they form purple flowers, with dark red to reddish-brown stigmas and yellowish-brown to yellowish-orange styles. The flowers of these plants have the characteristic of closing during cold weather (Hussain et al., 2019).

Culture management techniques

Saffron is gaining increasing attention as an alternative crop in sustainable agricultural systems due to its unique biological, physiological, and agronomic traits, such as the ability to exploit marginal lands and adapt to different environmental conditions. However, saffron cultivation can be affected by various factors, including rapid climate changes, light intensity, pH level, soil moisture, salinity level, and improper cultivation techniques (Khan et al., 2024).

The biological cycle of saffron lasts approximately 200 days, from early autumn to May-June. In spring, the bulbs begin to grow in size, reproduce, and during the summer enter a

state of vegetative dormancy; and flowering occurs in October - November (Mariani et al., 2025).

Cultivation methods

Numerous researchers have proposed several techniques to optimize saffron growth, focusing mainly on the management of planting dates, the effect of temperature and crop density or establishment times, the effect of fertilizers on growth and productivity, for crop success.

Molina et al. (2005) studied the impact of temperature on saffron growth and found that low temperatures during bulb development or high temperatures during flower bud initiation negatively impacted saffron production. Furthermore, different strategies were discussed to control the effect of temperature on saffron growth, which helps growers optimize cultivation techniques.

Most experts recommend planting saffron in August-September, at a depth of 10-15 cm, depending on the soil characteristics. Some growers claim that if saffron is planted deep, the bulbs multiply less, the harvest is smaller, but the quality of the flowers produced is higher. In a 4-year study, Karra et al. (2017) demonstrate that planting depth is directly correlated with flower production and bulb survival. The authors recommend 12-15 cm for sandy soils and 10-12 for clay soils. El Hajj et al. (2019) demonstrate similar results and state that too great a depth can delay the flowering period in compact soils.

Lachguer et al. (2025) show that a moderate density of 100-150 bulbs per square meter is suitable for most crop systems. This density allows good production correlated with the regeneration of biological material. A high density of about 180 bulbs per square meter leads to a significant increase in stigma production in the first years of cultivation, to the detriment of the development of daughter bulbs, as stated by Azizi Zohan et al. (2015). The study by El Hajj et al. (2019) indicates that a low density of about 80 bulbs per square meter allows good bulb development, but flower production is low.

Mollafilabi et al. (2010) initiated the application of a new method of saffron cultivation for four years, with several factors: planting time - early June, planting 8 g bulbs and summer irrigation in early August, compared to the traditional

method, with planting in September, without sorting the bulb weight and without summer irrigation. The results indicated that the average production for stigma + style and stigma was 11.61 and 8.64 respectively (48% superiority and approximately double) in the new method and 6.01 and 4.47 kg/ha in the traditional method, respectively. Therefore, with a confidence of 99%, the authors suggest that the new method can be recommended for saffron cultivation.

Another two-year (2017-2019) field study was conducted to compare annual and biennial cropping cycles of saffron, using three bulb size classes (D1: 2.0-2.5 cm, D2: 2.6-3.5 cm and D3: 3.6-4.5 cm) and the results showed that bulbs in class D3, planted in the annual cropping cycle, produced flowers with the largest stigma length of 42.2 mm, dry weight of stigmas of 7.4 mg, stamens of 11.4 mg and tepals of 40.7 mg. The highest stigma production was 20.7 kg ha⁻¹ when the bulbs belonged to class D2 and were planted in the biennial crop cycle, and the highest young bulb production was 35.9 t ha⁻¹ when the bulbs belonged to class D3 and were planted in the annual crop cycle. Regarding the quality of the spices, the color and bitter strength were positively influenced by the biennial crop cycle (Cordone et al., 2021).

In a study on saffron (Ralli Parthenopi et al., 2024) two corm sizes for planting were investigated: with a diameter of up to 2.5 cm and with a diameter greater than 2.5 cm, and five planting densities: 22, 27, 33, 44 and 67 corms/m², with planting distances of 30, 25, 20, 15 and 10 cm per plant row, respectively. The results showed that over the six-year study period, a gradual increase in flowering was observed from the first to the third year, followed by a decrease in the fifth and sixth years. The production of corms larger than 2.5 cm was higher in the first three years, while corms smaller than 2.5 cm) produced higher yields in the fourth to sixth years. Therefore, bulb size, together with the environment, are important factors that can influence crop productivity and longevity and should be considered when planting a new saffron crop.

In another experiment on flower production and stigma production in saffron depending on bulb size and spacing, three treatments were used for bulb size, namely: large (8-11 g or 2-2.5 cm in

diameter), medium ($5-7 \text{ g} \leq 2 \text{ cm}$ in diameter) and small (less than $4 < 1 \text{ cm}$ in diameter). In the second experiment, five treatments were used for row-to-plant spacing, namely: T1 = 15 cm, T2 = 20 cm, T3 = 25 cm, T4 = 30 cm and T5 = 35 cm. The results indicated that only large bulbs produced flowers, while medium and small bulbs failed to produce flowers. However, the fresh and dry weight of saffron stigma produced by large bulbs was recorded as 0.0354 and 0.0075 g, respectively. In terms of production, the highest (162.3) was recorded at a spacing of 15 cm. (Ahmed et al., 2024). According to Andabjadid et al. (2015), flower production increases with increasing row spacing.

Koocheki et al. (2014) also reported that planting density or sowing dates of corms in the first year are the most critical factors affecting saffron production. Selecting appropriate sowing dates of corms in the first year simultaneously determines the crop exploitation period, increases production and reduces the period between establishment.

Greenhouse (controlled environment) cultivation of saffron has increased significantly in recent years due to limited areas suitable for growing the species and the increasing demand for this valuable spice (Munjid et al., 2025). Recent studies have reported modern cultivation of saffron in soilless environments leading to optimized yields and quality (Nardi et al., 2025). Maggio et al. (2006) conducted research on saffron cultivation in different cropping systems, and the results showed that production doubled (22.5 kg ha^{-1}) in greenhouses and growth chambers, compared with traditional field cultivation (10 kg ha^{-1}).

Soil type and fertilization methods for saffron cultivation

Soil type influences the formation of stamens and flower petals, which can be further used in other sectors, increasing income and contributing to sustainable saffron cultivation. Deterioration of soil quality can lead to poor bulb quality, the occurrence of diseases and pests, and poor post-harvest management (Cardone et al., 2020). These same authors analyzed several soil types and concluded that sandy loam and loamy soils offer the best conditions for saffron growth. This crop also grows in calcareous clay soils, loose, low-

density, well-drained, and irrigated with a pH between 6.8 and 7.8 and electrical conductivity below 2 dS m^{-1} (Zarghani et al., 2016).

Several researchers have studied the effects of organic, biological, or synthetic fertilizers on saffron cultivation. Their application improves soil properties and nutrient availability, leading to higher saffron yields. Compost, manure, and vermicompost, as well as their integrated application, promote higher saffron growth (Hourani, 2023). Under drought conditions, the combined use of chicken manure and chemical fertilizers (3:1 ratio) improved saffron growth and production (Aboueshaghi et al., 2022; Hourani, 2023). Numerous studies have shown that the combined application of manure with synthetic fertilizers improves the quantitative and qualitative production and the content of microcrocins, safranal, and crocin in plants (Amiri et al., 2008; Esmacilian et al., 2022).

The use of nitrogen and phosphorus fertilizers had a significant effect on saffron production. Thus, in the study of Amiri et al. (2008) they showed that the highest production was obtained by applying 50 kg/ha urea and 40 kg/ha superphosphate, together with 20 t/ha manure. Other studies obtained the same results by combining fertilizers with 75 kg N/ha and 100 kg P/ha (Emam et al., 2012). Other authors demonstrated that the use of 35 kg ha⁻¹ of phosphorus increased the fresh weight of saffron bulbs and leaves, but decreased the number of bulbs (Chaji et al., 2013). Application of 200 kg ha⁻¹ of potassium sulfate increased the number and weight of flowers (by 5.5% and 5.6%, respectively) and the weight of fresh and dried stigmas (by 10% and 8.5%, respectively) (Akrami et al., 2015).

In the biology of saffron, the months of February and March are of considerable importance because the young bulbs that form on the mother bulbs by February have no roots, and for the continuation of vital activities in March and April, the plant relies on its leaves through photosynthesis and nutrient absorption. Therefore, the application of foliar fertilizers in March is very useful (Hosseini et al., 2004; Renau-Morata et al., 2012).

When fertilizing the soil with Italpolina organic fertilizer (granulated bird droppings) at a dose of 70 kg/day, applied before planting in the first year under specific conditions and in organic

farming, an average yield of raw stigmas of 435 g/1000 m² or 129 g/1000 m² of dried saffron (stigmas) was obtained (Zorovski, 2025). The application of vermicompost at a dose of 10.2 t ha⁻¹ positively influenced the increase in the number, weight, and N and P content of medium and large daughter bulbs per plant (Seyyedi et al., 2018). It was found that the application of humic acid to the saffron crop improves the production and quality of flowers (Mollafilabi and Khorramdel, 2016). Some researchers have evaluated the use of arbuscular mycorrhizal fungi and concluded that they increased saffron production and quality (Husaini, 2014; Caser et al., 2019).

Irrigation

Irrigation is an important sequence that influences vegetative development and crop production. In general, saffron has low water requirements and is usually grown under rainfall conditions in various Mediterranean areas. However, it was demonstrated that the use of irrigation systems improves the quantitative and qualitative production of saffron under drought conditions (Renau-Morat et al., 2012). Early autumn rainfall contributes to good flower production, and spring rainfall is beneficial for bulb production (Menia et al., 2018).

Some authors have investigated the effects of different irrigation levels, including 50, 75 and 100% of saffron water requirement (SWR) and dense corm planting (50, 100, 200 and 300 corms m⁻²) on saffron grown under arid and semi-arid conditions. They recommended that providing 75% of SWR and dense corm planting of 200 corms m⁻² achieves the desired corm and flower production (Koocheki et al., 2014). Saffron is considered a salt-sensitive species; however, it can grow in semi-saline irrigation up to 4 dS m⁻¹ (Khalilzadeh et al., 2025).

In some studies, the effect of using different irrigation methods, including sprinkler, drip, furrow, and basin, on the following factors was evaluated: dry weight of saffron stigma, quality, and irrigation water use efficiency, and the results showed that the maximum dry weight of stigma and water use efficiency were achieved using the drip irrigation method compared to the sprinkler or basin irrigation methods (Karimiferezh et al., 2018; Mollafilabi et al., 2021), and basin irrigation is preferred over furrow irrigation because a greater number of

large corms was obtained (Azizi-Zohan et al., 2009). According to Khazaei et al. (2013), a higher number of corms and a higher weight were recorded with the furrow irrigation method. It can be stated that irrigation is an important technological sequence for obtaining high and quality saffron production under drought conditions, regardless of the method. In addition, it is known that the drip irrigation method is recommended for lower water consumption. It should be taken into account that over-irrigation or heavy rainfall during flowering can lead to the appearance of pathogens and low production. Therefore, water management is essential for maintaining good soil moisture and preventing flooding (Mollafilabi et al., 2021).

Weed control

Saffron production is limited by several factors, including weed infestation, and for this reason it is important to manage weeds in the vegetation so as not to affect the crop, considering that it is a slow-growing plant and weeds grow much faster. Weed control can be done by several methods: manually, by weeding, by mulching, or chemically. In this regard, Mzabri et al. (2021) studied the effect of some mulching materials on weed control, and the results showed that the use of PE (polyethylene) mulch effectively reduced weed populations and improved vegetative growth and saffron production. The chemical approach is one of the most effective methods of weed control in saffron cultivation. There are studies that indicate chemical control, but this method is unpretentious to the environment. In the study by Asil et al. (2023), several treatments, both cultural and herbicides, were applied to control weeds in saffron, and the results showed that the application of pre-emergence methods to weeds was recorded for the applications of treatments U5 (pine sawdust + benfluralin) and U6 (textile mulch).

Pests and diseases

Saffron production can be affected by numerous pathogens. Among these, the occurrence of *Fusarium oxysporum*, *Rhizoctonia solani*, and *Sclerotium rolfsii* can affect the crop. The most frequent is *Fusarium oxysporum*. (Caligiore-Gei et al., 2023). Crop rotation, the use of pathogen-free bulbs, the use of resistant varieties, soil solarization, and the judicious use of chemical

treatments or biological control are important for the success of the crop (Sharma and Bora, 2025). The occurrence of diseases is also influenced by soil type. Thus, El Hajj et al. (2019) reported an increased incidence of bulb rot in heavy soils.

Harvesting and production

In general, production is affected by numerous factors: climatic conditions, soil type, disease and pest attack, crop cycle length, time, size, density, corms, and depth of planting or fertilization and irrigation. In Iran, the country that occupies large areas of saffron, historically, production per unit area was on average 3.5 kg ha⁻¹ in 2017 (Cardone et al., 2020). The highest stigma yield was obtained in locations with a temperature of 15-16°C and without excessive rainfall during the flowering period of the biennial cycle (Cardone et al., 2019). In Spain, the average saffron production was 6.5 kg ha⁻¹, and in Morocco it varies between 2 and 6 kg ha⁻¹, depending on the growing season (Mzabri et al., 2021). Some researchers have suggested a two- to three-year crop cycle to avoid a decrease in production during the four-year crop cycle (Temperini et al., 2009).

Yildirim et al. (2017) obtained the highest bulb unit weight (10,348 g) from small-sized bulbs harvested every two years, and regarding removal periods and planting depths, the highest bulb production from large bulbs (527.33 g m⁻²) was obtained when they were removed every two years and planted at a depth of 15 cm.

In all countries, in June and July, the bulbs are harvested, selected according to size, disinfected, and then planted. During bulb storage, temperature conditions influence growth and flowering, and the highest number of flowers was at temperatures of 23-27°C for 90-150 days (Molina et al., 2005).

CONCLUSIONS

The saffron crop has economic importance given the value of the dried stigmas used in the food, pharmaceutical, and cosmetic industries. To improve production, theoretical guides with recommendations for the respective crop area should be developed, and appropriate technology management should be implemented regarding the establishment of the emergence period, planting densities and depths depending

on the soil type, improvement of weed control techniques, and adequate fertilization management.

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MORPHOLOGICAL ADAPTATIONS OF *CHENOPODIUM QUINOA*'S ROOTS UNDER STRESS DURING VEGETATIVE DEVELOPMENT

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Abstract

The study evaluated the synergistic interaction between the nutritional and water regimes on the morphophysiological plasticity of two quinoa cultivars, Puno and Vikinga, assessed from the perspective of root system development. The research compares the effectiveness of microbial biostimulation and chemical fertilization under three irrigation regimes (50%, 75%, and 100%). The results indicate that the 75% irrigation regime was the ideal regulatory factor, successfully mitigating chemical stress through dilution and facilitating microbial mobility. In contrast, the 50% regime exacerbated salt toxicity, while the 100% regime limited growth by inducing hypoxia at the root level.

Biochemical profiling proved to be a highly accurate indicator of physiological status; the application of high-dose chemical fertilizers exerted a pronounced toxic effect, with inhibition exceeding 60% regardless of variety. This sharp decline indicates that a critical threshold of osmotic and saline stress was reached, caused by the high concentration of KSC® II.

Key words: biochemical profile, *Chenopodium quinoa*, microbial mobility, nutritional regimes.

INTRODUCTION

The species *Chenopodium quinoa* Willd. is native to the Andean region of South America and has been a staple for pre-Columbian civilizations for over 3 000 years. In recent decades, the recognition of its exceptional nutritional value and remarkable agronomic adaptability has facilitated its transition from a local crop to one of global interest. Against the backdrop of climate change, *Chenopodium quinoa* Willd. is of strategic interest for food security due to its resilience to abiotic stress and its exceptional nutritional profile (Bazile et al., 2016; Jacobsen, 2003; Stoleru et al., 2022a, 2022b). Furthermore, medical research has explored quinoa through computational chemistry, highlighting the potential of its bioactive compounds to inhibit or combat certain viral mechanisms, including those associated with COVID-19. According to recent international resolutions (e.g., UN General Assembly, 2022), quinoa is officially recognized as a functional food with preventive and therapeutic roles in human health management.

The species' adaptation to new areas is conditioned by the plasticity of its root system, whose architecture is decisive for water use efficiency (WUE) and nutrient use efficiency (NUE) under soil stress (González et al., 2015; Nieves-Cordones et al., 2019).

Quinoa is a facultative halophyte characterized by high hardiness, thanks to mechanisms that enable it to withstand various stress factors - such as salinity, drought, and frost. It possesses remarkable genetic diversity resulting from centuries of fragmented cultivation across diverse ecological zones in the Andean region, ranging from Ecuador to southern Chile and from sea level to high-mountain elevations (Vitaneascu et al., 2019; Zhang et al., 2026). This plant's tolerance to abiotic stress (drought, salinity) has led to the development of programs focused on increasing its productive potential.

The quinoa plant's tolerance to abiotic stress (salinity and drought) has led to the development of programs aimed at increasing its productive potential, as it is capable of withstanding soil salinity levels comparable to those of seawater (Manna et al., 2019; Stoleru et al., 2019).

In 1996, the FAO classified quinoa as one of the most promising crops for humanity, not only for its health benefits but also for its versatility, considering it an alternative solution to eradicate global hunger. Subsequently, 2013 was declared the “International Year of Quinoa” by the United Nations, following a proposal endorsed by the FAO (2026). Root development is influenced by fertilization management in two ways: chemical fertilizers can induce osmotic stress at excessive doses, while biofertilizers stimulate growth and mitigate stress through symbiotic mechanisms (Hinojosa et al., 2018; Ruiz et al., 2017). The effectiveness of these inputs critically depends on the water regime, which modulates nutrient solubility and microbial viability (Chirita et al., 2022; Rădeanu et al., 2025). The present study analyses root dynamics in the Vikinga and Puno varieties under the influence of the interaction between fertilization and water availability, aiming to optimize sustainable cultivation technologies.

MATERIALS AND METHODS

The research was carried out in a greenhouse of Iasi University of Life Sciences (IULS), Romania, during March to April 2021-2024. vegetation pots (27 cm³), chemically and organically fertilized substrate, resulted 42 variants, of 3 replicates, 10 plants for each repetition. The study focused on two varieties of quinoa, specifically Vikinga and Puno, examining their root systems. Both varieties were cultivated under controlled conditions, with temperatures maintained between 16-18°C during the night and 20-22°C during the day. Humidity levels were kept at 70-75% for Vikinga and 60-65% for Puno. Additionally, plants were exposed to natural light cycles of 13 hours of light and 11 hours of darkness. The substrate for experiments consisted of a standardized mixture of Kekkila[®] peat (600 L), Orgevit[®] biofertilizer (3.00 kg/m³), and perlite (3.00 kg/m³). This homogenized medium was designed to optimize *Chenopodium quinoa* germination and early root system establishment. Into the substrate was also added Osmocote[®] Pro, a second-generation controlled-release fertilizer with a high NPK

value and a rich content of magnesium and micronutrients (N11:P10:K19:2MgO+ME). It is particularly suitable for use as a base fertilizer mixed into the growing medium due to its steady-release system and a longevity of 5-6 months. Kekkila[®] is a specialized peat-based medium for seedling production (0-25 mm), featuring an NPK ratio of 14–16–18 + ME. Its fine-to-medium grain size ensures optimal seed-to-substrate contact, while the pH (5.5-5.9) maximizes micronutrient bioavailability and prevents phosphorus fixation, meeting the specific physiological needs of quinoa. Perlite[®] (3.00 kg/m³) was incorporated to optimize the water-to-air ratio. As a chemically inert and non-degradable expanded volcanic mineral, it ensured structural stability throughout the growing cycle. The fertilizers used were biologic fertilizer, Micoseed MB[®] and chemical fertilizer, KSC[®] II, in different quantities. To test the influence of fertilizer type on plant growth,the following amounts:

- Biological fertilization: 500g/m³ (F1); 1000 g/m³ (F2); 1500 g/m³ (F3) - Micoseed MB[®]. This biological product is formulated with a consortium of arbuscular mycorrhizal fungi-specifically *Claroideoglomus etunicatum*, *Funneliformis mosseae*, *Glomus aggregatum*, and *Rhizophagus intraradices* - supplemented with beneficial microbes from *Trichoderma*, *Streptomyces*, *Bacillus*, and *Pseudomonas*.
- Chemical fertilization: 1000 g/m³ (F4); 2000 g/m³ (F5); 3000 g/m³ (F6) KSC[®]. KSC II[®] is a water-soluble NPK complex (14-40-5 + 13SO₃ and ME) enriched with the Phyt-actyl biostimulator. Its application was prioritized during early vegetation to optimize root system architecture establishment.

The experiment was established using a split-plot, multifactorial design (2×7×3), conducted in pots with a diameter of 17 cm and a height of 12 cm.

Table 1. Experimental design and treatment structure

Cultivar - A	Fertilization - B	Irrigation regime - C
A1 - Vikinga	NF- Control	50%
		75%
		100%
	F1- Micoseed MB [®] (500 g/m ³)	50%
		75%
		100%

Cultivar - A	Fertilization - B	Irrigation regime - C
	F2 Micoseed MB® (1000 g/m³)	50%
		75%
		100%
	F3- Micoseed MB® (1500 g/m³)	50%
		75%
		100%
	F4- KSC I® (1000 g/m³)	50%
		75%
		100%
	F5 - KSC I® (2000 g/m³)	50%
		75%
		100%
A2 - Puno	NF- Control	50%
		75%
		100%
	F1- Micoseed MB® (500 g/m³)	50%
		75%
		100%
	F2- Micoseed MB® (1000 g/m³)	50%
		75%
		100%
	F3- Micoseed MB® (1500 g/m³)	50%
		75%
		100%
	F4- KSC I® (1000 g/m³)	50%
		75%
		100%
	F5- KSC I® (2000 g/m³)	50%
		75%
		100%
	F6- KSC I® (3000 g/m³)	50%
		75%
		100%

*(NF – control, F1 – 500 g/m³ MB, F2 – 1000 g/m³ MB, F3 – 1500 g/m³ MB, F4 – 1000 g/m³ KSC I, F5 – 2000 g/m³ KSC I, F6 – 3000 g/m³ KSC I).

For the watering of the substrate, water was used at different percentages quantities 50%, 75% and 100% of substrate capacity.

Table 2. Irrigation guidelines for *Chenopodium quinoa* cultivation

Irrigation (%)	Watering interval (days)	Watering rate (mL)
50	2	115-300
75	2	150-450
100	2	225-600

Data on taproot length, obtained through high-resolution 2D scanning and processed using Image Analyser software, were subjected to rigorous statistical analysis to evaluate the impact of the experimental treatments. After removing debris and washing the samples thoroughly, the roots were positioned on a flatbed scanner to capture clear, non-

overlapping images, thereby minimizing measurement errors. This digital mapping of root architecture ensured highly accurate quantification of longitudinal growth.

All reported values represent the arithmetic mean, expressed relative to the unfertilized control.

RESULTS AND DISCUSSIONS

An analysis of root system development in the Vikinga and Puno quinoa varieties under different fertilization regimes revealed a complex physiological response. The results showed that the length of the taproot was significantly influenced by both the type of fertilizer and the applied rate.

It is observed that the maximum root length values were recorded in the unfertilized (NF) treatment for both varieties (approximately 200 mm for Vikinga and 202 mm for Puno). This behaviour suggests an adaptation mechanism specific to *Chenopodium quinoa*, whereby, in the absence of sufficient nutrients, the plants allocate their energy resources toward the elongation of the main root in order to explore a larger volume of substrate and find the necessary macro- and microelements.

Biofertilizer applications resulted in a moderate reduction in root length compared to the control (NF), although significant variations were observed between the varieties. The Puno variety demonstrated superior growth performance under biostimulant treatment, maintaining a significantly greater root length in the F2 variant (approximately 157 mm) compared with Vikinga (approximately 105 mm). This difference indicates that the Puno variety responds more effectively to biological fertilization, leading to a stronger root system architecture.

The application of chemical fertilizers (KSC® II) had a marked inhibitory effect on the taproot, with the highest doses (F5, F6) resulting in an inhibition index of over 60% in both varieties. This response indicates a critical threshold for osmotic and salt stress. In contrast, biofertilization was better tolerated, particularly by the Puno variety, which exhibited greater stability (inhibition of only 22.07% in the F2 variant) compared to Vikinga (-47.68%).

Interestingly, in the case of Vikinga, the application of chemical fertilizer at the lowest concentration (F4) was less inhibitory than the average doses of biofertilizer (F2, F3). However, in the case of Puno, biofertilization proved to be better accepted by the plants than any form of chemical fertilization.

When chemical fertilizers were applied at the highest doses (F5, F6), regardless of the variety, inhibition exceeded 60%. This indicates a survival response, with main root growth limited by the high salinity of the substrate.

Correlating the data with irrigation regimes (50%, 75%, 100%) provides an essential perspective for the study, as water acts as a solvent that regulates salt concentration and the mobility of microorganisms in the root zone.

The results indicated that the lowest values were recorded in the treatments under a 50% irrigation regime. This case revealed that water deficiency concentrated salts from the chemical

fertilizers (KSC[®] II), leading to the lowest observed values in F5 and F6, with decreases exceeding 60% (Figure 1). These findings suggest that the roots are not only chemically inhibited, but also experience physiological stress due to water deficit. Under this regime, root length decreases drastically, as the plant cannot compensate for ion toxicity through water absorption (Kang et al., 2022).

The 75% irrigation regime proved to be the most favourable for quinoa, with the highest values. At this moisture level, the osmotic stress induced by chemical fertilizers (F5-F6) was partially mitigated by dilution, allowing the taproot to reach greater lengths than under the 50% regime (Figure 2). Biofertilization, combined with the 75% irrigation level, promoted microbial mobility, resulting in a low inhibition (-22%) for the Puno variety. In contrast, treatments under the 100% WHC exhibited intermediate values (Figure 3).

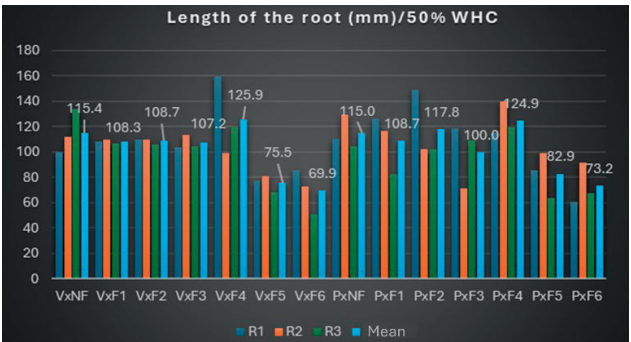


Figure 1. The combined influence of factors on primary root growth (mm)/ 50% WHC
 *(NF - control, F1 - 500 g/m³ MB, F2 - 1000 g/m³ MB, F3 - 1500 g/m³ MB, F4 - 1000 g/m³ KSC I, F5 - 2000 g/m³ KSC I, F6 - 3000 g/m³ KSC I)

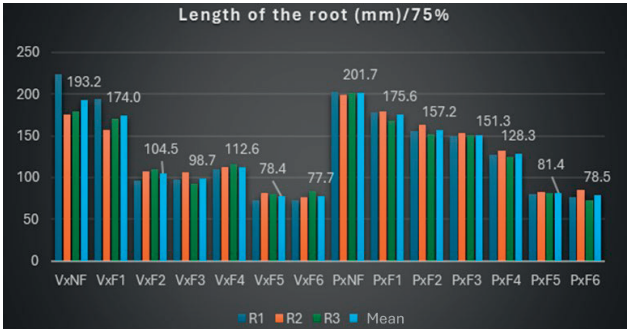


Figure 2. The combined influence of factors on primary root growth (mm)/ 75% WHC
 *(NF - control, F1 - 500 g/m³ MB, F2 - 1000 g/m³ MB, F3 - 1500 g/m³ MB, F4 - 1000 g/m³ KSC I, F5 - 2000 g/m³ KSC I, F6 - 3000 g/m³ KSC I)

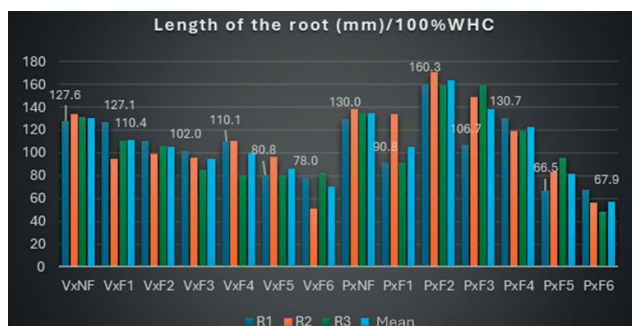


Figure 3. The combined influence of factors on primary root growth (mm)/ 100 % WHC
 *(NF – control, F1 – 500 g/m³ MB, F2 – 1000 g/m³ MB, F3 – 1500 g/m³ MB, F4 – 1000 g/m³ KSC I, F5 – 2000 g/m³ KSC I, F6 – 3000 g/m³ KSC I)

Variations in quinoa growth parameters under different irrigation regimes have been documented in previous studies. For instance, Khaled et al. (2021) identified that irrigation treatments applying 80% and 60% of reference evapotranspiration (ET₀) yielded the highest seed production. Furthermore, Mirsafi et al. (2024) noted an increase in the root-to-shoot ratio as the volume of applied irrigation water decreased. According to Mirsafi et al. (2024), the variation in root length is influenced by the irrigation regime since roots are the primary organs for absorbing water and nutrients. Consequently, their distribution in the soil is closely linked to the soil's water content and nutrient uptake capacity. Water scarcity often drives plants to develop deeper root systems by promoting elongation. Conversely, excessive water can lead to oxygen deprivation, which inhibits root growth and may cause decay. Mild drought conditions can enhance the root-to-shoot ratio as plants search for moisture, while prolonged water saturation restricts essential oxygen availability and may result in root rot (Ramachandran et al., 2020).

CONCLUSIONS

The absence of fertilization resulted in the greatest root elongation in both varieties. This behaviour confirms the high plasticity of *Chenopodium quinoa* and its ability to redirect energy resources toward substrate exploration when nutrient availability is limited. The Puno variety demonstrated superior stability and adaptability compared to Vikinga, maintaining a more robust root architecture

under biofertilization. Specifically, in the F2 variant, Puno exhibited an inhibition of only 22%, whereas Vikinga showed a drastic decrease of nearly 50%.

High doses of chemical fertilizers (F5, F6) exerted a pronounced toxic effect, with inhibition exceeding 60% across both cultivars. This significant decline indicates that a critical threshold for osmotic and saline stress, triggered by the concentration of KSC® II, was reached.

Optimal Regime (75%): proved to be the ideal regulatory factor, effectively mitigating chemical stress through dilution and enhancing microbial mobility within the root zone.

Limiting Regimes: In contrast, the 50% regime exacerbated salt toxicity due to concentration effects, while the 100% regime restricted growth by limiting oxygen availability at the root level (hypoxia).

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IN VITRO EVOLUTION OF SOME SWEET POTATO (*IPOMOEA BATATAS* [L.] LAM.) GENOTYPES UNDER THE INFLUENCE OF DIFFERENT PLANT GROWTH REGULATORS (AUXINS AND CYTOKININS)

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Abstract

A study was initiated with the aim of identifying an effective nutrient medium formulation capable of stimulating the growth and development of microplants. The in vitro development of five sweet potato genotypes (DK 19/1, DK 19/2, DCh 19/3, DK 19/4, and DK 19/5) was monitored. One month after culture initiation, the sweet potato microplants were evaluated for shoot length, number of leaves, and root length. Six nutrient media were formulated and compared with the control (Murashige-Skoog - without phytohormones). Regarding shoot length, number of leaves, and root length, the media variants V1 (MS+BAP 0.5 mg/L + IAA 0.5 mg/L), V4 (MS+KIN 0.5 mg/L+IAA 0.5 mg/L), and V0 (MS without phytohormones) showed the most notable effects. Media variants V6 (MS+KIN 0.5 mg/L+NAA 0.5 mg/L+IBA 1 mg/L) and V3 (MS+BAP 0.5 mg/L+NAA 0.5 mg/L+IBA 1 mg/L) had a positive influence on the number of roots. The study highlighted BAP 0.5 mg/L and IAA 0.5 mg/L, positively influencing the in vitro development of microplants across all five sweet potato genotypes.

Key words: auxin, cytokinin, genotype, plant tissue culture, sweet potato.

INTRODUCTION

Ipomoea batatas (L.) Lam., commonly known as sweet potato, is a resilient, nutritious, and versatile crop traditionally propagated using conventional methods. However, these methods have limitations, which has led to the adoption of micropropagation as an efficient alternative for producing healthy, cost-effective plantlets in a shorter time (Adu Donyina et al., 2025; Neja and Tileye, 2020).

Plant tissue culture has emerged as a powerful technique, offering the potential not only for rapid, clonal, and large-scale propagation of plant species needed in plantation programs, but also for the conservation of important and rare species. This technique has been widely applied across horticulture, plantation, and forestry sectors, significantly contributing to the enhanced production of high-quality planting material for the market (Siddique et al., 2015).

Plant growth regulators are chemical substances that play a vital role in the growth and development of plants (Singh, 2021; Zhang et

al., 2022; Woodward and Bartel, 2005). They act as chemical messengers that facilitate cell to cell communication to induce the growth and differentiation of plant cells, tissues, and organs (Feraru and Friml, 2008; Singh, 2021). Under *in vitro* conditions, plants can't synthesize hormones for their growth and development, and that's why exogenous hormones are required (Singh, 2021; Gerdakaneh et al., 2020; Bernula et al., 2020).

Auxins are considered the most important hormones because they play a role in all physiological processes of plants. They induce the formation of root buds, contribute to cell division, and are involved in tropic responses. Cytokinins influence cell division, thereby impacting plant growth, and they also promote the development of lateral buds while delaying the aging of organs and plant tissues (Sosnowski et al., 2023). Auxins are a growth-promoting substance that is involved in the shoot and root tips and promotes cell division, stem and root growth. In tissue culture conditions, auxins are used to induce root development (Falasca and

Altamura, 2003; Singh, 2021; El-Sherif, 2018). Sometimes, a high ratio of auxin to cytokinin is required for proper organ development in some species (Singh, 2021). The auxin response is concentration-dependent in most tissues, with different tissues exhibiting distinct responses to varying amounts of exogenous auxin (Thimann, 1937 cited by Zhang et al., 2022). Cytokinins play a role in the transport and accumulation of photosynthetic products and in regulating the activity of other enzymes, and they also have a significant impact on physiological and biochemical processes (Wang et al., 2022 cited by Sosnowski et al., 2023).

Auxins are produced by plants in the apical meristems of shoots, as well as in young leaves, seeds, and fruits. They promote the elongation of shoots and initiate the formation of adventitious and lateral roots. Cytokinins, on the other hand, are synthesized in root tips and in

unripe fruits and seeds. These hormones stimulate the growth of lateral shoots and also promote cell division (Sosnowski et al., 2023). This study aimed to identify an auxin-cytokinin combination that promotes the growth and development of sweet potato microplants, supporting the optimization of *in vitro* cultivation methods.

MATERIALS AND METHODS

Biological Material

Five sweet potato genotypes were chosen for this study: DK 19/1 (white flesh); DK 19/2 (yellowish-white flesh); DCh 19/3 (purple flesh); DK 19/4 (yellow flesh); DK 19/5 (pale yellowish flesh with pink streaks). These sweet potato genotypes belong to the Research and Development Station for Plant Culture on Sands Dăbuleni, Dolj County, Romania.



Figure 1. Source of explants for initiating *in vitro* cultures

To initiate *in vitro* cultures, sweet potato shoots obtained in the greenhouse were used as a source of nodal explants (Figure 1).

The *in vitro* regenerated microshoots (1.5-2 cm long) from these explants constituted the biological material for the experiment, which aimed to identify an optimal nutrient medium recipe to stimulate the *in vitro* growth and development of sweet potato microplants.

The experiment was conducted at the National Institute of Research and Development for Potato and Sugar Beet Brasov, Romania, Research Laboratory for Plant Tissue Culture.

Media and culture conditions

The aim was to identify an optimal culture medium formulation that would produce well-

developed sweet potato plantlets, characterized by larger leaves and a vigorous root system.

For this experiment, six artificial media formulations were designed as substrates for the *in vitro* cultivation of sweet potato explants and were compared with a control variant (V0), represented by the Murashige-Skoog Murashige & Skoog, 1962) medium without added phytohormones (Table 1).

The six medium variants were based on the MS formulation supplemented with a cytokinin (6-benzylaminopurine or kinetin) and auxins (either indole-3-butyric acid at different concentrations, or a combination of two other auxins - namely α -naphthalene acetic acid and indole-3- acetic acid).

Table 1. Composition of medium variants for *in vitro* cultivation of sweet potato

V0 (Control)	MS hormone-free
V1	MS + BAP 0.5 mg/l + IAA 0.5 mg/l
V2	MS + BAP 0.5 mg/l + IAA 1 mg/l
V3	MS + BAP 0.5 mg/l + NAA 0.5 mg/l + IBA 1 mg/l
V4	MS + KIN 0.5 mg/l + IAA 0.5 mg/l
V5	MS + KIN 0.5 mg/l + IAA 1 mg/l
V6	MS + KIN 0.5 mg/l + NAA 0.5 mg/l + IBA 1 mg/l

MS - Murashige-Skoog; BAP - 6-benzylaminopurine; KIN - kinetin; IAA - indole-3-acetic acid;
NAA - α -naphthalene acetic acid; IBA - indole-3-butyric acid.

Sucrose 20 g/l as a carbon source and agar 9 g/l were added to all medium variants. Prior to autoclaving, the pH of the medium was adjusted to 5.7. The nutrient medium was dispensed into glass test tubes (150 mm in height; 20 mm in diameter), previously sterilized in a drying oven for 2 hours at 180°C. After sealing the tubes with aluminum foil, the culture medium was sterilized in an autoclave at 120°C for 20 minutes.

The cultures were incubated in a growth room under artificial lighting conditions provided by neutral-white LED tubes (luminous flux of 2300 lm; 2000 lux light intensity) with a controlled temperature ($21 \pm 2^\circ\text{C}$) and photoperiod (16 hours light/8 hours dark). After one month from the initiation of the *in vitro* cultures, the sweet potato microplants were evaluated for shoot length (cm), number of leaves, number of roots, and root length (cm), depending on the genotype and the culture medium variant.

RESULTS AND DISCUSSIONS

Influence of growth regulators on the length of sweet potato microshoots

Regarding this parameter, the addition of 0.5 mg/l BAP to the nutrient medium along with 0.5 mg/l IAA (V1) led to the best result (1.98 cm), a favorable aspect in the micropropagation process, being the only variant that exceeded the control (Figure 2). On the other five medium variants, insignificant differences were recorded compared with the control (V0).

Comparing the evolution of the genotypes regarding microshoot length (Table 2), genotypes DCh 19/3 (2.56 cm) and DK 19/1 (1.78 cm) are distinguished, which recorded very significant differences (1.44 cm and 0.65 cm, respectively) compared to the control.

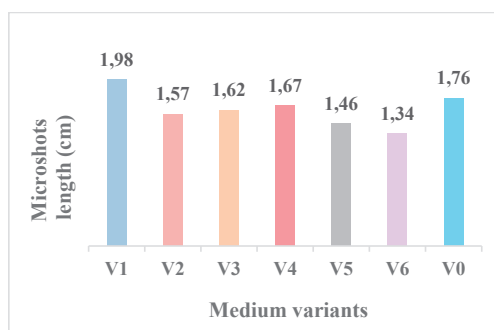


Figure 2. Effect of MS and plant growth regulators on sweet potato microshoot length after 1 month of culture

Table 2. Behavior of sweet potato genotypes regarding microshoots length

Genotype	Microshoots length (cm)		
	Mean	Diff.	Sign.
DK 19/1	1.78	0.65	***
DK 19/2	1.33	0.20	ns
DCh 19/3	2.56	1.44	***
DK 19/5	1.34	0.21	ns
DK 19/4 (Control)	1.13	-	-

LSD 5%: 0.33; LSD 1%: 0.43; LSD 0.1%: 0.56

After 1 month from the initiation of the cultures, the highest value of microshoot length was recorded in the DCh 19/3 genotype (3.23 cm) using the hormonal combination BAP (0.5 mg/l) + IAA (0.5 mg/l) in the culture medium (Table 3). In fact, this genotype also obtained the best results on all medium variants, except for the control variant (V0), where it was surpassed by the DK 19/1 genotype. Regarding the microshoot length parameter, the DCh 19/3 and DK 19/1 genotypes recorded distinctly significant and very significant differences compared to the DK 19/4 (control).

Table 3. Microshoot length of sweet potato genotypes, depending on the composition of the culture medium (after 1 month from culture initiation)

Genotype	Medium variants							
	V1		V2		V3		V4	
	Microshoot length (cm)	Diff./ Sign.	Microshoot length (cm)	Diff./ Sign.	Microshoot length (cm)	Diff./ Sign.	Microshoot length (cm)	Diff./ Sign.
DK 19/1	1.45	-0.43	1.55	0.08	1.60	0.58	2.15	1.23**
DK 19/2	1.63	-0.25	1.60	0.13	1.30	0.28	0.63	-0.30
DCh 19/3	3.23	1.35**	2.20	0.73	2.68	1.65***	3.13	2.20***
DK 19/5	1.73	-0.15	1.00	-0.48	1.48	0.45	1.53	0.60
DK 19/4 (Control)	1.88	-	1.48	-	1.03	-	0.93	-

Genotype	Medium variants					
	V5		V6		V0	
	Microshoot length (cm)	Diff./ Sign.	Microshoot length (cm)	Diff./ Sign.	Microshoot length (cm)	Diff./ Sign.
DK 19/1	1.80	1.20**	1.53	0.83	2.35	1.08*
DK 19/2	1.45	0.85	1.33	0.63	1.38	0.10
DCh 19/3	2.60	2.00***	2.13	1.43**	1.98	0.70
DK 19/5	0.83	0.23	1.03	0.33	1.80	0.53
DK 19/4 (Control)	0.60	-	0.70	-	1.28	-

LSD 5%: 0.86; LSD 1%: 1.14; LSD 0.1%: 1.47

The minimum value of microshoot length was determined in the DK 19/4 genotype (0.60 cm) by using the hormonal combination KIN (0.5 mg/l) + IAA (1 mg/l). In the DK 19/4, DK 19/5 and DK 19/2 genotypes the microshoots have the Lowest length in most medium variants, except for those that had the hormonal combination BAP (0.5 mg/l) + IAA (0.5 mg/l and 1 mg/l).

Influence of growth regulators on the leaves number

The average values recorded for this parameter varied between 5.50 on V4 and 4.00 on V2 (Figure 3).

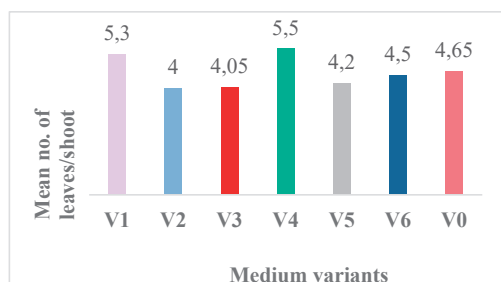


Figure 3. Effect of MS and plant growth regulators on the number of leaves/shoot after 1 month of culture

It can be observed that, irrespective of the cytokinin used (BAP or KIN), when the auxin concentration was higher (IAA 1 mg/L) or when

a combination of two auxins was applied (NAA 0.5 mg/L + IBA 1 mg/L), the number of leaves per plantlet was lower.

The hormonal combination consisting of KIN 0.5 mg/l and IAA 0.5 mg/l had a positive influence on the number of leaves, followed by the variants V1 and V0.

Regarding the influence of the genotype on the number of leaves/shoot (Table 4), the genotypes DK 19/1 (6.64), DK 19/5 (6.04) and DCh 19/3 (4.00) were noted. These were statistically different from the control, recording very significant positive differences (3.82 and 3.21), respectively significant (1.18).

Table 4. Behavior of sweet potato genotypes regarding number of leaves/shoot

Genotype	Number of leaves/shoot		
	Mean	Diff.	Sign.
DK 19/1	6.64	3.82	***
DK 19/2	3.50	0.68	ns
DCh 19/3	4.00	1.18	*
DK 19/5	6.04	3.21	***
DK 19/4 (Control)	2.82	-	-

LSD 5%: 0.93; LSD 1%: 1.24; LSD 0.1%: 1.60

Under the combined influence of genotype and culture medium composition, in terms of leaf number (Table 5), genotypes DK 19/1 and DK 19/5 were prominent across all medium variant.

Table 5. Behavior of sweet potato genotypes regarding the number of leaves/shoots, depending on the composition of the culture medium (1 month after culture initiation)

Genotype	Medium variants							
	V1		V2		V3		V4	
	Number of leaves	Diff./ Sign.	Number of leaves	Diff./ Sign.	Number of leaves	Diff./ Sign.	Number of leaves	Diff./ Sign.
DK 19/1	6.00	1.25	6.25	4.25***	4.75	2.75*	8.75	5.50***
DK 19/2	4.25	-0.50	3.75	1.75	3.00	1.00	2.75	-0.50
DCh 19/3	4.25	-0.50	3.50	1.50	3.75	1.75	5.00	1.75
DK 19/5	7.25	2.50*	4.50	2.50*	6.75	4.75***	7.75	4.50***
DK 19/4 (Control)	4.75	-	2.00	-	2.00	-	3.25	-

Genotype	Medium variants					
	V5		V6		V0	
	Number of leaves	Diff./ Sign.	Number of leaves	Diff./ Sign.	Number of leaves	Diff./ Sign.
DK 19/1	4.75	1.50	8.50	7.25***	7.50	4.25***
DK 19/2	4.00	0.75	3.75	2.50*	3.00	-0.25
DCh 19/3	4.25	1.00	4.00	2.75*	3.25	0.00
DK 19/5	4.75	1.50	5.00	3.75**	6.25	3.00*
DK 19/4 (Control)	3.25	-	1.25	-	3.25	-

LSD 5%: 2.46; LSD 1%: 3.27; LSD 0.1%: 4.22

The highest number of leaves was observed in genotype DK 19/1 (8.75) on medium variant V4, while the lowest value was recorded for genotype DK 19/4 (1.25) on medium variant V6. For genotypes DK 19/1 and DK 19/5, the hormonal treatment consisting of KIN 0.5 mg/L + IAA 0.5 mg/L had a positive effect on leaf number, resulting in the best outcomes (8.75 and 7.75, respectively). In contrast, genotypes DK 19/4 and DK 19/2 produced fewer leaves across all tested medium variants.

Both the culture medium without added phytohormones (V0-control) and the medium variants V1 and V4, in which an auxin (IAA) at a lower concentration (0.5 mg/L) was added alongside a cytokinin (BAP or KIN), resulted in

an average of more than 6 leaves per plantlet for genotypes DK 19/1 and DK 19/5.

Influence of growth regulators on the number of roots

Regarding the number of roots/shoots, the hormonal balance in the composition of the culture medium had a major influence. The results obtained in this study confirmed the role of auxins in inducing rhizogenesis (Fogaça and Fett-Neto, 2005; Kanungo et al., 2012; Singh, 2021; Shum and Dohm, 2003). The data presented in Table 6 highlight that medium variants containing a cytokinin (BAP or KIN) at 0.5 mg/L and two auxins (NAA 0.5 mg/L + IBA 1 mg/L) produced the best results in terms of root number.

Table 6. Effect of culture medium composition on the number of roots/shoots

Medium variants		Number of roots		
		Mean	Diff.	Sign.
V1	MS + BAP 0.5 mg/l + IAA 0.5 mg/l	5.65	2.05	ns
V2	MS + BAP 0.5 mg/l + IAA 1 mg/l	5.60	2.00	ns
V3	MS + BAP 0.5 mg/l + NAA 0.5 mg/l + IBA 1 mg/l	7.05	3.45	**
V4	MS + KIN 0.5 mg/l + IAA 0.5 mg/l	3.75	0.15	ns
V5	MS + KIN 0.5 mg/l + IAA 1 mg/l	4.60	1.00	ns
V6	MS + KIN 0.5 mg/l + NAA 0.5 mg/l + IBA 1 mg/l	8.25	4.65	***
V0	MS hormone-free (Control)	3.60	-	-

LSD 5%: 2.07; LSD 1%: 2.84; LSD 0.1%: 3.86

Sweet potato plantlets produced the highest number of roots on medium variants V6 (8.25) and V3 (7.05), showing highly significant (4.65) and significant (3.45) differences, respectively,

compared to the control (medium without added phytohormones). On the other medium variants, where a hormonal combination consisting of a cytokinin (0.5 mg/L) and an auxin (0.5 mg/L or

1 mg/L IAA) was applied, differences relative to the hormone-free medium were positive but not statistically significant.

Regarding the response of genotypes in terms of the number of roots/shoot, the most affected was genotype DK 19/4, which recorded a value of 2.50. All other genotypes showed highly significant differences compared with the control, with an average of more than 6 roots per shoot (Table 7).

Table 7. Behavior of sweet potato genotypes regarding the number of roots/shoot

Genotype	Number of roots/shoot		
	Mean	Diff.	Sign.
DK 19/1	6.21	3.71	***
DK 19/2	6.18	3.68	***
DCh 19/3	6.36	3.86	***
DK 19/5	6.25	3.75	***
DK 19/4 (Control)	2.50	-	-

LSD 5%: 1.74; LSD 1%: 2.31; LSD 0.1%: 2.98

Table 8. Behavior of sweet potato genotypes regarding the number of roots/shoot, depending on the composition of the culture medium (1 month after culture initiation)

Genotype	Medium variants							
	V1		V2		V3		V4	
	Number of roots	Diff./ Sign.	Number of roots	Diff./ Sign.	Number of roots	Diff./ Sign.	Number of roots	Diff./ Sign.
DK 19/1	6.75	4.50	6.25	5.25*	4.75	1.75	4.75	3.25
DK 19/2	7.25	5.00*	8.50	7.50**	5.50	2.50	3.00	1.50
DCh 19/3	5.00	2.75	7.00	6.00*	12.50	9.50***	2.75	1.25
DK 19/5	7.00	4.75*	5.25	4.25	9.50	6.50**	6.75	5.25*
DK 19/4 (Control)	2.25	-	1.00	-	3.00	-	1.50	-

Genotype	Medium variants					
	V5		V6		V0	
	Number of roots	Diff./ Sign.	Number of roots	Diff./ Sign.	Number of roots	Diff./ Sign.
DK 19/1	3.25	1.00	13.00	6.75**	4.75	3.50
DK 19/2	9.50	7.25**	6.00	-0.25	3.50	2.25
DCh 19/3	4.00	1.75	9.50	3.25	3.75	2.50
DK 19/5	4.00	1.75	6.50	0.25	4.75	3.50
DK 19/4 (Control)	2.25	-	6.25	-	1.25	-

LSD 5%: 4.60; LSD 1%: 6.11; LSD 0.1%: 7.89

Influence of growth regulators on root length

According to the results obtained (Table 9), regarding the composition of the culture medium, the variant in which no growth regulators were added (the control) determined the best results in terms of root length (6.51 cm). This was followed by the variants in which, along with cytokinin (BAP or KIN at a

Under the combined influence of genotype and the culture medium composition, the best results were recorded for genotype DK 19/1 (13.00) on the medium supplemented with KIN 0.5 mg/L + NAA 0.5 mg/L + IBA 1 mg/L, and for genotype DK 19/3 (12.50) on the medium supplemented with BAP 0.5 mg/L + NAA 0.5 mg/L + IBA 1 mg/L (Table 8).

As regards the number of roots per shoot, the medium variant containing KIN 0.5 mg/L + NAA 0.5 mg/L + IBA 1 mg/L promoted root formation in all sweet potato genotypes, resulting in an average of at least 6 roots per plantlet. On this medium, even genotype DK 19/4 had an average root number greater than 6, whereas on the other medium variants this parameter did not exceed 3 roots per shoot (Table 8).

concentration of 0.5 mg/l), a lower concentration of auxin (0.5 mg/l IAA) was used. And on these two variants, the average root length exceeded 6 cm (6.10 cm on variant V1, respectively 6.03 cm on variant V4), but without statistically significant differences compared to the control.

Table 9. Effect of culture medium composition on root length/shoot

Medium variants		Root length (cm)		
		Mean	Diff.	Sign.
V1	MS + BAP 0.5 mg/l + IAA 0.5 mg/l	6.10	-0.40	ns
V2	MS + BAP 0.5 mg/l + IAA 1 mg/l	3.47	-3.04	oo
V3	MS + BAP 0.5 mg/l + NAA 0.5 mg/l + IBA 1 mg/l	2.72	-3.79	oo
V4	MS + KIN 0.5 mg/l + IAA 0.5 mg/l	6.03	-0.47	ns
V5	MS + KIN 0.5 mg/l + IAA 1 mg/l	4.22	-2.29	o
V6	MS + KIN 0.5 mg/l + NAA 0.5 mg/l + IBA 1 mg/l	2.19	-4.32	ooo
V0	MS hormone-free (Control)	6.51	-	-

LSD 5%: 2.16; LSD 1%: 2.96; LSD 0.1%: 4.03

Table 10. Behavior of sweet potato genotypes regarding root length, depending on the composition of the culture medium (1 month after culture initiation)

Genotype	Medium variants							
	V1		V2		V3		V4	
	Root length (cm)	Diff./ Sign.	Root length (cm)	Diff./ Sign.	Root length (cm)	Diff./ Sign.	Root length (cm)	Diff./ Sign.
DK 19/1	8.90	0.73	7.10	6.80**	4.30	3.20	8.18	3.78
DK 19/2	5.83	-2.35	4.73	4.43	2.70	1.60	4.93	0.53
DCh 19/3	1.40	-6.78 ^{oo}	2.83	2.53	2.20	1.10	5.53	1.13
DK 19/5	6.20	-1.98	2.38	2.08	3.30	2.20	7.13	2.73
DK 19/4 (Control)	8.18	-	0.30	-	1.10	-	4.40	-

Genotype	Medium variants					
	V5		V6		V0	
	Root length (cm)	Diff./ Sign.	Root length (cm)	Diff./ Sign.	Root length (cm)	Diff./ Sign.
DK 19/1	6.85	3.85	4.30	3.60	12.33	8.63***
DK 19/2	4.30	1.30	2.50	1.80	5.10	1.40
DCh 19/3	3.33	0.33	0.75	0.05	3.63	-0.07
DK 19/5	3.60	0.60	2.68	1.98	7.78	4.08
DK 19/4 (Control)	3.00	-	0.70	-	3.70	-

LSD 5%: 4.73; LSD 1%: 6.27; LSD 0.1%: 8.10

Analyzing the data presented in Table 9, it can also be observed that the addition to the culture medium of the auxin combination NAA 0.5 mg/L + IBA 1 mg/L together with kinetin (0.5 mg/L) had a negative influence on root length, with the minimum value (2.19 cm) recorded for this medium variant and a very significant negative difference (-4.32 cm) compared with the control (V0). Although, on this medium variant (V6) the sweet potato genotypes obtained the best results in terms of the number of roots, the root length was strongly affected. From the analysis of sweet potato genotype performance, genotype DK 19/1 stands out, having recorded the greatest root length (7.42 cm) and showing a highly significant difference (4.37 cm) compared with the control (Table 11). It was followed by genotypes DK 19/5 (4.72 cm) and DK 19/2 (4.30 cm), although the differences between them were not statistically significant.

Table 11. Behavior of sweet potato genotypes regarding root length

Genotype	Root length (cm)		
	Mean	Diff.	Sign.
DK 19/1	7.42	4.37	***
DK 19/2	4.30	1.24	ns
DCh 19/3	2.81	-0.25	ns
DK 19/5	4.72	1.67	ns
DK 19/4 (Control)	3.05	-	-

LSD 5%: 1.79; LSD 1%: 2.37; LSD 0.1%: 3.06

The combined influence of genotype and culture-medium composition on root length (Table 10) highlights the V1 medium variant, in which the hormonal treatment consisting of 0.5 mg/l BAP + 0.5 mg/l IAA exerted a positive effect on root elongation in most genotypes, with the exception of genotype DCh 19/3 (1.40 cm), which showed a highly significant negative difference (-6.78 cm) compared with the control.

The hormone-free medium variant (V0) is also noteworthy, where the minimum root length recorded was 3.63 cm (DCh 19/3) and the maximum was 12.33 cm (DK 19/1). Genotype DK 19/1 stood out with respect to root length, achieving the highest values regardless of the culture-medium composition.

A negative effect on root elongation was observed in the V6 medium variant, where the minimum value was recorded in genotype DK 19/4 (0.70 cm), while the highest value, 4.30 cm, was obtained for genotype DK 19/1.

The use of BAP was more effective compared with KIN in terms of microshoot development, an aspect also observed in other studies (Tsemah et al., 2022; Koleva et al., 2012; Prabhakaran and Abraham, 2025).

In this study, the hormonal combination of a cytokinin (BAP or KIN) and IAA, in lower concentrations (0.5 mg/l), had a positive influence on leaf number. This aspect has also been observed in other research (Tsemah et al., 2022; Ali et al., 2015; Mayada and Seliem, 2025).

Regarding the length of microshoot roots, in our study the best results were obtained on the medium variant without the addition of phytohormones, followed by those with a low concentration of cytokinins or auxin. Inhibition of *in vitro* rooting due to the presence of cytokinins in the environment has also been observed by other researchers (Dobránszki et al., 2010; Dewir et al., 2016; Tahir et al., 2024; Luna et al., 2014). Also, as stated by Van Staden et al. (2008), the presence of cytokinins in high concentrations in the culture medium inhibits *in vitro* rooting, root growth, or delays the formation of roots. These results were previously demonstrated by Huetteman and Preece, who stated that cytokinins inhibit root formation, especially when used in shoot proliferation medium at high concentrations or for long periods of time.

CONCLUSIONS

In this study, seven medium variants and five sweet potato genotypes were tested with the aim of identifying a medium formulation that would promote the growth and development of microplants under controlled conditions. The *in vitro* performance of the five genotypes was

assessed by measuring growth parameters such as shoot length, number of leaves/shoot, number of roots/shoot, and root length.

Regarding the influence of culture-medium composition on shoot length, the addition of 0.5 mg/l BAP together with 0.5 mg/l IAA (V1) led to the highest value observed (1.98 cm).

Concerning the average number of leaves per shoot, the hormonal combination of 0.5 mg/l KIN and 0.5 mg/l IAA had a positive effect on this parameter, followed by variants V1 and V0. When a higher auxin concentration was used (1 mg/l IAA) or a combination of two auxins (0.5 mg/l NAA + 1 mg/l IBA) was applied, the number of leaves per shoot was lower, regardless of the cytokinin used (BAP or KIN). Regarding root length, the hormone-free medium variant produced the best results (6.51 cm). This was followed by variants in which a cytokinin (BAP or KIN at 0.5 mg/l) was combined with a lower concentration of auxin (0.5 mg/l IAA). Thus, the use of a cytokinin in combination with a lower concentration of an auxin in the culture medium, as well as the use of a hormone-free medium, led to the best outcomes in terms of shoot length, leaf number, and root length.

The hormonal balance in the culture medium had a major influence on the average number of roots per shoot. The results highlighted the medium variants containing a cytokinin (BAP or KIN at 0.5 mg/L) combined with two auxins (0.5 mg/L NAA + 1 mg/L IBA). Sweet potato microshoots produced the highest number of roots in medium variants V6 (8.25) and V3 (7.05), showing highly significant (4.65) and distinct significantly (3.45) differences compared with the control (medium without added phytohormones).

Among the seven culture-medium variants tested in this study, the hormonal combination of 0.5 mg/L BAP and 0.5 mg/L IAA (medium V1) was particularly effective, producing a positive response in all five sweet potato genotypes for every growth parameter measured. This medium consistently supported optimal *in vitro* development of the microplants. Research on culture-medium composition, particularly the presence of phytohormones, their combinations and the concentrations used, is of great importance for establishing an

effective *in vitro* cultivation protocol for sweet potato.

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COMPARATIVE EVALUATION OF EGGPLANT PLANT ESTABLISHMENT METHODS: CASE STUDY ON MIRVAL AND BLACK PEARL HYBRIDS

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Abstract

Efficient cultivation of eggplants is gaining renewed importance in sustainable farming strategies, as rising demand forces producers to manage land, water, and labour with a degree of precision that earlier systems seldom required. Given the crop's thermophilic nature, its performance depends strongly on the establishment method, either open-field planting or cultivation in unheated solar tunnels, an agronomic choice that has implications for both productivity and longer-term resource sustainability. Between 2019 and 2021, at the “V. Adamachi” Experimental Station, two hybrids, Mirval F1 and Black Pearl F1, were grown under two systems. Transplanted seedlings were maintained under relatively uniform irrigation and fertilisation regimes, allowing analysis of the factors that shape yield formation and resource-use efficiency, even though minor seasonal variations still occurred in practice. The comparative results indicated a consistent advantage for the protected environment: yields increased by approximately 50–70% relative to open-field production, largely due to a higher fruit load per plant and, to a smaller extent, slightly greater fruit mass. The hybrids displayed distinct adaptive patterns as well. Mirval F1 reached commercial maturity earlier and set more fruits per plant, whereas Black Pearl F1 produced larger fruits and expressed its yield potential most fully under the moderated microclimate of the tunnel structure.

Key words: Eggplant (*Solanum melongena* L.), high tunnel cultivation, open-field production, yield components, sustainable vegetable systems.

INTRODUCTION

Eggplant (*Solanum melongena* L.) has gradually assumed a prominent role among vegetable species, not only because of its nutritional profile and the concentration of antioxidant compounds it contains, but also due to the way it seems to integrate into culinary traditions (Gürbüz et al., 2018; Koley et al., 2019; Salamatullah et al., 2021; Silva et al., 2020). Currently, the eggplant crop occupies roughly 1.8 million hectares worldwide, from the milder Mediterranean zones to humid tropical regions, and even in places that challenge the species beyond its comfortable thermal limits (Rasouli et al., 2023).

Eggplant now ranks, according to Rasouli et al. (2023), in fourth place among the top greenhouse-grown vegetables. However, the exact position tends to fluctuate depending on the dataset consulted, and this variation appears linked to the crop's marked sensitivity to

stabilized, moderated environments (Shimira and Taşkın, 2022).

Unheated greenhouses and simple plastic tunnels enable growers to extend the season beyond what open fields usually permit, while also adjusting the microclimate in ways that outdoor systems simply cannot (Sharaf-Eldin et al., 2023). These advantages have become more salient as weather patterns show an increasingly erratic behaviour from one year to the next, pushing agriculture toward forms of “sustainable intensification” that are not always easy to implement (Bell et al., 2024).

Despite these benefits, a significant number of farmers continue to rely on open-field production, partly due to familiarity and partly because the upfront costs remain far lower (Gan et al., 2022). This method demands fewer technological inputs, yet exposes the crop to abrupt temperature declines, uneven rainfall, and a range of pest pressures. Each of these factors may curtail, at times quite severely, the

level of productivity that eggplant can achieve when grown under more buffered conditions. Selecting an appropriate cultivation system has become a rather persistent challenge in sustainable agriculture, as producers must attain high yields while avoiding the gradual depletion of the resources on which the system relies (Khan et al., 2021). In temperate continental regions, the NE part of Romania offers a familiar example: summers generally supply adequate heat, yet the frost-free period remains unexpectedly short (Rothacher, 2021). This compressed window limits the growth trajectory of thermophilic crops such as eggplant when they are placed directly into open soil. Recent research reinforces the advantages of protected systems. For example, Shaik et al. (2023) showed that organic eggplants cultivated in tunnel greenhouses produced roughly 84% more than those in the open field, largely due to improved fruit set and a modest increase in average fruit mass (Ciubotarita et al., 2022). Resource management can be notably improved, particularly in semi-arid regions. In Egypt, for example, Fan et al. (2023) have shown that greenhouse-grown eggplants required nearly 90% less irrigation than their field counterparts, while net profit increased by more than 80%, even if seasonal costs did not remain fully constant, as so often happens. Taken together, these observations indicate that protected cultivation offers considerable potential for improving both economic outputs and resource efficiency, especially in areas where open-field production is repeatedly pressured by climatic instability. The benefits of protected cultivation for eggplant are often discussed, but its performance compared with open-field systems is not well understood, particularly in Romania's specific climate and with recent hybrids. Existing evidence is often limited, lacking comprehensive multi-year data to accurately compare yield and quality between the two methods, especially regarding economic sustainability. Excessive heat in greenhouses can hinder production without proper ventilation, and a lack of natural pollinators may negatively impact fruit set (Abbas et al., 2025). One study found that a controlled greenhouse yielded smaller harvests than nearby field plots due to inadequate pollination and low light penetration

(Dingley et al., 2022). This raises the unresolved question of how the advantages of protected cultivation translate into actual field-level gains, considering the differing responses of eggplant hybrids to contrasting environments.

This study aimed to clarify ambiguities by comparing eggplant grown in a tunnel versus open-field conditions, focusing on total yield, fruit number per plant, and average fruit weight. The hybrids selected, Mirval F1 and Black Pearl F1, are part of a more recent cohort of high-performance genetic lines. Both are marketed for protected and outdoor cultivation, though their profiles differ: Mirval F1 is generally classified as very early and relatively stable across environments (Abdelnaby et al., 2024), whereas Black Pearl F1 is usually recommended for open-field production (Gaccione et al., 2023), producing medium-to-large fruits with a teardrop shape and a high degree of uniformity, even if its performance shifts a bit from year to year.

MATERIALS AND METHODS

The research was carried out from 2020 to 2021 in the "V. Adamachi" Research and Training Station Iași, Romania. The area is characterised by a temperate-continental climate; summers tend to be warm, with July means around 21–22°C, although a fair number of days rise well above 30°C. Rainfall occurs irregularly and, rather often, at moments that complicate open-field management, influencing crop performance more than producers usually care to admit (Istrate et al., 2023).

Each experimental cycle began in early spring and continued into autumn, with harvests collected through summer and the early part of autumn. The same design was implemented during 2020 and 2021, allowing the datasets to remain comparable and, at least in principle, to provide more dependable trends, even if small seasonal shifts did appear from one year to the next.

For ease of interpretation, Table 1 groups the principal experimental factors and indicates how each was applied in practice, including brief notes from field observations. Table 1 summarises the principal elements of the experimental design, indicating how each factor was defined and applied across the two cultivation systems, showing how the hybrids could

diverge in their behaviour, and allowing the yield outcomes to be read against the broader agronomic tendencies that emerged, even if a section of year-to-year drift was, unavoidably, still present. The greenhouse used in the trial was a straightforward, unheated tunnel covered with

PE film, equipped with manually operated side vents, and occupying roughly 250 m². The open-field plots assigned to the unprotected variant were situated only a short distance away, on the same well-drained cambic chernozem, ensuring that soil variability would not interfere with comparisons.

Table 1. Overview of the experimental framework

Factor	Practical meaning	Examples from the study
Cultivation method	Compares crop performance in a protected environment and in open field	Solar greenhouse setting; conventional field plots
Hybrid used	Two modern hybrids assessed for their contrasting traits:	Mirval F1, Black Pearl F1
Seedling production	Seedlings grown in trays with sterile substrate under controlled temperature	Transplanting performed once plants had 5 to 6 true leaves
Planting calendar	Transplant dates adapted to climatic risks in each system	Greenhouse: mid to late April; Field: early May
Phenological advantage in greenhouse	Plants start growing earlier under protection than outdoors	Roughly a three-week advance in early development
Mirval F1 traits	Very early type, good adaptability, and stable yield potential	Earlier fruit initiation and higher fruit number
Black Pearl F1 traits	Vigorous hybrid producing larger fruits, performs strongly in open field	Higher fruit mass and more robust vegetative growth
Expected hybrid differences	Anticipated variability in response to environmental conditions	Mirval favoured by field timing; Black Pearl enhanced in greenhouse

Soil preparation followed the standard routine: autumn ploughing, disking, and bed formation. Inside the tunnel, the beds ended up somewhat wider to make slightly better use of the protected surface, although planting density was kept the same in both systems, at least in theory; small deviations do slip in during fieldwork. Plants were spaced 70 cm between rows and 40 cm on the row, a configuration equivalent to approximately 35,000 plants/ha. Each experimental plot, corresponding to one A×B combination, contained four rows with ten plants per row, yielding forty plants over an effective surface of a little under 11 m². The study employed a two-factor randomized block arrangement with three replications. Factor A, the cultivation environment, could not be fully randomized, since each block necessarily included one greenhouse section and a corresponding open-field section positioned nearby.

Overall crop management

Crop management was maintained as closely as practicable between the two cultivation systems, since the intention was for any differences to arise from the experimental factors rather than from extra technological variability. Prior to planting, the soil received well-decomposed farmyard manure-about 30 t/ha, incorporated in

autumn to provide a reasonably uniform nutrient background. At transplanting, a small starter dose was placed directly in each planting hole, roughly 5 g of a 14-14-14 NPK formulation, a routine measure many growers use to help seedlings settle a bit quicker. Throughout the vegetation period, both systems followed the same top-dressing schedule. Ammonium nitrate was applied three weeks after transplanting at around 50 kg/ha, followed by two monthly applications of a 20-20-20 fertilizer, close to 5 kg/ha each time. Irrigation relied on drip lines, one per row, in both the tunnel and the open field, all connected to the same water source so that no hidden discrepancies would creep in unnoticed. Under typical weather conditions, watering occurred twice weekly; during hotter intervals, it increased to three times, supplying roughly 25-30 mm per week, with open-field adjustments depending on rainfall.

To reduce water loss and suppress weeds, black polyethylene mulch was laid across the soil surface in both environments. It maintained slightly higher soil temperatures early in the season, benefiting eggplant establishment, though this advantage diminishes as summer warms the soil.

Disease and pest control

Disease and pest management was approached through a mix of preventive steps and direct interventions, whenever the situation genuinely required it, following the general principles of Integrated Pest Management (Deguine et al., 2021). Yellow sticky traps were used for monitoring, and treatments relied mainly on potassium soap or neem-based formulations, avoiding synthetic substances in the enclosed space whenever reasonably possible.

To improve pollination, we opened side vents and doors for insect access and introduced bumblebee hives (*Bombus terrestris*) during flowering. Studies show that using bumblebees can increase yields by 20-25% in protected systems (Khalifa et al., 2021).

Data recording and statistical analysis

Data were collected throughout the season, with observations recorded whenever a developmental stage appeared, such as seedlings taking hold after transplanting, the onset of flowering, the first visible fruit set, and later when fruits reached a marketable size. A marked difference in earliness emerged: plants inside the tunnel began flowering roughly 7-10 days ahead of those in the open field. Between the two hybrids, Mirval F1 maintained its reputation for very early maturity, usually reaching the first harvest about 5 days before Black Pearl F1. This tendency was observed in both systems, although the exact calendar dates drifted slightly from one year to the next.

Harvesting was done manually in successive rounds, once fruits showed the usual indicators of commercial maturity, glossy skin and the firmness typical for each hybrid. Harvests continued until yields fell to negligible levels: in early October inside the tunnel, when night temperatures dropped below 10°C, and by mid-September outside, after the first light frosts stopped growth.

For each plot, the total yield in kilograms and converted to a per-hectare basis, was recorded. Fruit counts were also taken to estimate the mean number of fruits per plant. Average fruit weight was determined both from the ratio of total yield to fruit number and from a 20-fruit sample weighed at peak harvest; a procedure that helped smooth out the usual small irregularities in fruit size.

RESULTS AND DISCUSSIONS

The comparison between the two cultivation systems showed marked and consistent differences in total yield per hectare across the three study years. Plants grown in the solar tunnel outperformed those in the open field in every season, and this held true for both hybrids without any real exception. When the averages were considered together, the protected environment produced close to 60% higher yields than the outdoor plots, which more or less aligned with the expectations formed at the outset.

For Mirval F1, the mean yield reached roughly 65.3 t/ha in the tunnel, whereas the same hybrid produced around 45.1 t/ha under open-field conditions. The contrast was sharper for Black Pearl F1, which recorded about 70.4 t/ha under protection but only 40.2 t/ha outside. Expressed in relative terms, Black Pearl gained nearly 75% over its open-field yield, while Mirval showed an increase of about 45%. The ANOVA confirmed the strong effect of the Environment factor ($p < 0.05$), underscoring how a moderated microclimate can, quite literally, reshape production levels (Table 2).

Table 2. Yield performance of eggplant hybrids

Hybrid	Greenhouse yield (t/ha)	Open-field Yield (t/ha)	Yield increase (%)
Mirval F1	65.3	45.1*	45%
Black Pearl F1	70.4*	40.2	75%
Mean (both hybrids)	67.85	42.65	59%

*significance for $p \leq 0.05$

These patterns echo findings reported elsewhere. A study from South Texas observed that organic eggplant grown in high tunnels achieved an 84% yield increase relative to outdoor plants, driven mostly by approximately 63% more fruits per plant and a smaller increase in average fruit weight of about 9% (Shaik et al., 2023). Comparable tendencies were visible in our dataset as well, though the specific components are examined in the next sections. The yield figures from the trial indicate the advantage of protected cultivation, with both hybrids performing better in the tunnel than in the open field. The extent of the gain did not follow the same pattern across hybrids. The yield comparison of the two hybrids suggests that Black Pearl depends more heavily on a steady microclimate and may cope less well

with the small but persistent stresses that accumulate in open-field settings.

Taken together, the two hybrids produced close to 60% higher yields in the protected environment than in the field plots. This difference appeared each year for both hybrids, despite their distinct growth habits, supporting the idea that controlled structures offer a stable, almost dependable advantage for eggplant cultivation, even if field conditions, as growers know too well, seldom behave exactly the same from one year to another.

Number of fruits and average fruit weight

A closer examination of the yield components revealed distinct differences between the two cultivation systems, as well as between the hybrids themselves. Plants grown inside the greenhouse tended to carry more fruits per plant, and those fruits were, on average, marginally heavier than the ones obtained outdoors. Across years and hybrids, the protected environment produced roughly 30% more fruits per plant, a tendency that stayed surprisingly steady, even if the exact figures varied a little from one season to the next (Table 3).

For Mirval F1, the greenhouse conditions resulted in about 6.8 fruits/plant, compared with roughly 5.0 in the open field. Black Pearl F1 showed the same directional shift but with a sharper contrast: around 5.8 fruits/ plant in the tunnel and only 3.8 outdoors. This wider difference suggests that the shorter and more irregular field season likely restricted Black Pearl from forming a higher fruit load.

Despite these contrasts, Mirval F1 remained the hybrid with the higher fruit number in both environments. Its advantage, about 1.2 fruits/plant in the tunnel and outdoors alike, fits its known morphology of short internodes and a notably strong fruit-setting ability, although in some plots the pattern appeared a bit less tidy than the numbers might imply.

Table 3. Eggplant fruits number from experiment

Hybrid	Greenhouse fruit number	Open-field fruit number	Differences (%)
Mirval F1	6.8*	5*	36%
Black Pearl F1	5.8	3.8	53%
Mean	5.8	3.8	43%

*significance for $p \leq 0.05$

Average fruit weight

The examination of average fruit weight revealed a pattern almost opposite to that observed for fruit number. Black Pearl F1 consistently produced heavier fruits than Mirval F1, both in the tunnel and under field conditions. In the open field, Black Pearl averaged roughly 350 g, whereas Mirval remained closer to 280 g, a difference of about 25%. Under the steadier conditions of the greenhouse, both hybrids increased in weight: Black Pearl reached around 400 g, while Mirval settled near 320 g. The gap between them, usually in the 70-80 g range, stayed surprisingly constant, suggesting a strong genetic control over this trait. Black Pearl is bred as a large-fruited type, typically around 19 × 10 cm with an ovoid or slightly teardrop profile, while Mirval produces more elongated fruits of roughly 20 cm, most often falling in the 250-300 g interval in repeated observations (Table 4). What emerges quite clearly is that the cultivation environment influenced the fruit number far more than the weight of individual fruits.

Table 4. Eggplant fruit weight from experiment

Hybrid	Greenhouse fruit weight (g)	Open-field fruit weight (g)	Differences (%)
Mirval F1	320	280	14.3%
Black Pearl F1	400*	350*	14.3%
Mean	320	280	14.3%

*significant for $p \leq 0.05$

In proportional terms, average fruit weight increased by about 14% in the greenhouse, roughly 40 g for Mirval and about 50 g for Black Pearl. By contrast, fruit number rose by nearly 36% in Mirval and close to 53% in Black Pearl. This pattern indicates that the tunnel enhanced yield chiefly by extending the period during which plants could set fruits and by buffering late-season stress, rather than by making individual fruits substantially heavier.

Debates among growers focus on whether markets prefer smaller or larger fruits, with local demand dictating the preference. Both systems produced sizes within commercial expectations, with the greenhouse increasing both weight and fruit count, which is ideal. The weight differences between hybrids reflect their breeding strategies. Mirval emphasizes early maturity and a heavier fruit load, leading to

smaller fruits. In contrast, Black Pearl produces fewer fruits but allows for greater mass due to a later production peak. This pattern is common: genotypes with high fruit counts yield smaller fruits, while those with moderate counts often produce larger ones. Both can achieve similar total yields but require different management - heavy setters may need fruit thinning, while large-fruited types need careful nutrient management to prevent flower drop.

Earliness and harvest dynamics

Plants grown inside the tunnel-entered production notably sooner, roughly two to three weeks ahead of those in the open field, an advance that carries real weight for growers, since early-season fruit usually commands higher prices.

Mirval F1, owing to its quicker maturity, reached its first marketable stage toward the end of June in the tunnel, at least in 2020 and 2021 when spring came somewhat warmer than expected. Black Pearl F1 followed only shortly after, with initial harvests appearing in the first days of July. In the open field, the calendar shifted backwards: Mirval generally began around 10-15 July, while Black Pearl tended to reach usable maturity closer to the month's end, sometimes even a bit beyond, depending on the weather swings.

These contrasts in maturity rate shaped the seasonal distribution of yield as well. Mirval placed a larger share of its production in the early half of the harvest window, whereas Black Pearl maintained a steadier output and continued fruiting into the later weeks. The divergence was especially clear inside the greenhouse, where Black Pearl still produced reasonably well until late September, while Mirval had already shown a decline, most likely because it had pushed much of its growth and assimilates earlier in the cycle.

In practical terms, Mirval tended to "finish" its main period of productivity sooner, whereas Black Pearl stretched its output across a longer span.

CONCLUSIONS

The comparative assessment of eggplant grown in solar-tunnel structures and in open-field plots, using the hybrids Mirval F1 and Black Pearl F1,

brought into view several aspects relevant for sustainable vegetable production. The unheated tunnels created a more buffered microclimate, and the plants, almost predictably, responded with yields roughly 50-70% higher than those obtained outdoors.

The hybrids themselves did not respond identically. Mirval F1, being a very early type, performed reliably even outdoors and maintained a stable number of medium-sized fruits despite the more erratic conditions. Black Pearl F1 expressed its potential far more fully in the extended, calmer tunnel environment, where it produced larger fruits and generally higher yields once the plants had enough time to progress through their cycle. For growers, the results indicate that unheated tunnels represent a practical investment in temperate climates, increasing productivity while softening the impact of weather fluctuations. Even simple structures require attention: ventilation to prevent overheating and ensuring adequate pollination, at times with bumblebees, so fruit set does not suffer. Techniques such as drip irrigation and mulching, applied consistently in this study, ought to be seen as standard components rather than optional additions.

From a wider sustainability perspective, the move toward efficient protected systems advances ecological and economic aims simultaneously. Higher yields per area reduce pressure on land, water is used more sparingly since direct evaporation is minimal, and nutrients remain available instead of being washed away by rain. Although tunnels bring an initial cost, the steadier and higher returns typically balance this investment within a few seasons, strengthening longer-term resilience on the farm.

Stripped of the smaller details, the central point is that choosing the cultivation environment is not merely a technical adjustment but a strategic decision. A basic solar tunnel paired with a hybrid that fits its growth rhythm can lift production considerably without imposing notable ecological burdens. Further economic analysis or work on nutritional traits would refine the picture, although the core finding remains firm: protected cultivation offers a distinct advantage when managed according to the crop's requirements.

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IN VITRO CULTURE, ACCLIMATIZATION AND BIOCHEMICAL TRAITS OF *HYPERICUM PERFORATUM* L.

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Abstract

The present study aims to develop an efficient in vitro culture and acclimatization protocol for Hypericum perforatum and to provide a preliminary biochemical assessment of micropropagated plants compared to acclimatized plants. In vitro cultures were initiated from nodal explants collected from greenhouse-grown plants and established on Murashige and Skoog (MS) medium. Shoot multiplication was evaluated on Driver and Kuniyuki Walnut (DKW) medium supplemented with different cytokinins, while rooting was assessed on DKW medium with and without indole-3-butyric acid. Ex vitro acclimatization was performed in perlite under controlled humidity conditions, followed by transfer to a peat substrate under greenhouse conditions. The highest shoot multiplication rate was obtained on medium supplemented with benzyladenine, while the best rooting response was recorded on auxin-free medium, which resulted in high explant viability. Acclimatization rates were high for both rooted and non-rooted shoots, with superior growth performance observed in plantlets rooted in vitro. The biochemical analysis revealed comparable levels of phenolic compounds and flavonoids in both plant categories, suggesting that in vitro-derived biomass may represent a potential source of phenolic compounds.

Key words: micropropagation, 6-benzyladenine, total phenolic content (TPC), total flavonoid content (TFC).

INTRODUCTION

Hypericum perforatum L. (St. John's wort) is a medicinal plant of significant pharmacological interest due to its rich content of bioactive secondary metabolites, especially phenolic compounds and flavonoids, which contribute to antioxidant, anti-inflammatory, and therapeutic effects (Al-haliem et al., 2025; Al Nooh et al., 2025; Amooaghaie & Rajaie, 2025; Clapa et al., 2025; Kilibarda et al., 2025; Mišina et al., 2025; Rusanova et al., 2025). The continued scientific interest in *H. perforatum* and related *Hypericum* species is reflected in recent research on in vitro propagation and phytochemical analysis, highlighting the need for robust protocols to produce uniform plant material with consistent biochemical profiles (Kwiecień et al., 2023; Ravindran et al., 2023; Shasmita et al., 2023; Tzatzani et al., 2023; Ahmed et al., 2025).

Conventional propagation and wild harvesting may lead to variable phytochemical profiles in *H. perforatum*, depending on environmental factors, genotype, and developmental stage (Barnes et al., 2001; Southwell & Bourke, 2001; Zobayed et al., 2024; Kilibarda et al., 2025). Additionally, intensive harvesting from natural habitats can raise sustainability concerns (Chen et al., 2016). These limitations emphasize the need for alternative propagation strategies that ensure both uniformity in plant material and stability in phytochemical composition (Selvakesavan & Franklin, 2021).

In vitro culture techniques are widely adopted for rapid micropropagation of medicinal plants, offering advantages such as year-round production, genetic uniformity, and pathogen-free material (Hasnain et al., 2022; Anuruddi et al., 2023; Rahimi et al., 2023; Thiem et al., 2023; Samridha & Chandra, 2024). Recent studies

have demonstrated efficient shoot initiation and multiplication in several *Hypericum* species, including *H. perforatum*, with optimized responses to plant growth regulators and improved acclimatization strategies under controlled conditions (Khakpour et al., 2015; Sood et al., 2015; Abdollahpoor et al., 2017; Fascella et al., 2017; Syahid & Wahyuni, 2019; Ravindran et al., 2023).

In addition to morphological development, *in vitro* conditions and acclimatization may influence secondary metabolism (Espinosa-Leal et al., 2018; Grzelak et al., 2024). Phenolic compounds and flavonoids are important for plant defense and stress adaptation and are often used as indicators of biochemical stability in medicinal plants (Reshi et al., 2023). Assessing total phenolic and flavonoid contents in both *in vitro* cultured and acclimatized plants provides insight into the impact of micropropagation on phytochemical traits and biomass quality (Moraes et al., 2012; Kikowska et al., 2020; Custódio et al., 2022).

Therefore, the present study aims to develop an efficient *in vitro* culture and acclimatization protocol for *H. perforatum* and to perform a preliminary assessment of total phenolic and total flavonoid contents in *in vitro* cultured and acclimatized plants.

MATERIALS AND METHODS

In vitro culture conditions

All *in vitro* culture stages were performed under the same controlled environmental conditions. Culture initiation was carried out in test tubes containing 5 mL of culture medium, while shoot multiplication and rooting stages were conducted in 720 mL culture jars containing 100 mL of medium. All culture media were supplemented with 30 g/L sucrose and solidified with 5 g/L plant agar. The pH of the media was adjusted to 5.8 prior to autoclaving at 120°C for 20 min. Cultures were maintained at $23 \pm 1^\circ\text{C}$ under a 16-h light/8-h dark photoperiod, with a light intensity of $32.4 \mu\text{mol m}^{-2} \text{s}^{-1}$. Unless otherwise stated, all chemicals and culture media components were purchased from Duchefa Biochemie B.V. (Haarlem, The Netherlands).

Initiation of *in vitro* cultures

In vitro cultures of *H. perforatum* were initiated using nodal explants excised from juvenile

shoots collected from greenhouse-grown plants. The explants were surface-sterilized using a 20% (v/v) commercial bleach solution (5% active chlorine) for 20 minutes and rinsed three times with sterile distilled water under aseptic conditions. Single-node explants were inoculated individually onto Murashige and Skoog (MS) (Murashige & Skoog, 1962) basal medium without plant growth regulators (PGRs). The cultures were maintained under the environmental conditions described above. After four weeks of culture, the percentage of culture initiation was recorded based on the number of viable, regenerated explants.

In vitro shoot multiplication

For the shoot multiplication stage, Driver and Kuniyuki Walnut (DKW) (Driver and Kuniyuki, 1984) basal medium was supplemented with different plant growth regulators (PGRs): 2 mg/L kinetin (Kin), 2 mg/L meta-topolin (mT), 1 mg/L zeatin (Z), 5 mg/L 2-isopentenyladenine (2iP), 0.1 mg/L forchlorfenuron (CPPU), and 0.1 mg/L benzyladenine (BA). Five nodal explants were inoculated per culture vessel. After 30 days of culture, the multiplication rate (number of shoots per initial explant) and shoot length were recorded.

In vitro rooting and *ex vitro* acclimatization

For the *in vitro* rooting stage, explants with apical buds of *H. perforatum* were cultured on DKW basal medium supplemented with indole-3-butyric acid (IBA) at concentrations of 0.5, 1.0, and 1.5 mg/L, as well as on an auxin-free control medium. Fifteen explants were inoculated per culture vessel. After 30 days of culture, rooting percentage, number of roots per explant, root length, and shoot viability were recorded.

Ex vitro acclimatization was performed using both *in vitro*-rooted plantlets and non-rooted shoots. The plant material was transferred to trays containing moistened perlite and maintained under high humidity conditions, which were gradually reduced over two weeks. During acclimatization, plantlets were regularly misted to prevent dehydration. After six weeks, acclimatized plants were transferred to peat substrate in alveolar trays and maintained under greenhouse conditions. Survival rates were assessed after 30 days.

Biochemical analysis (TPC and TFC)

Total phenolic content (TPC) and total flavonoid content (TFC) were determined spectrophoto-

metrically in *in vitro* cultured and acclimatized plants of *H. perforatum*. Fresh plant material (0.5 g) was extracted with acidified methanol (1% HCl) and the obtained extracts were used for biochemical analyses.

TPC was determined using the Folin-Ciocalteu method (Singleton et al., 1999; Puia et al., 2025) with gallic acid as the standard, and the results were expressed as μg gallic acid equivalents per g fresh weight (μg GA/g FW). TFC was determined using the aluminium chloride colorimetric method, with quercetin as the standard, and the results were expressed as μg quercetin equivalents per g fresh weight (μg Q/g FW) (Zhishen et al., 1999; Molnar et al., 2024). The concentration of TPC and TFC was calculated using standard calibration equations derived from gallic acid and quercetin, respectively. For TPC, the calibration equation was $y = 0.0011x + 0.1319$, with $R^2 = 0.9981$. For TFC, the calibration equation was $y = 0.001x + 0.0313$, with $R^2 = 0.9952$, where y represents the absorbance and x represents the concentration of standard equivalents used for calibration. Absorbance was measured using a UV-Vis spectrophotometer.

Statistical analysis

Experiments were arranged in three replicates, and data were expressed as mean $\pm$ standard error. Statistical analysis was performed using OriginPro software.

RESULTS AND DISCUSSIONS

In vitro culture initiation

H. perforatum responded satisfactorily to the applied *in vitro* initiation protocol. After four weeks of culture, an initiation rate of 64.44% was recorded, while 8.89% of explants showed microbial contamination (Figure 1).

A relatively high percentage of non-developing but non-contaminated explants (26.67%) was also observed. Similar responses have been reported in *H. perforatum*, where explant sensitivity to surface sterilization and oxidative stress may negatively affect culture establishment (Tzatzani et al., 2023). Nevertheless, the obtained initiation rate enabled the successful establishment of viable *in vitro* cultures for subsequent multiplication stages.

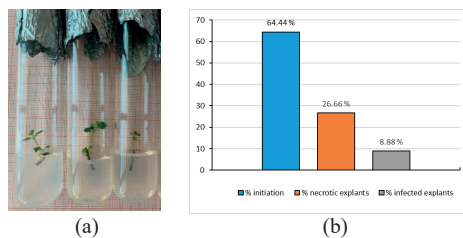


Figure 1. Initiation of *in vitro* culture of *H. perforatum*: (a) - initiation of *in vitro* culture from mini-cuttings with one node; (b) - Percentage of initiation

In vitro shoot multiplication

Shoot multiplication was significantly influenced by the type of cytokinin supplemented in the culture medium. The lowest number of shoots per explant was recorded in the presence of meta-topolin (1.6 ± 0.17), while the highest number (6.6 ± 0.50) was obtained on DKW medium supplemented with benzyladenine (Figure 2a, Figure 3). These results confirm the high responsiveness of *H. perforatum* to benzyladenine, which has been widely reported as an efficient cytokinin for shoot proliferation in this species (Savio et al., 2012).

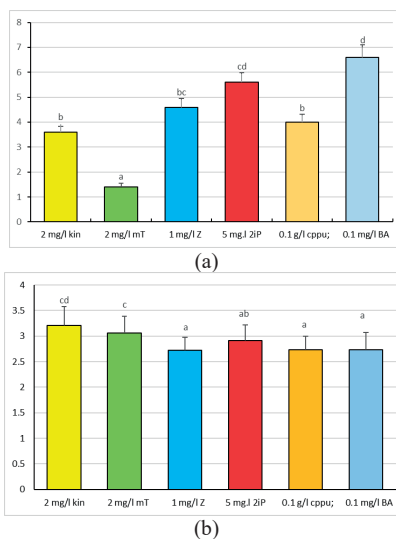


Figure 2. Influence of growth regulators on the number of shoots/explant (a) and shoot length (b), in *H. perforatum*, cultivated *in vitro* on DKW medium supplemented with the following growth regulators: kinetin (2 mg/L kin); metatopolin (2 mg/L mT); zeatin (1 mg/L Z); 2-isopentenyladenine (5 mg/L 2iP); forchlorfenuron (0.1 mg/L CPPU); benzyladenine (0.1 mg/L BA)

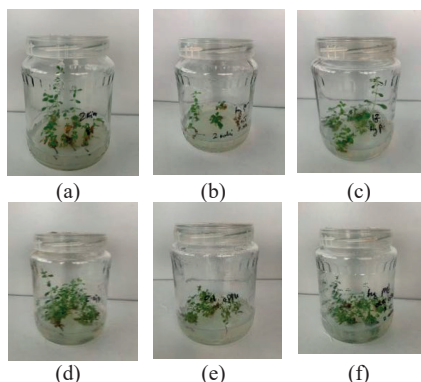


Figure 3. *In vitro* cultures of *H. perforatum* on DKW medium supplemented with different growth regulators: (a) - 2 mg/4L kinetin (kin); (b) - 2 mg/L metatopolin (mT); (c) - 1 mg/L zeatin (Z); (d) - 5 mg/L 2-isopentenyladenine (2iP); (e) - 0.1 mg/L forchlorfenuron (CPPU); (f) - 0.1 mg/L benzyladenine (BA)

Shoot length was less affected by cytokinin type, showing relatively uniform values across treatments. The longest shoots were obtained in the presence of kinetin, while slightly shorter but morphologically uniform shoots were observed on media supplemented with zeatin, CPPU, and benzyladenine (Figures 2b and 3).

The balance between shoot number and shoot quality indicates that benzyladenine is the most suitable cytokinin for efficient multiplication of *H. perforatum* under the tested conditions (Santarém & Astarita, 2003; Tzatzani et al., 2023).

In vitro rooting and ex vitro acclimatization

Rooting response was influenced by the presence and concentration of IBA (Figures 4 and 5). Interestingly, the highest rooting percentage (83.33%) and the longest roots were obtained on auxin-free medium, where explant viability reached 100%. Similar auxin-independent rooting has been reported in *Hypericum microshoots*, indicating that rooting can occur on hormone-free media depending on genotype and culture conditions (McCoy & Camper, 2002). In contrast, media supplemented with IBA resulted in lower rooting percentages, which is consistent with reports indicating that the effect of exogenous auxins on *in vitro* rooting may be genotype- and medium-strength-dependent, and that IBA may reduce rooting performance under certain conditions (Coste et al., 2012; Fascella et al., 2017).

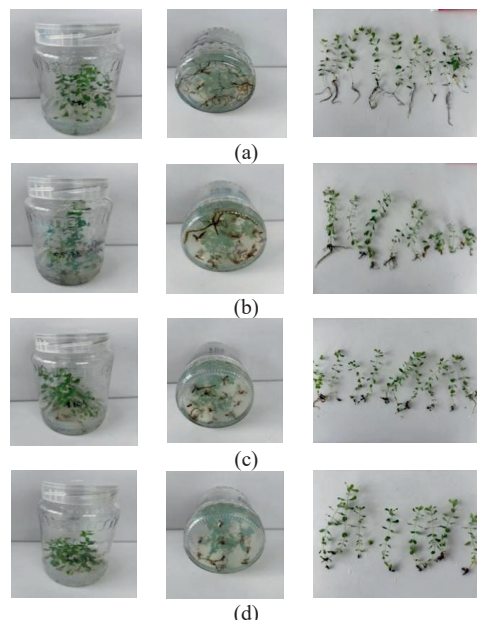


Figure 4. *In vitro* rooting of explants with apical bud in *H. perforatum* on DKW culture medium without auxins (a) and supplemented with 0.5 mg/L IBA (b), 1 mg/L IBA (c), 1.5 mg/L IBA (d)

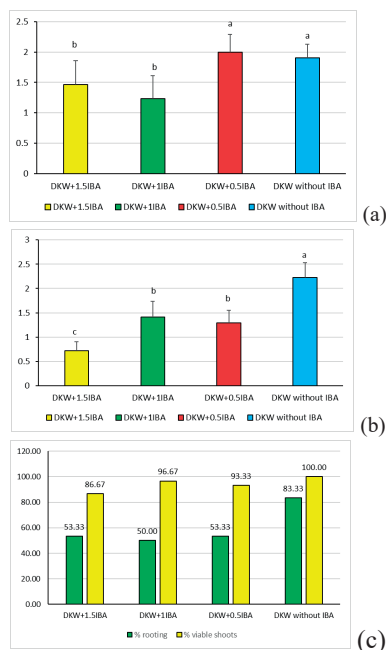


Figure 5. *In vitro* rooting on DKW culture medium without IBA and supplemented with 0.5 mg/L IBA, 1 mg/L IBA, and 1.5 mg/L IBA in *H. perforatum*: (a) average number of roots/initial explant; (b) average root length; (c) rooting percentage and percentage of viable shoots

These results suggest that endogenous auxin levels and physiological status of explant can play a major role in *in vitro* rooting, while exogenous auxin supplementation may not always be required (Koperdakova et al., 2009). *Ex vitro* acclimatization in perlite (Figure 7a,b) was successful for both in vitro-rooted plantlets and non-rooted shoots. However, significantly higher survival rates were recorded in plantlets rooted in vitro, which showed an acclimatization percentage of 98.66%, compared with 85.15% in non-rooted shoots (Figure 6a).

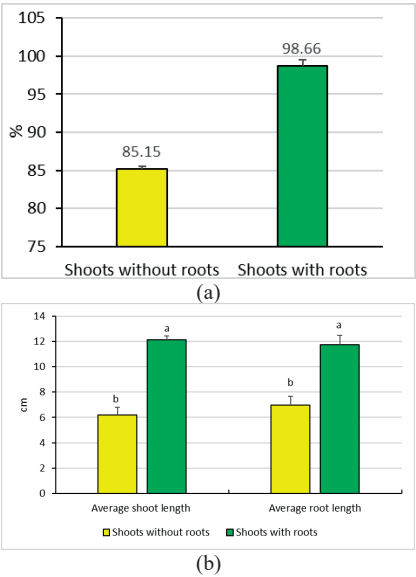


Figure 6. Perlite acclimation of rooted and unrooted shoots of *H. perforatum*: (a) percentage of acclimatization; (b) average shoot and root length

In addition, plantlets rooted in vitro exhibited superior growth performance, expressed by greater shoot and root length, compared with non-rooted shoots (Figure 6b).

After transfer to peat substrate and greenhouse conditions (Figure 7c), acclimatized plants derived from in vitro-rooted plantlets showed a high establishment rate, with 93% successful establishment in alveolar trays.

These results demonstrate the efficiency of the proposed micropropagation and acclimatization protocol and highlight the advantage of in vitro rooting prior to *ex vitro* transfer.

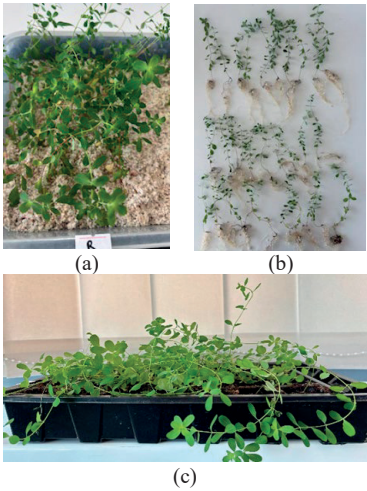


Figure 7. Rooting and acclimatization in *H. perforatum*: shoots acclimatized in perlite (a); acclimatized plants transferred to peat (b, c)

Biochemical traits (TPC and TFC)

As shown in Table 1, TPC and TFC exhibited comparable values in samples obtained from in vitro cultures and potted plants of *H. perforatum*.

Table 1. Total phenolic content (TPC) and total flavonoid content (TFC) in *H. perforatum* samples obtained from in vitro cultures and potted plants. Values are expressed as mean $\pm$ standard error (n = 3)

Parameter	Potted plants	In vitro plants
Total phenolic content ($\mu\text{g GA g}^{-1}\text{ FW}$)	6539.67 $\pm$ 30.46	6395.17 $\pm$ 29.55
Total flavonoid content ($\mu\text{g Q g}^{-1}\text{ FW}$)	5882.33 $\pm$ 36.19	5846.10 $\pm$ 21.39

TPC reached $6539.67 \pm 30.46 \mu\text{g GA g}^{-1}$ fresh weight in potted plants and $6395.17 \pm 29.55 \mu\text{g GA g}^{-1}$ fresh weight in *in vitro*-derived plants. Similarly, TFC showed close values between the two plant categories, with $5882.33 \pm 36.19 \mu\text{g Q g}^{-1}$ fresh weight in potted plants and $5846.10 \pm 21.39 \mu\text{g Q g}^{-1}$ fresh weight in in vitro cultures. In contrast to the present results, other studies have reported significant differences between in vitro derived and field-grown plants. For instance, Yaman et al. (2022) reported significantly lower phenolic and flavonoid contents in in vitro samples compared to field-grown plants, with all samples differing statistically at $p \leq 0.01$.

In their study, *in vitro* plantlets and callus exhibited the lowest TPC and TFC values, while field-grown plants showed substantially higher levels of secondary metabolites.

The larger differences observed in that study may be attributed to the use of mature field-grown plants, which are known to accumulate higher levels of phenolic compounds due to prolonged development and exposure to environmental stimuli. In the present study, both *in vitro* and potted plants originated from micropropagated material and were at relatively early developmental stages, which may explain the smaller differences observed between the two plant categories. These findings suggest that plant developmental stage and growth conditions play a major role in secondary metabolite accumulation in *H. perforatum*.

The present study demonstrated that *H. perforatum* can be efficiently micropropagated through *in vitro* culture using nodal explants, followed by successful rooting and *ex vitro* acclimatization. Among the tested cytokinins, benzyladenine proved to be the most effective for shoot multiplication, while auxin-free medium ensured the highest rooting percentage and explant viability.

Ex vitro acclimatization was achieved with high survival rates for both rooted and non-rooted shoots, although superior growth performance was observed in plantlets rooted *in vitro*. The high establishment rate after transfer to greenhouse conditions confirms the reliability of the proposed micropropagation protocol for producing healthy planting material.

Biochemical analysis revealed comparable levels of total phenolic and flavonoid contents in *in vitro* cultured and acclimatized plants, indicating that secondary metabolism was not negatively affected by *in vitro* conditions or by the acclimatization process. These results suggest that biomass obtained through *in vitro* culture may represent a potential source of phenolic compounds.

CONCLUSIONS

The developed protocol provides a practical and efficient approach for the propagation of *H. perforatum*, while also highlighting the potential of *in vitro*-derived biomass for further applications. Additional studies focusing on

detailed phytochemical profiling and biomass yield optimization are required to further explore this potential.

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RESEARCH ON THE IDENTIFICATION OF THE NEW IDEOTYPE OF POTATO, COMPETITIVE IN THE AGRICULTURAL SECTOR IN SOUTHERN OLTENIA

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Abstract

The research was carried out at R.D.S.P.C.S. Dabuleni in the agricultural years 2024 and 2025 and aimed at comparing potato lines originating from the National Institute for Research and Development for Potato and Sugar Beet Brașov, at different stages of the improvement process, with the aim of identifying the new potato ideotype, competitive in the agricultural sector of the southern area.

The results obtained highlighted increased tolerance to thermohydric stress in lines 1927/1 and 22-1941/8, in 2024 and in line 1901/6 in 2025, a line differentiated positively, with statistically assured values that were very significant compared to the control. In terms of potato line productivity, in 2024, the 1895/4 line stood out, with an average production of 38.49 t/ha, which was 5.45 t/ha above the average of the varieties. In 2025, the 2008/4 lines stood out, with 48.75 t/ha and 22-1895/4, which achieved 44.05 t/ha.

Also, in the two years of study, environmental factors had a direct influence on the physiological, biochemical, and production processes.

Key words: potato, sandy soils, abiotic stress, production, quality.

INTRODUCTION

Potato is one of the most important food crops worldwide, ranking fourth in the hierarchy of staple crops, after rice, wheat and maize, and being cultivated on an area of over 17 million hectares (FAO, 2023). With a global production of over 370 million tons annually, potato contributes significantly to food security, especially in temperate and mountainous regions, being an essential source of food and income for millions of farmers (FAO, 2023). Due to its high nutritional value, ecological adaptability, and high yield per unit area, the potato is considered a strategic crop in the face of the climatic and demographic challenges of the 21st century (FAO, 2023).

In recent decades, climate change has required a profound reassessment of genetic improvement strategies in agriculture, especially for staple crops such as potatoes (CIP, 2024). The creation of new potato varieties is a strategic priority both globally and regionally, especially in areas

vulnerable to climate change, such as southern Oltenia. This region frequently faces pedological drought, high temperatures during the growing season, and sandy soils with low fertility, which requires the development of genotypes adapted to these restrictive conditions. The International Potato Center (CIP), based in Peru, conducts research in over 25 countries to develop potato varieties adapted to diverse agroecosystems. Internationally, CIP promotes the use of local varieties and wild relatives of the potato to introduce traits of resistance to drought, disease, and extreme temperatures (Nature, 2019; CIP, 2024). Researchers from the University of Hawaii and the Royal Botanic Gardens, Kew, are exploring the potential of wild potato to expand the genetic base of the cultivated species. These genetic resources provide valuable traits for adaptation to emerging ecological niches, especially in regions affected by rapid climate change (Fumia et al., 2021). In Europe, research is focused on integrating traits of drought resistance, high

temperature tolerance, and nutrient use efficiency into modern ideotypes, with the aim of maintaining the competitiveness of potato cultivation in southern and Mediterranean regions. In the case of potatoes, the agronomic ideotype translates into an ideal plant profile consisting of:

- a deep and branched root system, capable of exploring large volumes of soil for the absorption of water and minerals;
- a moderate leaf surface, with increased efficiency in photosynthesis and controlled transpiration;
- a high capacity for allocating assimilates to tubers;
- physiological resistance to water and thermal stress;
- uniform and compact tubers, with firm skin and high dry matter content, over 20% (Caliskan & Demirkaya, 2019).

The sand region of southern Oltenia is characterized by psamosol and arenosol soils, with a sandy-loamy texture, high permeability, and low humus content (below 0.6%), and because of this texture, the water retention capacity is two times lower than in chernozems, and leaching losses are high (Velu & Popescu, 2022).

Studies conducted in the Dăbuleni region have demonstrated that the application of drip irrigation and manure (30 t/ha) improves water retention and leads to increased production of up to 28-30 t/ha (Velu & Popescu, 2022). The creation and exploitation of an ideotype adapted to the sands of Oltenia presents multiple benefits, both ecologically and economically.

By integrating genetic and physiological approaches into breeding programs, the ideotype becomes a biological target capable of redefining potato cultivation in sandy ecosystems – offering a sustainable agronomic and ecological solution. At the genetic level, contributions from the wild species *Solanum chacoense*, *Solanum commersonii*, and *Solanum berthaultii* represent the basis of modern breeding for traits such as drought tolerance and vascular disease (Machida-Hirano, 2015).

Research conducted on sandy soils in southern Oltenia has highlighted the role of variety, fertilization, and irrigation in obtaining economically efficient and high-quality potato production (Croitoru et al., 2014; Dima et al.,

2014). Early potato ideotypes intended for cultivation under stress conditions, such as drought, frost, or attacks by pests and diseases, must present a series of specific characteristics to ensure healthy development and optimal production.

In order to select and develop early potato ideotypes that are resistant to stress conditions, it is essential to integrate these characteristics into the selection and breeding process. The purpose of this paper is to analyze the pedoclimatic context of the southern Oltenia region, the main challenges related to potato cultivation on sandy soils, and the genetic and technological characteristics necessary to define a new potato ideotype adapted to abiotic stress. Understanding the complexity of the relationship between genotype and environment is essential for building a sustainable, productive, and efficient agriculture on the sandy lands of Romania.

MATERIALS AND METHODS

In 2024, technical work was carried out corresponding to the planting of the breeding material for the establishment of the experimental field. For the study of potato lines, four lines subjected to the breeding process by NIRDPSB Braşov were studied, namely 1901/12, 1895/4, 1927/1 and 22-1941/8, and the Ervant variety was taken as the control, while in 2025, five potato lines were studied, respectively 22-1895/4, 1897/2, 1901/6, 1930/3 and 2008/4, the same Ervant variety as the control. The monofactorial experiment was located on a sandy soil with low fertility, under sprinkler irrigation conditions, according to the randomized block method. The planting of the tubers was carried out on February 22, 2024 and March 11, 2025, the works consisted of opening the mechanized trenches, manually planting the sprouted tubers, and then covering the trenches mechanically. The distance between the rows was 70 cm, and between the tubers in a row was 25 cm, achieving a planting density of about 57,000 nests/ha. The basic fertilization was done the day before planting, using mineral-type NPK 15.15.15 fertilizers in a quantity of 400 kg/ha. In vegetation, fertilization was carried out with ammonium nitrate at a dose of 200 kg/ha. In order to ensure an early harvest, the tuber-

germination work was carried out one month before planting in the field. Thus, by planting the sprouted tubers, conditions were ensured for harvesting the tubers in June, thus avoiding the crop being subjected to the drought and heat periods in July-August characteristic of the southern area of the country. Optimal light and temperature conditions were ensured for the germination of the seed tubers. Vigorous tubers were obtained at a temperature of 16-18°C for 12-14 days (pre-germination), after which the temperature was reduced to 12-15°C and maintained for 20-25 days (germination itself). Maintenance work was carried out during vegetation to prevent the occurrence of, and to manage, fungal diseases or harmful insects that appeared in the crop. Observations and experimental determinations were carried out regarding:

- early potato production;
 - sensory evaluation of vegetation parameters.
- In order to evaluate the sensory parameters of vegetation, photosynthesis processes were analyzed using modern study methods. To determine the physiological activity of potato plants, the portable LC Pro SD system was used, with the help of which the gas exchange between plants and the environment was analyzed using the broad leaf chamber.

RESULTS AND DISCUSSIONS

Year 2024 can be characterized as a dry year. Climatic data recorded at the RDSPCS Dābuleni Weather Station during February-May 2024 showed an average temperature of 8.5°C in February, which allowed potato planting in this month (Table 1).

The average temperature recorded in the four months exceeded the multiannual average (1956-2023) by +3.4°C, and the amount of precipitation was higher by 14.7 mm, but these were unevenly distributed.

Potato plants were influenced by these climatic conditions both during the emergence period and during the initiation and development of tubers.

In 2025, tuber planting was done in the second decade of March because the average monthly temperature was between 9.37°C in March and 24.7°C in June, and the months of March and April were colder compared with previous

years, with minimums of -6.0°C in March, -3.97°C in April, and 1.53°C in May (Table 2). During April 07-11, 2025, the air temperature dropped significantly, with average values ranging between 3.73-6.83°C. The minimum temperatures during this period ranged between +0.94 and -3.97°C.

Table 1. Analysis of the climatic conditions at RDSPCS Dābuleni during February - May 2024

Climatic conditions	II	III	IV	V	Average/ Sum
Average temperature, °C	8.5	9.25	15.2	16.7	12.41 +3.4
The absolute maximum, °C	22	29	34.1	28.6	28.6
The absolute minimum, °C	-7.1	-3.1	2.7	7.4	-7.1
Rainfall, mm	11.2	36.6	36	114	197.8 +14.7
Moisture, %	100	100	99.6	100	99.92
Multiannual average temperature (1956-2023), °C	1.299	5.919	11.916	16.943	9.01
Rainfall Multi-year monthly average (1956-2023), mm	32.104	40.611	46.967	63.415	183.09

Table 2. Analysis of the climatic conditions at RDSPCS Dābuleni during March 10-June 10, 2025

Climatic conditions	III	IV	V	VI	Average/ Sum
Average temperature, °C	9.37	12.54	16.07	24.05	15.5
The absolute maximum, °C	24.1	28.97	29.87	32.9	29.0
The absolute minimum, °C	-5.99	-3.97	1.53	15.86	1.9
Rainfall, mm	28.8	21.0	55.4	7.6	112.8
Multiannual average temperature (1956-2023), °C	5.91	11.91	16.95	21.53	16.79
Rainfall Multi-year monthly average (1956-2023), mm	40.61	46.96	63.41	10.43	161.41

The precipitation recorded during these months amounted to 112.8 mm, lower than the multiannual amount for the respective months (between 1956-2024), the accumulation of water in the soil being very important for the normal development of the plant throughout the vegetation period. In 2024, the average production of marketable tubers obtained at the final harvest ranged between 23.99 t/ha for the 22-1941/8 line and 41.97 t/ha for the Ervant

variety, with an average production of 33.05 t/ha (Figure 1).

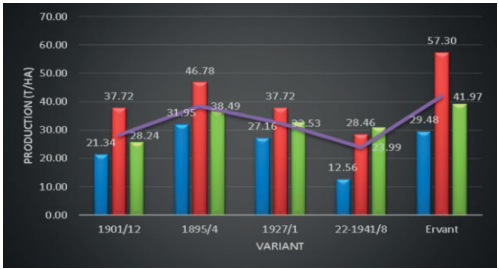


Figure 1. Average production of tubers in 2024

The potato genotypes studied showed different results in terms of their biological potential. Marketable yields above the average of the five genotypes were achieved by the 1895/4 line (by 5.45 t/ha) and the Ervant variety (by 8.92 t/ha), the marginal differences being statistically insignificant (Table 3).

Table 3. Statistical analysis of average production in 2024

Genotype	Production (t/ha)	Difference (t/ha)	Significance
1901/12	28.24	-4.80	ns
1895/4	38.49	5.45	ns
1927/1	32.53	-0.52	ns
22-1941/8	23.99	-9.05	ns
Ervant	41.97	8.92	ns
Production Average (t/ha)	33.05	Mt.	
LSD 5%=9.00; LSD 1%=13.09; LSD 0,1 %=19.63			

Regarding the average production obtained in 2025, it ranged between 29.26 t/ha for the 1901/6 line and 63.12 t/ha for the Ervant variety, with the average production being 42.68 t/ha (Figure 2).

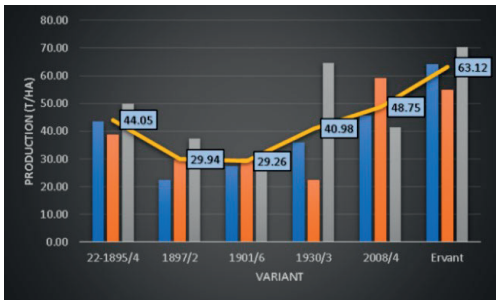


Figure 2. Average production in tubers in 2025

Marketable yields above the average of the six genotypes were achieved in lines 22-1895/4 (by

1.37 t/ha) and 2008/4, where production exceeded the average by 6.06 t/ha, the marginal differences being statistically insignificant. In Ervant variety, production obtained in 2025 exceeded the average production of the genotypes by 20.44 t/ha, this difference being statistically ensured as positively significant (Table 4).

Table 4. Statistical analysis of average production in 2025

Genotype	Production (t/ha)	Difference (t/ha)	Significance
22-1895/4	44.05	1.37	ns
1897/2	29.94	-12.75	ns
1901/6	29.26	-13.42	ns
1930/3	40.98	-1.70	ns
2008/4	48.75	6.06	ns
Ervant	63.12	20.44	*
Production Average (t/ha)	42.68	Mt.	
LSD 5%=18.62; LSD 1%=26.47; LSD 0.1 %=38.33			

The intensity of the photosynthesis process of potato genotypes cultivated on sandy soils from Dăbuleni varied depending on the genotype and their degree of adaptability to severe thermal stress, ranging between 7.57 $\mu\text{mol CO}_2/\text{m}^2/\text{s}$ at Ervant and 15.46 at 22-1941/8 (Table 5).

Table 5. Photosynthesis rate in potatoes grown on sandy soils in Dăbuleni - 2024

Genotype	Photosynthesis ($\mu\text{mol CO}_2/\text{m}^2/\text{s}$)	Difference	Significance
1901/12	9.34	1.77	ns
1895/4	11.55	3.98	*
1927/1	15.19	7.62	***
22-1941/8	15.46	7.89	***
Ervant	7.57	Mt.	
LSD 5%=2.77; LSD 1%=4.03; LSD 0.1 %=6.05			

The genotypes 1927/1 and 22-1941/8 were noted with have increased tolerance to thermal stress, which differed very significantly from a statistical point of view compared to the Ervant variety.

At RDSPCS Dăbuleni, in 2025, research undertaken aimed at monitoring the physiological reactions of the 6 potato genotypes studied, by vegetation phenophases, in order to determine their tolerance to the climate and soil conditions specific to the sandy soil area in southern Romania; the determinations were carried out during the vegetation period between the appearance of the floral bud and the full flowering of the potato.

The intensity of the photosynthesis process of potato genotypes grown on sandy soils from Dăbuleni varied between 11.69 $\mu\text{mol CO}_2/\text{m}^2/\text{s}$

at line 2008/4 and 23.17 $\mu\text{mol CO}_2/\text{m}^2/\text{s}$ at line 1901/6 (Table 6).

Table 6. Photosynthesis rate in potatoes grown on sandy soils in Dăbuleni - 2025

Genotype	Photosynthesis is ($\mu\text{mol CO}_2/\text{m}^2/\text{s}$)	Difference	Significance
22-1895/4	18.75	-0.23	ns
1897/2	20.30	1.32	ns
1901/6	23.17	4.19	***
1930/3	20.40	1.42	ns
2008/4	11.69	-7.29	ooo
Ervant	18.98	0.00	Mt
LSD 5%=1.55; LSD 1%=2.21; LSD 0.1 %=3.20			

Comparing the results obtained with the average values of the Ervant variety (control variant) developed at NIRDPSB Braşov, it was found that the 1901/6 line differentiated positively, with values statistically assured as being very significant, and showed an increased tolerance to severe thermal stress.

CONCLUSIONS

Research conducted in 2024 and 2025 monitored the behaviour of potato lines originating from INCDCSZ Braşov at different stages of the improvement process.

Marketable production above average of the five genotypes was achieved in 2024 in the 1895/4 line (by 5.45 t/ha) and in the Ervant variety (by 8.92 t/ha), the marginal differences were statistically insignificant, and in 2025, it was achieved in the 22-1895/4 lines (by 1.37 t/ha) and 2008/4, in which the production exceeded the average by 6.06 t/ha, the marginal differences being statistically insignificant.

In Ervant variety, the production obtained in 2025 exceeded the average production of the genotypes by 20.44 t/ha, this difference being statistically ensured as positively significant.

With an increased withlerance to thermal stress in 2024, the genotypes 1927/1 and 22-1941/8 were noted, which differed very significantly from a statistical point of view compared to the Ervant variety, and in 2025 the line 1901/6 differed positively, with values statistically ensured as being very significant, having an increased tolerance to accentuated thermal stress.

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THE BEHAVIOR OF 'VALIN 24' PUMPKIN CULTIVAR IN THE PEDOCLIMATIC CONDITIONS OF SANDY SOILS FROM DĂBULENI RDSPCSS

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Abstract

During the two years 2024-2025 the 'Valin 24' pumpkin cultivar was studied at the Dăbuleni Research and Development Station for Plant Cultivation on Sandy Soils (RDSPCSS), as a result of the creative breeding process. The characters that play a determining role in the agronomic value of a cultivar, such as fruit weight and pulp thickness, remained constant during the study period. Also, the pumpkin cultivar presented good stability for characters like fruit height, fruit diameter, seminal cavity diameter, and shape index, given by the ratio between the height of the fruit and its diameter. Regarding the biochemical content of pumpkin fruits analyzed during the 2024-2025 study period, it was found that the highest average values were recorded in 2024 in terms of total dry matter content (%), soluble dry matter (%), starch (%), titratable acidity (g malic acid/100 g fresh matter) and vitamin C (mg/100 g fresh matter). Following statistical analysis of the characters measured over the two years of study, it was found that the pumpkin variety developed at Dăbuleni RDSPCSS performed well under the pedoclimatic conditions in the sandy soil area.

Key words: pumpkin, cultivar, sandy soils, conservative selection.

INTRODUCTION

Pumpkin (*Cucurbita maxima*) belongs to the Cucurbitaceae family and is a vegetable with green leaves and fruits of different sizes, colors, shapes, and weights. The Cucurbitaceae family is one of the most important botanical families for humans, constituting a favorable crop. Pumpkin ($n=20$) exhibits great variability in shape and color depending on species, being a member of the Cucurbitaceae family, which includes zucchini, cucumbers, and melons (Nanu et al., 2022).

There are three species of *Cucurbita*: *Cucurbita pepo* L., *Cucurbita maxima* Duch., and *Cucurbita moschata* Duch., which have different climatic adaptations and are widely distributed in agricultural regions worldwide (Robinson & Decker-Walters, 1997; Paris & Brown, 2005; Wu et al., 2007).

Cucurbita spp. is collectively ranked among the top 10 vegetable crops worldwide (Rakcejeva et al., 2011).

In Brazil, the breeding programs carried out by Embrapa were based on a varied germplasm of

pumpkin collected from the northeastern region of the country (Borges et al., 2019).

In Romania, the first writings mentioning pumpkin were identified in "Practical Agriculture" by D. Alexandrescu, published in 1973, in which the following varieties were listed: yellow pumpkins with hard skin and white pumpkins with thin skin (Vinătoru et al., 2019).

Edible pumpkin is cultivated for its fruits, which are consumed at physiological maturity in various forms: baked, boiled, candied, jellied, and used for the preparation of pies, cakes, juices, etc. (Ciofu et al., 2003).

Because pumpkin cultivars differ in terms of nutritional values, producers and researchers are searching for the most valuable and nutritionally appropriate genotypes (Dinu et al., 2016, Frătuțu et al., 2024).

The genetic diversity of germplasms and of *Cucurbita* populations, including shape, size, and color variation of fruit, seed number and size, fruit quality, and fruit pulp thickness, is high (Montes-Hernandez et al., 2005).

Traditional local pumpkin crops represent important genetic resources for plant breeding

due to their considerable genotypic variability. These variations are maintained through the deliberate selection of specific traits by farmers (Nanu et al., 2022).

Selection is a recommended method to streamline and improve the characteristics of a population and to maintain genetic variability (Wilyanne et al., 2024).

The constantly changing climatic conditions in southern Oltenia require the creation of new genotypes that are notable for their earliness, quality, and resistance to area-specific climatic factors.

In order to maintain the authenticity and biological uniformity of the pumpkin genotype 'Valin 24', the aim was to restrict the variability of the main characters analyzed within the limits of small and medium coefficients of variation. The purpose of this study was the selection and maintenance of the genetic structure within the limits established by the author/maintainer of the pumpkin cultivar 'Valin 24'.

MATERIALS AND METHODS

The study was carried out at the Dăbuleni Research and Development Station for Plant Cultivation on Sandy Soils during 2024-2025 and aimed to improve the pumpkin cultivar 'Valin 24' (Figure 1).



Figure1. 'Valin 24' pumpkin cultivar

The biological material used to set up the experiment was represented by the pumpkin seedlings obtained from the selected elites of the 'Valin 24' variety. When setting up the experiment in the research field, the seedlings were planted at a distance of 100 cm between plants per row and 180 cm between rows.

The biometric determinations and morphological observations were carried out on the main

quantitative and qualitative characters of 100 fruits obtained in a selection field.

The physical properties of the fruits, determined in the two years, were represented by the height and diameter of the fruits (cm), the diameter of the seminal cavity (cm), the shape index, fruit weight (kg) and pulp thickness (cm), according to the methodology described by Ionică (2014).

The chemical properties of pumpkin fruits were determined according to the methodology described by Croitoru (2021).

Soluble dry matter (SUS) was determined by the refractometric method, the results being expressed as percentages (%). To determine the content of total dry matter (SUT), the gravimetric method was applied based on the removal of water by evapotranspiration from the average analytical sample used, keeping it in the oven at a temperature between 85-105°C.

The results were expressed as a percentage of total dry matter (%).

The determination of titratable acidity (TA) was carried out using the method described by Ionică (2014), the results were expressed in grams of malic acid/100 g of fresh substance.

For the determination of vitamin C content, the iodometric method described by Croitoru (2021), which is based on the oxidation of ascorbic acid with excess iodine, and the results were expressed in mg of ascorbic acid.

Carbohydrates were determined according to the Fehling Soxhelt method described by Croitoru (2021), the result being expressed in percentages.

The obtained data were processed statistically using the statistical analysis program (StatPoint Technologies, Warrenton, VA, USA), calculating for each character the arithmetic mean (X), the standard deviation (SD), and the coefficient of variation (CV; 0-15 very low variability; 15-30 medium variability; >30 very high variability).

Relationships between fruit physical characteristics were quantified using correlations.

RESULTS AND DISCUSSIONS

The data in Table 1 show that the variability of fruit height is small, with average values between 8.83 cm (2025) and 9.97 cm (2024), which indicates the uniformity of the pumpkin cultivar 'Valin 24' from this point of view.

Table 1. Variability of physical characteristics of fruits from the pumpkin cultivar, 'Valin 24'

Characteristics	Statistical indicators	Year of observation	
		2024	2025
Fruit height (cm)	Mean	9.97	8.83
	Standard Deviation	1.11	1.09
	Minimum/Maximum	7.90 / 13.00	7.00 / 12.20
	CV %	11.15	12.29
Fruit diameter (cm)	Mean	19.90	22.30
	Standard Deviation	1.99	1.85
	Minimum/Maximum	16.70 / 26.50	13.40 / 28.30
	CV%	10.00	8.29
Seminal cavity diameter (cm)	Mean	13.03	14.84
	Standard Deviation	1.98	1.69
	Minimum/Maximum	10.00 / 18.00	11.00 / 23.80
	CV%	12.20	11.37
Shape index	Mean	0.50	0.40
	Standard Deviation	0.05	0.06
	Minimum/Maximum	0.40 / 0.66	0.29 / 0.67
	CV%	10.13	15.22

The cultivar 'Valin 24' also shows good stability in terms of fruit diameter, with average values between 19.90 cm (2024) and 22.30 cm (2025), the diameter of the seminal cavity between 13.03 cm (2024) and 14.84 cm (2025) and the shape index with 0.50 cm (2024) and 0.40 cm (2025).

Thus, the year 2025 stands out with the highest values recorded for fruit diameter, seminal cavity diameter, and shape index, and the year 2024 for the highest average value of fruit height. In both study years, a small coefficient of variation was calculated for the characteristics analyzed.

Table 2 presents the variability of fruit weight and fruit pulp thickness. Fruit weight in 2024 had an average value of 2.60 kg, and in 2025 of 3.47 kg, which shows a very high variability for this characteristic. The results obtained regarding fruit weight are similar to those reported by Nanu et al. in 2022, namely 2.46-3.70 kg. This highlights the need to continue the selection process for standardizing the material

through repeated purifications of the plant material, both at the seedling stage with 2-3 true leaves and in the selection field by removing diseased or non-compliant plants, but also by taking into account the shape, size, and external appearance of the fruits, the color of the pulp, and the shape, size, and color of the seeds of the elites.

Another characteristic of particular importance is the pulp thickness, which recorded close average values in the 2 years of study: 3.92 cm in 2024 and 3.95 cm in 2025, the coefficient of variation having an average value in 2024 (17.90%) and low in 2025 (13.36%).

The results obtained during the studied period are relatively similar to those reported by Nanu et al. in 2022, who reported an average value of pulp thickness between 3.55-4.31 cm.

On average over the 2-year study, the coefficient of variation ranged from 15.63% (pulp thickness) to 24.68% (fruit weight), indicating that for these characters the 'Valin 24' genotype is uniform (stable).

Table 2. Variability of quantitative characteristics of fruits from pumpkin cultivar 'Valin 24'

Characteristics	Statistical indicators	Year of observation		Average / years of study
		2024	2025	
Fruit weight (kg)	Mean	2.60	3.47	3.03
	Standard Deviation	0.78	0.67	0.72
	Minimum/Maximum	1.61 / 5.57	2.23 / 6.08	1.92/5.83
	CV %	29.94	19.43	24.68
Pulp thickness (cm)	Mean	3.92	3.65	3.79
	Standard Deviation	0.70	0.49	0.60
	Minimum/Maximum	2.00 / 6.40	2.50 / 4.80	2.25/5.60
	CV%	17.90	13.36	15.63

The relationships between the main characteristics analyzed in pumpkin fruits in the two years of study were quantified using correlations. Thus, according to the data in Table 3, for the study period, positive correlations were calculated between weight and the length and diameter of the fruit ($r=0.61$ in 2024) and between weight and the diameter of the fruit ($r=0.90$ in 2024, $r=0.71$ in 2025).

Table 3. Correlations between the main characteristics of the fruits of the cultivar 'Valin 24' studied in the period 2024-2025

Characteristics	Fruit weight (kg)		Fruit height (cm)		Fruit diameter (cm)		Seminal cavity diameter (cm)		Pulp thickness (cm)		IF	
	2024	2025	2024	2025	2024	2025	2024	2025	2024	2025	2024	2025
The year	2024	2025	2024	2025	2024	2025	2024	2025	2024	2025	2024	2025
Fruit weight (kg)	1	1										
Fruit height (cm)	0.61	0.14	1	1								
Fruit diameter (cm)	0.90	0.71	0.54	0.06	1	1						
Seminal cavity diameter (cm)	0.45	0.41	0.19	0.11	0.54	0.56	1	1				
Pulp thickness (cm)	0.53	0.38	0.31	-0.11	0.51	0.37	0.10	0.03	1	1		
IF	-0.18	-0.29	0.58	0.76	-0.36	-0.57	-0.30	-0.23	-0.17	-0.28	1	1

Positive cor relation was also calculated between the seminal cavity diameter and the the fruit weight ($r=0.45$ in 2024 and $r=0.41$ in 2025), and fruit diameter ($r=0.54$ in 2024 and $r=0.56$ in 2025). The shape index was positively cor related with fruit length during study period, with $r=0.58$ in 2024 and $r=0.76$ in 2025. Tables 4 and 5 present the distribution of pumpkin fruits of the cultivar 'Valin 24', in terms of the fruit weight values recorded in the study years 2024-2025.

Table 4. Distribution of 'Valin 24' pumpkin fruits selected according to fruit weight in 2024

Bin	Frequency	Cumulative, %
1.605	1	1.00%
2.001	19	20.00%
2.397	34	54.00%
2.793	14	68.00%
3.189	11	79.00%
3.585	13	92.00%
3.981	3	95.00%
4.377	1	96.00%
4.773	1	97.00%
5.169	1	98.00%
More	2	100.00%

Based on the data in Table 4, over 90% of the analyzed fruits had weights between 2.00 kg and 5.169 kg. Most fruits, namely 78, weighed between 2.397 kg and 5.169 kg in 2024.

In 2025, according to Table 5, over 90% of the analyzed fruits had weights between 2.23 kg and 4.912 kg.

Based on the data in Table 5, over 90% of the analyzed fruits had weights between 2.23 kg and 4.912 kg. Most fruits, namely 86, weighed between 2.99 kg and 4.537 kg.

Tables 6 and 7 present the distribution of pumpkin fruits of the elites 'Valin 24', in terms

of the thickness of the pulp of the fruits analyzed in the period 2024-2025.

Analyzing the data in Table 6, it is observed that in 2024, 82 of the 100 fruits analyzed had a pulp thickness between 3.32 cm and 4.64 cm, and 14% had a thickness between 4.64 cm and 5.96 cm.

Table 5. Distribution of fruits of the pumpkin variety 'Valin 24' selected according to fruit weight in 2025

Bin	Frequency	Cumulative, %
2.23	1	1.00%
2.6145	7	8.00%
2.999	18	26.00%
3.3835	24	50.00%
3.768	22	72.00%
4.1525	11	83.00%
4.537	11	94.00%
4.9215	5	99.00%
5.306	0	99.00%
5.6905	0	99.00%
More	1	100.00%

Table 6. Distribution of 'Valin 24' pumpkin fruits selected according to pulp thickness in 2024

Bin	Frequency	Cumulative, %
2	1	1.00%
2.44	0	1.00%
2.88	2	3.00%
3.32	12	15.00%
3.76	33	48.00%
4.2	23	71.00%
4.64	14	85.00%
5.08	11	96.00%
5.52	1	97.00%
5.96	2	99.00%
More	1	100.00%

Based on the data in Table 7, it can be concluded that in 2025, 82 of the analyzed fruits had pulp thicknesses between 2.614 cm and 4.115 cm, and 5% had thicknesses between 4.537 cm and 5.306 cm.

Table 7. Distribution of fruits of the pumpkin variety 'Valin 24' selected according to pulp thickness in 2025

Bin	Frequency	Cumulative, %
2.23	1	1.00%
2.6145	7	8.00%
2.999	18	26.00%
3.3835	24	50.00%
3.768	22	72.00%
4.1525	11	83.00%
4.537	11	94.00%
4.9215	5	99.00%
5.306	0	99.00%
5.6905	0	99.00%
More	1	100.00%

Regarding the biochemical composition of pumpkin fruits, Table 8 presents data on their quality collected during the 2024-2025 study period.

Analyzing the data in Table 8, it was found that during the study period, the year 2024 stood out with the highest average values in terms of total dry matter content (22.17%), soluble dry matter

(17.32%), starch (14.03%), titratable acidity (0.38 g malic acid/100 g fresh matter) and vitamin C (26.22 mg/100 g fresh matter).

From the point of view of the coefficient of variability calculated for each characteristic in both years of the study, it ranged between 2.92% and 21.38% in 2024 and between 2.53% and 17.62% in 2025.

These data indicate low variability in the characteristics of the analyzed fruits, suggesting the uniformity of the cultivar 'Valin 24' and, at the same time, its good stability.



Figure 2. 'Valin 24' pumpkin cultivar in section

Table 8. Biochemical characteristics of pumpkin fruits from the 'Valin 24' elites analyzed in the period 2024-2025

Year	Statistical analysis	Characteristics					
		Water (%)	Total dry matter (%)	Soluble dry matter (%)	Starch (%)	Titratable acidity (g malic acid/100 g of fresh matter)	Vitamin C (mg/100 g of fresh matter)
2024	Mean±SD	77.83±2.31	22.17±2.13	17.32±3.70	14.03±1.18	0.38±0.05	26.22±3.49
	Variation limits	75.85/81.05	18.95/24.15	11.60/20.40	12.46/15.15	0.32/0.45	22.00/30.80
	CV%	2.97	10.42	21.38	8.41	12.84	13.30
2025	Mean±SD	78.05±1.98	21.95±1.98	17.08±3.01	13.83±1.12	0.31±0.05	25.42±2.69
	Variation limits	75.96/81.15	18.85/24.04	12.60/20.20	12.45/15.15	0.23/0.36	22.00/28.80
	CV%	2.53	9.00	17.62	8.13	15.60	10.57

CONCLUSIONS

In the process of conservative selection, the pumpkin cultivar 'Valin 24' recorded the highest average fruit weight of 3.47 kg in 2025, with variation limits between 2.23 kg and 6.08 kg.

The results obtained with the pumpkin cultivar 'Valin 24' showed that both the fruit weight and the pulp thickness have constant values from year to year.

The pumpkin cultivar 'Valin 24' presents low variability in the fruit height characteristic, with average values between 8.83 cm (2025) and 9.97cm (2024), which indicates the uniformity of the pumpkin cultivar 'Valin 24' from this point of view.

The analysis of the characteristics measured over 2 years (2024-2025) demonstrates that the pumpkin cultivar 'Valin 24' can be

recommended to growers passionate about pumpkin cultivation.



Figure 3. 'Valin 24' pumpkin cultivar at physiological harvest maturity

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IMPACT ASSESSMENT OF PASSIVE ELECTROMAGNETIC DEVICES ON THE GROWTH PERFORMANCE OF SELECTED LETTUCE VARIETIES

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Abstract

The study was conducted at the Faculty of Horticulture in Bucharest under greenhouse conditions with LED lighting on two Lollo-type lettuce cultivars, aiming to evaluate the effect of electromagnetic frequencies (5-30 MHz) on morphological, productive, and quality parameters. The results highlighted differentiated responses depending on frequency compared with the control variant. Vegetative growth parameters, such as plant height and rosette diameter, showed small variations, suggesting low sensitivity to the applied treatments. The number of leaves remained relatively constant, with slight increases at 7 and 30 MHz, indicating a moderate influence on leaf development. In contrast, productive parameters were significantly influenced. The highest total plant weight values were recorded at 28 MHz and 30 MHz. At the same time, root system development was stimulated, with maximum values of root weight and root volume observed at 28 MHz. Root length was greater at lower frequencies and reduced at higher frequencies, suggesting a redistribution of resources toward volumetric growth. Frequencies of 28-30 MHz demonstrated a stimulatory effect on biomass accumulation and root system development, indicating potential applicability in optimizing production.

Key words: Lettuce, LED Light, Electromagnetic Fields, Nutrient Film Technique, Bio Stimulation, Frequency-Dependent Effects.

INTRODUCTION

In the context of sustainable agricultural development and the imperative to reduce chemical inputs in crop production, research into alternative plant growth stimulation methods has become increasingly vital. Among these approaches, the application of passive electromagnetic devices and electromagnetic fields represents a prominent physical factor capable of influencing the biological processes of plant organisms.

In studies conducted in recent years, Abdel Latef et al. (2020) highlighted that exposing plants to magnetic or electromagnetic fields can alter processes such as seed germination, water and nutrient uptake, enzymatic activity, photosynthesis, and biomass development. Concurrently, Tran et al. (2023) demonstrated that exposing lettuce plants to radiofrequency electromagnetic fields (Wi-Fi and DECT) reduced photosynthetic efficiency and stress tolerance, negatively impacting plant development and physiological responses, particularly under field conditions. "

De Souza et al. (2008) demonstrated that applying magnetic treatments to lettuce seeds prior to sowing enhanced plant development across all growth stages, stimulating root length, plant height, leaf area, and ultimately, crop yield and fresh biomass. Similarly, Abdel Latef et al. (2020) showed that exposing lettuce seeds to a static magnetic field led to an increase in vegetative parameters and antioxidant system activity, contributing to a yield enhancement of up to 50% compared to the control group. Shabrangi and Majd (2009) highlighted that magnetic pretreatment of lentil seeds stimulated the growth of roots and shoots, increased biomass and the activity of antioxidant enzymes (APX and SOD), and also contributed to enhanced plant tolerance to water stress. Putti et al. (2023) highlighted that utilizing magnetically treated water for lettuce crop irrigation improved nutrient uptake, plant development, and yield, while also contributing to enhanced water-use efficiency and the optimization of irrigation management under water stress conditions.

El-Ssawy et al. (2020) evaluated the effect of utilizing magnetized water across different hydroponic systems for lettuce cultivation, finding that higher magnetic field intensities improved the properties of the irrigation water, reduced water consumption, and increased water productivity. The best results regarding lettuce yield and quality were obtained by combining the NFT system with the maximum water magnetization level (6300 Gauss), thereby highlighting the potential of utilizing magnetized water to enhance water-use efficiency and crop yield in hydroponics.

El-Sayed (2022) demonstrated that treating wheat seeds with synchronized electro-magnetization and microwave radiation significantly improved their germination parameters, vigour, and physiological characteristics compared with the control, highlighting the efficiency of this method as a sustainable alternative for seed priming.

Kim and Oh (2024) reported that applying electric fields to lettuce plants stimulated growth, mineral uptake, and physiological processes. Furthermore, the direction of the electric field differentially influenced leaf development and plant physiological responses, suggesting the potential of utilizing these fields to enhance crop productivity.

Ramalingam (2019) highlighted that the application of magnetic fields represents a promising strategy for stimulating plant growth and enhancing resistance to both biotic and abiotic stresses, while simultaneously underscoring the need for further research to elucidate the underlying mechanisms of action. Similarly, Nyakane Neo et al. (2019) showed that utilizing magnetic fields in agriculture has the potential to contribute to increased crop productivity and improved water-use efficiency; however, they highlighted the necessity of additional studies to establish optimal application parameters and clarify the contradictory results reported in the literature.

The utilization of electromagnetic fields (EMFs) across various frequencies represents an innovative approach in modern agrotechnology, aiming to stimulate germination, accelerate growth rates, and enhance the vegetative vigor of agricultural crops. The present study analyzes the effects of exposing the 'Lollo Bionda' lettuce cultivar (*Lactuca sativa* L. var. *crispa*) to

different electromagnetic frequencies ranging from 5 MHz to 30 MHz, compared with an untreated control group. The primary objective of this research is to identify the optimal frequency that maximizes the development of both the shoot and root systems, as well as fresh biomass accumulation.

Tong et al. (2013) and Cai et al. (2024) highlighted that optimizing lighting and HVAC systems, alongside integrating renewable energy sources, represents essential pathways for reducing energy consumption and enhancing the sustainability of plant factory with artificial light (PFAL) systems.

This study aims to evaluate the impact of passive electromagnetic devices operating at different frequencies on the growth performance, biomass accumulation, and quality of selected lettuce cultivars grown in an NFT system under LED lighting.

To this end, relevant morphological and biochemical parameters were analyzed to determine the efficacy of these devices in enhancing crop yield and nutritional quality.

MATERIALS AND METHODS

Experimental Site and Growth Conditions

The study was conducted at the Faculty of Horticulture within the University of Agronomic Sciences and Veterinary Medicine of Bucharest, inside an experimental 'plant factory' greenhouse.

The facility was equipped with a hydroponic Nutrient Film Technique (NFT) system and controlled LED lighting.

Environmental conditions within the experimental greenhouse (air temperature, relative humidity, photoperiod, and light intensity) were continuously monitored and maintained at constant parameters throughout the research to ensure the uniformity of growth factors and to eliminate experimental errors.

Biological Material

The biological material consisted of two lettuce (*Lactuca sativa* L.) cultivars evaluated to determine the impact of passive electromagnetic treatments on their growth and development. The selected varieties were Aleppo (a Lollo Bionda type with green, wrinkled leaves) and Carmesi (a Lollo Rossa type with reddish, wrinkled leaves).

Experimental Layout

The study incorporated nine experimental treatments based on the varying operational frequencies of the tested passive electromagnetic devices, organized as follows: V1 - 5.0 MHz; V2 - 7 MHz; V3 - 10 MHz; V4 - 14 MHz; V5 - 21 MHz; V6 - 25 MHz; V7 - 28 MHz; V8 - 30 MHz și V9 - Control (plants cultivated under identical conditions, but without exposure to electromagnetic devices).

Biometric Determinations and Statistical Analysis

At the end of each cultivation cycle, upon reaching commercial maturity, plants were harvested for biometric analysis. The evaluated morphological traits included shoot parameters (plant height, rosette diameter, and total leaf count per plant), fresh biomass (mean plant weight), and root system characteristics (root length, total root weight, and root volume). Arithmetic means were calculated across three replicates for each treatment over both crop cycles. Data were statistically analyzed to evaluate the significance of differences between the treated groups and the untreated control (V9).

RESULTS AND DISCUSSIONS

The results regarding average plant height revealed differential responses between the two analyzed cultivars depending on the frequency of the applied devices, compared with the control variant (V9), which recorded 18.50 cm for the Aleppo cultivar and 18.00 cm for Carmesi.

For the Aleppo cultivar, the highest values were recorded in treatment V7 - 28 MHz (20.50 cm), representing an increase of +10.8% compared with the control, followed by V4 - 14 MHz (20.30 cm; +9.7%) and V8 - 30 MHz (20.25 cm; +9.5%). Treatment V1 - 5 MHz resulted in a moderate increase (19.30 cm; +4.3%), whereas V2 - 7 MHz showed a slight reduction (18.25 cm; -1.4%) relative to the control. Overall, the Aleppo cultivar exhibited a positive response to medium and high frequencies, with the most pronounced effect observed at 28 MHz.

For the Carmesi cultivar, the response was more variable. The highest value was also recorded in treatment V7 - 28 MHz (20.0 cm), corresponding to an increase of +11.1% compared with the control, followed by V4 - 14

MHz and V5 - 21 MHz (19.0 cm; +5.6%). Treatment V6 - 25 MHz resulted in a decrease relative to the control (16.5 cm; -8.3%), whereas V3 - 10 MHz exhibited values similar to those of the control (18.0 cm). In comparison between cultivars, Aleppo demonstrated greater stability and slightly higher values across most treatments, suggesting a more consistent adaptation to the applied treatments (Figure 1).

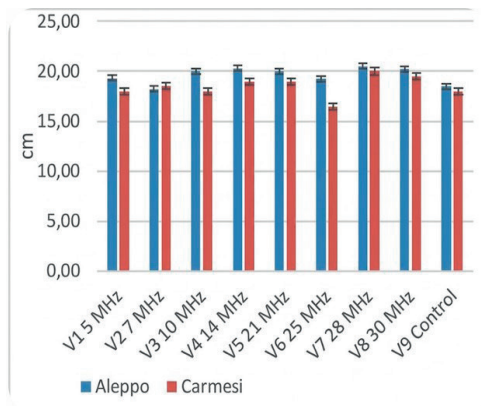


Figure 1. Average plant height of lettuce plants

Regarding rosette diameter, the experimental treatments exhibited moderate differences across both frequencies and cultivars when compared to the control groups-which measured 30.50 cm for Aleppo and 29.50 cm for Carmesi (Figure 2).

For the Aleppo cultivar, the largest rosette diameters were obtained in treatments V4 - 14 MHz and V7 - 28 MHz (33.50 cm), corresponding to an increase of +9.8% compared with the control. Treatment V2 - 7 MHz also produced a favorable effect (32.50 cm; +6.6%), while V8 - 30 MHz resulted in an increase of +7.4%. Treatments V1 - 5 MHz and V6 - 25 MHz showed only slight increases (+1.6% and +3.3%, respectively), whereas V5 - 21 MHz recorded a value lower than that of the control (30.00 cm; -1.6%).

For the Carmesi cultivar, the most favorable response was observed in treatments V4 - 14 MHz and V8 - 30 MHz (34.0 cm), representing an increase of +15.3% compared with the control.

Treatment V7 - 28 MHz also showed a positive effect (+10.2%), while treatments V2 - 7 MHz and V6 - 25 MHz resulted in moderate increases

(+5.1% and +6.8%, respectively). Treatments V1 - 5 MHz and V5 - 21 MHz exhibited values close to those of the control (+1.7%), whereas V3 - 10 MHz showed a relatively small increase (+5.1%).

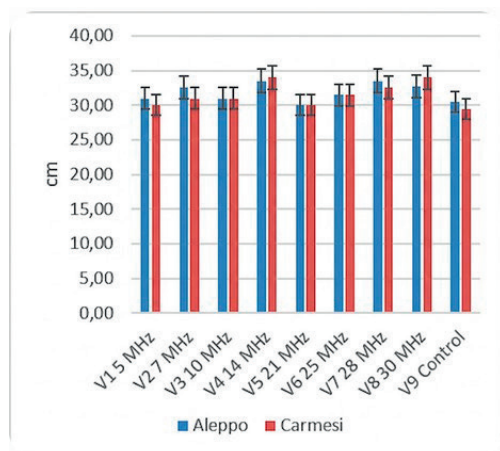


Figure 2. Rosette Diameter

Compared with the control group (27.00 leaves for Aleppo and 24.00 leaves for Carmesi, the experimental treatments induced distinct responses depending on both the cultivar and the applied frequency.

Regarding the Aleppo cultivar, the highest values were recorded in treatments V7 (28 MHz) and V8 (30 MHz), both yielding 30 leaves, which corresponds to an 11.1% increase relative to the control. These were followed by treatment V2 (7 MHz), with 29 leaves (+7.4%), V5 (21 MHz), with 28.50 leaves (+5.6%), and V3 (10 MHz), with 28.00 leaves (+3.7%). In contrast, treatments V1 (5 MHz) and V6 (25 MHz) fell below the control baseline, both recording 26.00 leaves (-3.7%), while V4 (14 MHz) exhibited only a minor increase (+1.9%). In the case of Carmesi, the variations were more constrained, with values remaining close to the control baseline in most instances. Certain treatments induced slight reductions, indicating a higher sensitivity of this cultivar to the applied treatments. The most favorable outcomes were achieved in treatments V2 (7 MHz) and V8 (30 MHz), both yielding 24.0 leaves, matching the control exactly. Conversely, treatments V1 (5 MHz), V6 (25 MHz), and V7 (28 MHz) recorded moderate declines (22.0 leaves; -8.3%), while the lowest values were observed in

V3 (10 MHz) with 21.0 leaves (12.5% below the control). Furthermore, treatment V4 (14 MHz) resulted in an average of 20.0 leaves, representing a decrease of 16.7% relative to the control baseline. Comparative analysis between the two cultivars revealed that Aleppo responded more favorably to the frequency applications, particularly within the 28-30 MHz range. In contrast, Carmesi exhibited higher sensitivity, with the majority of the treatments leading to a reduction in leaf number compared to the control (Figure 3).

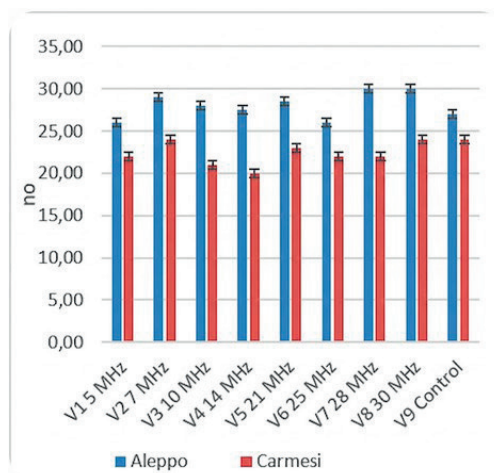


Figure 3. Number of leaves on plants

Root length analysis revealed that the response of the lettuce plants varied depending on both the cultivar and the applied frequency. For Aleppo, root length ranged between 22.50 and 33.25 cm. The highest value was recorded in treatment V1 (5 MHz), yielding 33.25 cm, which represents a +41.5% increase compared with the control group (23.50 cm). Relatively high values were also observed in treatments V2 (7 MHz) and V8 (30 MHz), with averages of 27.75 cm and 25.75 cm, respectively. Conversely, the lowest values were recorded in treatments V6 (25 MHz) and V7 (28 MHz), where root lengths reached only 23.00 cm and 22.50 cm - levels that were close to or even below the control baseline. In the case of Carmesi, the recorded values were generally higher, ranging from 28.0 to 36.0 cm, with the maximum achieved in treatment V2 (7 MHz), which reached 36.0 cm (a +38.5% increase relative to the control). Overall, low frequencies

(5-7 MHz) promoted root development in both cultivars.

In the case of the Carmesi cultivar, the development of the root system was more pronounced compared with the Aleppo cultivar. The maximum root length was recorded in treatment V2 (7 MHz), which achieved an average value of 36.0 cm, representing an increase of approximately 38.5% relative to the control group (26.0 cm). Furthermore, treatments V1 (5 MHz) and V4 (14 MHz) also induced high values for this parameter, reaching 35.5 cm and 32.0 cm, respectively. The lowest value was observed in treatment V7 (28 MHz), where root length was measured at 28.0 cm; however, this value remained superior to that of the control baseline.

Comparative analysis between the cultivars established that Carmesi exhibited higher root length values across the majority of the experimental treatments than Aleppo. This indicates both a higher capacity for root system development and greater sensitivity to the applied treatments. Concurrently, the results demonstrate that low frequencies, particularly within the 5-7 MHz range, promoted root growth in both cultivars. Conversely, frequencies ranging between 21 and 28 MHz exerted a more limited effect on this morphological trait (Figure 4).

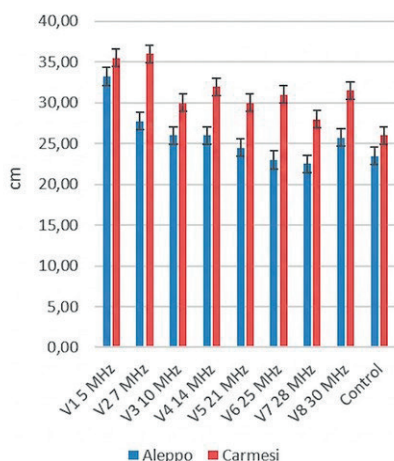


Figure 4. Root length

Regarding fresh biomass accumulation, the data presented in Tables 1 and 2 reveal a general increase in lettuce plant mass across all treated

variants compared with the control, thereby indicating a favorable effect of the applied treatments on vegetative biomass accumulation. Concurrently, the intensity of this response varied according to both the cultivar and the applied frequency.

In the case of the Aleppo cultivar (Lollo Bionda type), the average plant mass of the control group was 95.4 g, whereas all experimental variants recorded higher values, ranging between 106.4 g and 121.3 g. The highest value was observed in variant V2 (7 MHz), where the average mass reached 121.3 g, representing an increase of 25.9 g (+27.1%) relative to the control. Furthermore, variants V3 (10 MHz) and V4 (14 MHz) exhibited substantial biomass increases of 23.7% and 21.7%, respectively, compared to the control group. These differences were highly statistically significant, demonstrating the high efficiency of frequencies between 7 and 14 MHz in stimulating plant growth.

Variants corresponding to higher frequencies - specifically 25 MHz, 28 MHz, and 30 MHz - recorded gains of 19.1%, 16.4%, and 15.4% over the control, with these differences being distinctly statistically significant. In contrast, at frequencies of 5 MHz and 21 MHz, growth enhancements were more limited, reaching 11.5% and 12.1%, respectively, and lacked statistical significance (ns). Overall, these results suggest the existence of an optimal frequency window for stimulating biomass accumulation in the Aleppo cultivar, situated between 7 and 14 MHz, where the highest average mass values and the greatest increases relative to the control were achieved.

For the Carmesi cultivar (Lollo Rosa type), the average plant mass in the control group was 106.91 g. In comparison, all treated variants displayed superior values, fluctuating between 115.50 g and 142.17 g.

The maximum average mass was recorded in variant V8 (30 MHz; 142.17 g), followed closely by V7 (28 MHz; 136.70 g). These values corresponded to increases of 35.26 g (32.98%) and 29.79 g (27.86%), respectively, compared with the control, with the differences being highly statistically significant.

These findings indicate that high frequencies exerted the strongest stimulatory effect on biomass accumulation for this specific cultivar.

Variants V5 (21 MHz), V6 (25 MHz), V4 (14 MHz), and V3 (10 MHz) induced increases ranging between 11.93% and 16.28% relative to the control; all of these were statistically significant. At frequencies of 5 MHz and 7 MHz, the gains were more modest (8.03% and 10.78%, respectively), yet they remained statistically significant.

These outcomes suggest that the Carmesi cultivar responds favorably across the entire tested frequency range, although the maximum effect is observed at high frequencies, particularly between 28 and 30 MHz.

A comparative analysis of the two cultivars highlights clear discrepancies in their response mechanisms to the treatments. The Aleppo cultivar exhibited the most robust biomass development at intermediate frequencies (between 7 and 14 MHz), whereas Carmesi manifested a superior response to high frequencies (28-30 MHz).

The maximum mass increase was 27.1% for Aleppo and 32.98% for Carmesi, indicating a higher sensitivity of the Carmesi cultivar to high-frequency stimulation. These differences suggest the presence of distinct genetic and physiological traits that influence each cultivar's specific capacity of each cultivar to respond to the applied treatments.

Table 1. Average fresh weight of lettuce plants

Variant	Weight (g)	Difference from Control (g)	Percentage Difference (%)	Significance
V1 - 5 MHz	106.4	11	11.5	ns
V2 - 7 MHz	121.3	25.9	27.1	***
V3 - 10 MHz	118	22.6	23.7	***
V4 - 14 MHz	116.1	20.7	21.7	***
V5 - 21 MHz	106.9	11.5	12.1	ns
V6 - 25 MHz	113.6	18.2	19.1	**
V7 - 28 MHz	111	15.6	16.4	**
V8 - 30 MHz	110.1	14.7	15.4	**
V9 - Control	95.4	0	0	Ct.

Table 2. Average fresh biomass and statistical significance for the Carmesi lettuce cultivar (Lollo Rosa type) under different frequency treatments

Variant	Weight (g)	Difference from Control (g)	Percentage Difference (%)	Significance
V1 - 5 MHz	115.50	8.59	108.03	*
V2 - 7 MHz	118.44	11.53	110.78	*
V3 - 10 MHz	119.66	12.75	111.93	**
V4 - 14 MHz	122.25	15.34	114.35	**
V5 - 21 MHz	124.31	17.40	116.28	**
V6 - 25 MHz	121.48	14.57	113.63	**
V7 - 28 MHz	136.70	29.79	127.86	***
V8 - 30 MHz	142.17	35.26	132.98	***
V9 - Control	106.91	0.00	100.00%	control

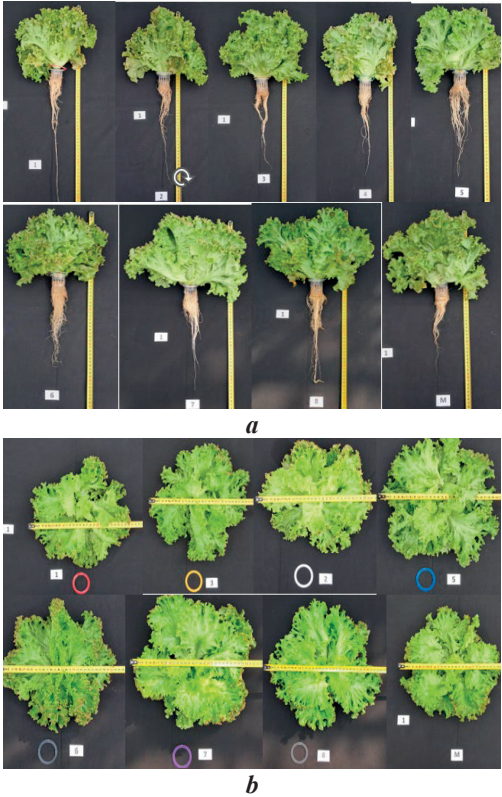


Figure 5. The appearance of lettuce plants in the Aleppo (a) and Carmesi (b) cultivars



Figure 6. Appearance of plants grown under LED lighting

Analysis of Root System Parameters

In the Aleppo variety, root length ranged between 22.50 and 33.25 cm (Figure 7).

The highest value was recorded for V1 - 5 MHz (33.25 cm), followed by V2 - 7 MHz (27.75 cm). Compared with the control (23.50 cm), most variants showed superior values, indicating a favorable effect of low frequencies on root system development. The lowest value was observed for V7 - 28 MHz (22.50 cm), slightly lower than the control.

In the Carmesi variety, root length was greater across all variants compared with the Aleppo, with values ranging between 28.0 and 36.0 cm. The maximum value was recorded for V2 - 7 MHz (36.0 cm), followed by V1 - 5 MHz (35.5 cm) and V4 - 14 MHz (32.0 cm). Compared with the control (26.0 cm), all treatments led to increases in root length, highlighting a superior capacity of the Carmesi variety to respond to the applied stimuli.

It was noted that low frequencies (5-14 MHz) favored the development of root length in both varieties.

In the Aleppo variety, root mass ranged between 3.91 g and 7.83 g. The highest value was obtained in V7 - 28 MHz (7.83 g), significantly exceeding the control (4.74 g). Additionally, variants V5 - 21 MHz (5.54 g), V6 - 25 MHz

(5.22 g), and V8 - 30 MHz (5.70 g) presented high values. These results suggest that high frequencies stimulated root thickening.

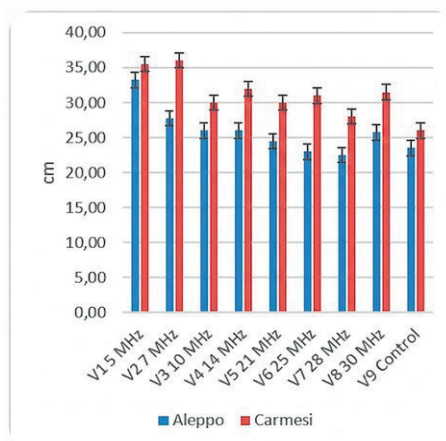


Figure 7. Root length in the analyzed cultivars

In the Carmesi variety, root mass ranged between 4.1 g and 8.9 g. The maximum value was also recorded for V7 - 28 MHz (8.9 g), followed by V6 - 25 MHz (6.5 g) and V8 - 30 MHz (6.2 g) (Figure 8).

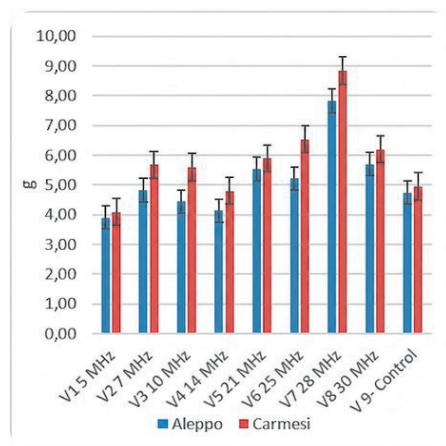


Figure 8. Root mass of lettuce plants

For the Aleppo variety, root volume ranged between 4.50 and 8.25 cm³. The maximum value was observed in V7 - 28 MHz (8.25 cm³), followed by V5 - 21 MHz (6.00 cm³) and V8 - 30 MHz (6.00 cm³). The control presented a value of 5.40 cm³. In the Carmesi variety, the values were higher, ranging between 5.0 and 9.0 cm³. The highest value was recorded in V7 - 28 MHz

(9.0 cm³), followed by V6 – 25 MHz and V5 - 21 MHz (6.8-6.5 cm³). The control had a value of 5.8 cm³.

These results show that the frequency of 28 MHz stimulated root volume most strongly in both varieties (Figure 9).

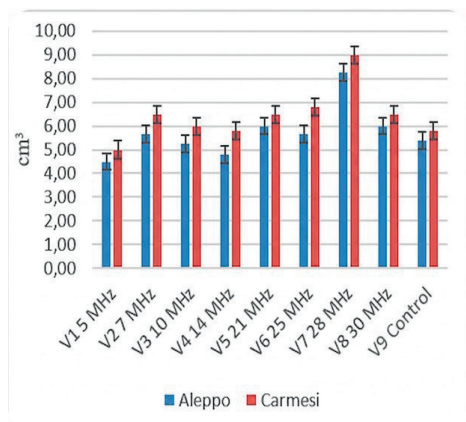


Figure 9. Root volume in the analyzed varieties

Nitrate content

The nitrate content in the Aleppo lettuce variety was influenced by the applied frequencies, with the determined values ranging between 1010.0 and 1730.0 mg/kg. The control variant (V9) recorded the highest nitrate content of 1730.0 mg/kg, while all treated variants exhibited lower values, highlighting a favorable effect of the treatments on reducing nitrate accumulation in the leaves. According to European regulations regarding maximum residue limits for nitrates in lettuce, the values obtained in this experiment are below the maximum allowable limits. This indicates that the resulting yield is safe for consumption and complies with food quality requirements. Compared to the control, the greatest reduction in nitrate content was recorded in variant V7 (28 MHz), where the concentration was 1010.0 mg/kg, representing a 41.6% decrease. Additionally, variant V3 (10 MHz) led to a major reduction of 38.0%, with a recorded value of 1073.3 mg/kg. Significant reductions were also observed in variants V8 (30 MHz), V2 (7 MHz), V4 (14 MHz), and V1 (5 MHz), where the nitrate content was 27.7-36.4% lower than in the control. In contrast, variants V5 (21 MHz) and V6 (25 MHz) presented values closer to the control, namely 1500.0 and 1,600.0 mg/kg, indicating a lower efficiency of

these frequencies in limiting nitrate accumulation. These results suggest that the application of specific frequencies, particularly 28 MHz and 10 MHz, favored a more efficient utilization of nitrogen in the plant's metabolic processes, thereby reducing nitrate accumulation in plant tissues. From a product quality standpoint, these variants are highly valuable, as reduced nitrate content is an important indicator of food safety and the nutritional value of lettuce (Figure 10).

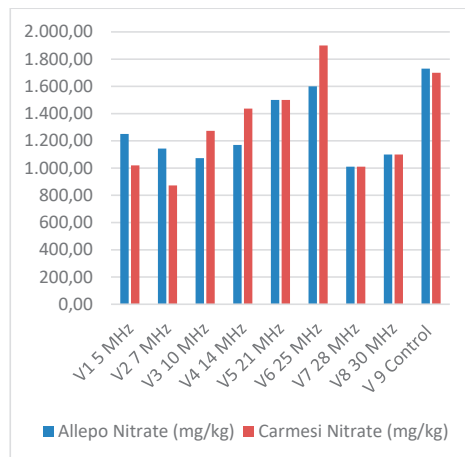


Figure 10. Nitrate content in lettuce leaves

Carbohydrate Content

The carbohydrate content showed less variation compared to nitrates, with values ranging between 2.80% and 3.50%. The control variant (V9) recorded a content of 3.00%, and most treated variants showed equal or superior values. Thus, variants V1 (5 MHz), V2 (7 MHz), and V4 (14 MHz) presented the same carbohydrate level as the control (2.80%). The highest values were determined in variants V6 (25 MHz) and V5 (21 MHz), where the carbohydrate content reached 3.50% and 3.40%, respectively, representing increases of 16.7% and 13.3% compared to the control. Similarly, variants V7 (28 MHz) and V8 (30 MHz) recorded a content of 3.20%, which is 6.7% higher than the untreated variant. The only reduction was observed in variant V3 (10 MHz), where the value of 2.80% was 6.7% lower than that of the control. The increase in carbohydrate content across most treated variants may indicate an intensification of the photosynthetic process and a higher accumulation of storage substances in the leaves. From a food quality

perspective, a higher carbohydrate level contributes to improving the nutritional value and organoleptic characteristics of the product. Variant V6 (25 MHz) stood out particularly, recording the highest carbohydrate content, which suggests that this frequency favored the accumulation of organic compounds resulting from photosynthesis. Overall, the results demonstrate that the applied treatments had a favorable effect on carbohydrate content while simultaneously contributing, in most cases, to a reduction in nitrate content compared to the control variant. Cambria variety (Lollo Rossa Type) - Nitrate Content. In the Lollo Rossa type lettuce, the Cambria variety, the nitrate content ranged between 873 and 1900 mg/kg, depending on the applied frequency. The control variant (V9) recorded a content of 1700 mg/kg, a value that falls within the maximum limits allowed by European legislation for lettuce. Furthermore, all experimental variants presented values below the maximum allowable thresholds for human consumption, confirming the quality and safety of the obtained product. Compared to the control, the greatest reduction in nitrate content was observed in variant V2 (7 MHz), where the value of 873 mg/kg represents a decrease of approximately 48.6%. Major reductions were also recorded in variants V7 (28 MHz), at 1010 mg/kg, and V1 (5 MHz), at 1020 mg/kg, corresponding to decreases of 40.6% and 40.0% compared to the control, respectively. Variant V8 (30 MHz) also led to a significant reduction in nitrate accumulation, with its value of 1100 mg/kg being 35.3% lower than the control. In the case of variant V3 (10 MHz), the nitrate content was 1273 mg/kg, representing a 25.1% reduction, while variant V4 (14 MHz) recorded 1437 mg/kg, which is 15.5% less than the control. Variant V5 (21 MHz) presented a value close to the control, namely 1500 mg/kg, corresponding to an 11.8% reduction. The only variant that exceeded the control value was V6 (25 MHz), where the nitrate concentration reached 1900 mg/kg, representing an increase of approximately 11.8% compared to V9. This result suggests that the 25 MHz frequency favored nitrate accumulation in plant tissues, unlike the other experimental variants. Overall, the results indicate that frequencies of 7 MHz, 28 MHz, and 5 MHz had the most pronounced effect on reducing nitrate accumulation in

Cambria lettuce, contributing to improved product quality and yielding values considerably lower than the control variant (Figure 11).

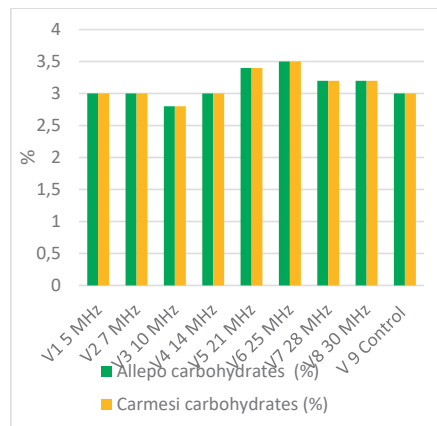


Figure 11. Carbohydrate Content

CONCLUSIONS

The application of treatments using passive electromagnetic devices exerted a general stimulatory effect on biomass accumulation and morphological parameters in lettuce. The biological response is strictly dependent on the genotype (cultivar) and the applied frequency. The Aleppo variety shows an affinity for medium frequencies (7-14 MHz), optimizing its biomass at 7 MHz.

Conversely, the Carmesi variety exhibits higher sensitivity to high frequencies (28-30 MHz), achieving a biomass increase of +32.98% at 30 MHz. Root system development follows a dual pattern: low frequencies (5.0-7 MHz) stimulate root elongation, whereas high frequencies (especially 28 MHz) stimulate root density, mass, and volume, generating a more vigorous anchoring and absorption system.

Passive electromagnetic technology represents a sustainable and promising alternative for optimizing horticultural production in Plant Factory-type systems.

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SYSTEMIC GENE EXPRESSION OF DEFENSE-RELATED ENZYMES INDUCED BY PLANT GROWTH-PROMOTING RHIZOBACTERIA

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Abstract

Plant growth-promoting rhizobacteria can induce both local and systemic plant defense responses. As in the case of local resistance, systemic resistance is associated with the activation of antioxidant enzymes, such as catalase (CAT), superoxide dismutase (SOD), and peroxidase (POX), and the synthesis of other defense-related enzymes, such as phenylalanine ammonia-lyase (PAL). This study aimed to evaluate the effect of bacterial treatment on transcriptional changes of genes encoding five defense-related proteins in distal tissues of Cucumis sativus L. plantlets. Suspensions of Pythium debaryanum Hesse (Py) and antagonistic bacteria, such as Bacillus licheniformis (B40), Bacillus amyloliquefaciens (Bw), Bacillus sp. (Bw1) and Pseudomonas aeruginosa (P14) were applied on cucumber plantlets. Total RNA was isolated, and reverse transcription-PCR (RT-PCR) was performed. A semi-quantitative method was used to determine alterations in the expression of five defense-related genes in treated plants. Plants pretreated with the selected bacterial strains showed overexpression of genes encoding two defense-related enzymes: one involved in the primary antioxidant system and the other a key enzyme in the phenylpropanoid pathway.

Key words: plant defence-related enzymes, systemic gene expression, beneficial microorganisms.

INTRODUCTION

In their natural environment, plants interact with multiple types of stressors, and to cope with these diverse and constant challenges, plants have evolved diverse defense strategies. Besides preformed defense mechanisms, which are passive innate characteristics (Ding et al., 2022; Flors et al., 2024), plants have also developed inducible defense strategies. Plant growth promoting rhizobacteria (PGPR) represent a group of valuable microorganisms that contribute to plant growth stimulation but also to defense system activation (Shores et al., 2005; Yu et al., 2022; Kour et al., 2024). Induced resistance is achieved through an active, highly energy consuming process and is defined by substantial gene regulation changes with significant impact on both primary and secondary metabolism. As a result of changes in gene regulation, plant-induced defense reactions can be manifested both locally and systemically. Systemic resistance is characterized by the triggering of defense signals in distal areas from

the treatment site in response to various elicitors, whereas local resistance involves the induction of defense reactions primarily at the site of application.

Plant induced resistance relies on two main pathways: one dependent on salicylic acid (SA), and another on jasmonic acid (JA) and ethylene (Et). Although it was previously considered that resistance induced by beneficial microorganisms is solely dependent on the JA/Et pathway, subsequent studies have shown that both SA and JA/Et are activated during resistance induction in plant-microbe interaction (Yu et al., 2022; Jung et al., 2024).

Systemic resistance is achieved through a very complex and diverse set of signaling pathways, being associated with the systemic synthesis of enzymatic and non-enzymatic antioxidants, antimicrobials, pathogenesis related proteins (PR) and signaling molecules in healthy plant tissues (Pieterse et al., 2014; Yang et al., 2023). In the case of a further pathogen attack, plants manifest accelerated and stronger structural defense responses, such as cell wall

reinforcement and the obturation of intercellular spaces (Helepciuc et al., 2013), and biochemical defense responses, such as the induction of phenylalanine ammonia-lyase (PAL) and the synthesis of antioxidant enzymes (Mauch-Mani et al., 2017; Jung et al., 2024).

In the current study, we aimed to induce a systemic defense response in cucumber plants treated with previously tested beneficial bacterial strains and pathogenic fungi. These bacterial strains were tested as candidates for PGPR and proved to synthesise indole acetic acid (Helepciuc et al., 2008), have antifungal activity (Mitoi et al., 2012), activate different types of structural and biochemical defense responses in plants (Helepciuc et al., 2013; Helepciuc et al., 2014), but also managed to colonize cucumber roots (Helepciuc et al., 2019). In this study, the systemic expression of genes encoding antioxidant enzymes (superoxide dismutase, peroxidase, and catalase) and phenylalanine ammonia-lyase was tested to evaluate the plant defense response.

MATERIALS AND METHODS

The **biological material** consisted of *C. sativus* var. Marketer plantlets, the fungal phytopathogen *Pythium debaryanum*, and suspension cultures of the bacterial strains *Bacillus licheniformis* (B40), *Bacillus amyloliquefaciens* (Bw), *Bacillus* sp. (Bw1), and *Pseudomonas aeruginosa* (P14) (Collection of the Faculty of Biotechnology, USAMV of Bucharest). The seeds were sterilised with 1% sodium hypochlorite for 1h, washed three times, and aseptically germinated in glass culture dishes (13/8 cm) on wet filter paper at 25°C with a 16/8 h photoperiod.

To obtain a fungal suspension, potato dextrose (PD) liquid medium was inoculated with 3 pieces of 1 cm² of actively growing *P. debaryanum* mycelium and cultured at 28°C for three days.

Bacterial suspensions were obtained by cultivating the bacterial strains on LB broth at 28°C, 150 rpm for 24 h.

Plant treatment

The three weeks old plantlets were treated with 10⁸ CFU/ml bacterial suspension or with three

days old fungal suspension. Also, in some variants, mixed treatment was applied, consisting of both bacterial and fungal suspension.

Plant roots were treated by application on the filter paper from culture dishes of 5 ml of fungal pathogen for the Py variant or each bacterial suspension for B40, P14, Bw and, respectively, BW1 variants. The mixed treatment was performed by applying the bacterial suspension followed by infection with the pathogenic fungus after 24 h for B40-Py, P14-Py, Bw-Py and Bw1-Py experimental variants.

RNA isolation

After 24 h from fungal infection and 48 h from bacterial suspension treatment, cucumber leaves were collected and used for total RNA extraction with the InuPREP Plant RNA Kit (AnalytikJena). The purity and total RNA concentration were estimated using a NanoDrop 1000 spectrophotometer (Thermo Scientific).

Reverse transcription-PCR amplification

RT-PCR amplifications were performed in a 25 µl reaction volume containing 5 µl AMN/Tfl reaction buffer, 0.5 µl AMV reverse transcriptase, 0.5 µl Tfl DNA polymerase, a concentration of 1 µM for each of the forward and reverse primers, 1mM Mg SO₄, 0.2 mM dNTP Mix (Promega, USA) and 4 µl of 125 ng/µl total RNA.

The five sets of primers were documented from the literature: Cu/Zn-superoxide dismutase (EF121763) and Mn-superoxide dismutase (EF203086) (Gao et al., 2009), peroxidase (M91374) (Shores et al., 2005), catalase (AY274258) (Xia et al., 2009) and phenylalanine ammonia-lyase (AF529240) (Bradley, 2009).

The cycling conditions were as follows: reverse transcription for 45 min at 45°C, reverse transcriptase inactivation and RNA primer denaturation for 2 min at 94°C, followed by 45 cycles of 30 s at 94°C (denaturation), 1 min at the specific annealing temperature (Table 1), 2 min at 68°C (extension), and 1 cycle for 7 min final extension step at 68°C. The amplifications were performed in an Eppendorf Mastercycler gradient (Germany).

Table 1. The specific tested primer sequences and annealing temperatures for mARN of the studied genes

Interest gene	RT-amplified fragment (bp)	Forward and reverse primer sequences	T _a (°C)
<i>CAT</i>			
Xia et al., 2009	732	5'-ATGCTGGAAGAGGAGGCTAT-3' 5'-ATGGTGAGGACATTTGGGAG-3'	58
<i>Cu/Zn-SOD</i>			
Gao et al., 2009	297	5'-GACTGGGCCACATTCAACC-3' 5'-GCCTTGCCATCTTCACCAA-3'	55.8
<i>Mn-SOD</i>			
Gao et al., 2009	330	5'-CAATGGCGGAGGTCACATTA-3' 5'-AGAGCAAGCCACCCATC-3'	55.8
<i>POX</i>			
Shoresh et al., 2005	1135	5'-AGAGCAACAAGTCGGTTTCA-3' 5'-GTGCCGACATCCTAGCTCAAG-3'	60
<i>PAL</i>			
Bradley, 2009	468	5'-AGTTGCATGAAATGGATCCTC-3' 5'-TTATGTTGCTCGGCACCTTG-3'	54

Electrophoretic separation

Amplicons were separated on 1.5% agarose gel, and multiple molecular weight and concentration markers, such as GeneRuler 50 bp DNA Ladder (Thermo Scientific), Gene Ruler Low Range DNA Ladder (Thermo Scientific), and DNA Standard 100 bp DNA Ladder (Serva) were used. For DNA band visualization, a 0.01% SYBR Safe solution was used.

Estimation of amplicons molecular weight and concentration

The gels were visualized using GENi (Syngene), equipment, and the DNA molecular weight and DNA concentrations were estimated using the GeneTools (Syngene) software, having as reference the above-mentioned molecular weight and concentration markers.

RESULTS AND DISCUSSIONS

In the current research, the systemic expression of cucumber enzymes Cu/Zn superoxide dismutase (*Cu/Zn-SOD*), Mn superoxide dismutase (*Mn-SOD*), peroxidase (*POX*), catalase (*CAT*), and phenylalanine ammonia-lyase (*PAL*) was analysed. In the case of superoxide dismutases and peroxidase, the separation of the amplification mixture on an agarose gel evidenced multiple reaction products, indicating low specificity of the primers used. In the case of some studied genes, the interest RT-PCR products are found among the multitude of amplified fragments, such as the 330 bp amplicon of *Mn-SOD* (Gao et al., 2009), 297 bp amplicon of *Cu/Zn-SOD* (Gao et al., 2009). In case of *POX* (Shoresh et al., 2005), the interest amplicon of 1135 bp, was not identified. Of the five defense-related genes tested, only *CAT* and *PAL* were considered for further

analyses, as the amplified products had an appropriate molecular weight.

Catalase gene expression

The reverse transcription of the catalase gene using the set of primers mentioned by Xia et al. (2009) showed a single amplification product with a molecular weight between 433 and 461 bp (Figure 1), the entire sequence as presented in the GeneBank database having 732 bp.

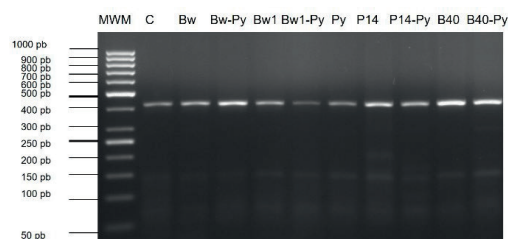


Figure 1. The UV visualization of *CAT* RT-product on 1.5% agarose gel electrophoresis: MWM - molecular weight marker; C - untreated control variant; Bw, Bw1, P14, B40 - experimental variants treated with beneficial bacterial suspensions; Py - infected variant with *P. debaryanum* phytopathogen; Bw-Py, Bw1-Py, P14-Py, B40-Py - experimental variants treated both with beneficial bacterial strains and phytopathogen

The treatments with different types of bacterial suspensions, such as B40, Bw, Bw1, and P14, induced an increase in *CAT* transcript concentrations compared to the control (Figure 1). The best stimulation was produced by B40 strain (7.001 ng cDNA/μl), followed by P14 and Bw, while the Bw1 strain had the weakest induction effect (3.78 ng cDNA/μl). The application of the phytopathogen determined a decrease in the expression of this *CAT* gene for all infected experimental variants, with the exception of the Bw-Py variant, in which pathogen administration caused an increase in this *CAT* gene expression (Table 2). Higher

CAT amplicon concentrations than in the control were observed in all treated variants, except for the Py variants Bw1-Py and (Table 2). The variants treated only with the pathogen (Py) and Bw1-Py variant, which was stimulated with the least inductive bacterial strain Bw1, registered the lowest CAT amplicon concentrations. The highest concentrations were recorded in B40- treated variants (B40, respectively B40-Py).

Table 2. The estimation of CAT cDNA amplicon concentrations in leaves of cucumber plantlets treated with beneficial bacterial suspensions and/or infected with *P. debarianum* phytopathogen

Experimental variant	CAT RT-amplicon concentration (ng/μl)
C	3.149
Bw	4.325
Bw-Py	5.926
Bw1	3.780
Bw1-Py	1.153
Py	2.921
P14	5.644
P14-Py	4.853
B40	7.001
B40-Py	6.001

The concentration of ROS in plants rises rapidly during plant-microbe interaction, reaching significantly higher concentrations than in normal conditions (Ding et al., 2022). The better expression of CAT amplicon shows the enzymatic antioxidant system, which is involved in counteracting the abundance of ROS in plant cells as a result of plant-microbe interaction. In a previous work, we also showed an increased catalase activity compared with other H₂O₂-degrading enzymes (guaiacol peroxidase) in cucumber plants treated with both beneficial and pathogenic microorganisms (Helepciuc et al., 2014). Similar results were obtained in a long-term experiment: maize embryos that showed increased pathogen resistance had a higher catalase activity (Magbanua et al., 2007). However, other studies reported a decrease in antioxidant enzymes, for example, in *in vitro* strawberry regenerants treated with anatoxic sera obtained from different phytopathogenic *Botrytis cinerea* or non-phytopathogenic *Trichoderma viride* and *Penicillium chrysogenum* fungal strains (Cogălniceanu et al., 2010). Besides their role in excess H₂O₂ neutralization, catalases can also

regulate various defense signaling pathways, influencing the (Riseh et al., 2024). Catalase expression is particularly important in plant-microbe interaction due to its dual role in plant protection. First, catalase is a highly efficient hydrogen peroxide neutralizer (Scandalios et al., 1997; Mhamdi et al., 2010; Rao et al., 2025) being directly involved in maintaining the balance between reducing reactions associated with defense responses and oxidizing reactions responsible for oxidative damage within cells. Besides cellular redox homeostasis, catalases are also involved in defense signaling pathways, interfering with both jasmonic acid and salicylic acid signaling pathways and shaping the plant’s response in beneficial and pathogenic interactions (Riseh et al., 2024).

Phenylalanine ammonia-lyase gene expression

In the case of the phenylalanine ammonia-lyase gene (Bradley, 2009) it was also obtained a main reversetranscription product with a molecular weight of 437-520 bp (Figure 2) comparing with the 533 bp sequence shown in the GenBank database.

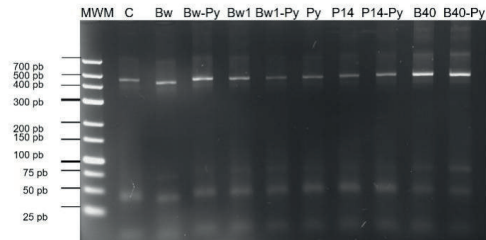


Figure 2. The UV visualization of PAL RT-product on 1.5% agarose gel electrophoresis: MWM - molecular weight marker; C - untreated control variant; Bw, Bw1, P14, B40 - variants treated with beneficial bacterial suspensions; Py - infected variant with *P. debaryanum* phytopathogen; Bw-Py, Bw1-Py, P14-Py, B40-Py - variants treated with both beneficial bacterial strains and phytopathogen

The concentration of PAL amplicon increased in all bacterial-treated variants compared with the control, except for P14 (Figure 2). The best stimulatory effect was observed in the variant treated with the B40 strain, followed by Bw and Bw1, while P14 inhibited PAL expression (Table 3). Also, infection with *P. debaryanum* decreased the PAL concentration in the variant untreated with beneficial bacteria. The amplicon

corresponding to the PAL gene had the highest concentration in the infected B40-Py (4.910 ng/μl), while the lowest concentration was registered in Bw1-Py variant (0.864 ng/μl) (Table 3).

Table 3. The estimation of PAL cDNA amplicon concentrations in leaves of cucumber plantlets treated with beneficial bacterial suspensions and/or infected with *P. debarianum* phytopathogen

Experimental variant	PAL RT-amplicon concentration (ng/μl)
C	1.825
Bw	3.651
Bw-Py	4.413
Bw1	2.794
Bw1-Py	0.864
Py	1.151
P14	1.098
P14-Py	1.744
B40	4.659
B40-Py	4.910

Except for Bw1-Py variant, the concentration of PAL amplicon increases in variants with mixed treatment (beneficial bacteria and fungal phytopathogen) compared with bacterial pretreated variants or with the pathogen-treated variant (Py) (Table 3). These results suggest that in the case of pretreatments with beneficial bacteria, the *PAL* gene is overexpressed in subsequent interaction with the pathogen.

A previous study also reported that overexpression of a *PAL* gene (*OsPAL1*) induces resistance in rice to both *Magnaporthe oryzae* and *Xanthomonas oryzae* pv *oryzae* (Zhou et al., 2018). Also, in soybean, PAL positively regulates plant resistance to *Phytophthora sojae* (Zhang et al., 2017).

PAL is involved in the first step of the phenylpropanoid pathway, catalyzing the non-oxidative L-phenylalanine deamination with the formation of trans-cinnamic acid (Liu et al., 2024, Amjad et al., 2024). Trans-cinnamic acid is a crucial player in plant defense responses, as it is the precursor of multiple defense-related compounds such as lignin, suberin, monolignols, flavonoids, coumarins, phytoalexins, phenolic acids, and salicylic acid (Zhang et al., 2017; You et al., 2020; Liu et al., 2024). In plants, these compounds can have structural roles; they can act as antioxidants and

antimicrobial compounds, but they can also play major roles as signaling molecules in plant defense pathways. The variety of defense-related compounds highlights the crucial role of PAL in plant biotic stress responses. The enhanced expression of *PAL* was correlated with an increase in lignin content in pathogen-resistant rice, pointing out the involvement of PAL in lignin biosynthesis, an important non-specific structural defense mechanism which can confer resistance to a wide range of pathogens (Zhou et al., 2018). In addition to its role in structural compounds, antimicrobials, and antioxidants, PAL also plays a major role in the plant hormone salicylic acid biosynthesis through the phenylpropanoid pathway, an alternative to the well described isochorismate synthase pathway (Liu et al., 2024; Wang et al., 2025).

Various studies have documented phenylalanine-derived salicylic acid biosynthesis in different plant species, highlighting, for example, its importance in *Brachypodium distachyon* resistance to *Panicum mosaic virus* (PMV) and its satellite virus (SPMV) (Pant et al., 2021). Also, Ullah et al. (2023) mentioned the reliance of *Populus* sp.'s on phenylalanine-derived salicylic acid biosynthesis for both constitutive and induced pathways.

In this study, the bacterial treatment managed to activate different components of the cucumber plants' defense system. First, the catalase gene shows the activation of the enzymatic antioxidant system to neutralize the excess of reactive oxygen species generated in plants, which is perhaps one of the earliest defense responses in plant-microbe interactions. Additionally, the overexpression of phenylalanine ammonia-lyase, a key enzyme in the phenylpropanoid pathway, primarily creates the premises for the synthesis of important defense-related compounds with structural, antimicrobial, and antioxidant properties. Furthermore, the initiation of the phenylpropanoid pathway enables the production of important signaling molecules, such as salicylic acid, compared with the untreated variant.

CONCLUSIONS

RT-PCR analysis showed a differential response in pretreated plants with bacterial suspensions compared with the untreated variant. Plants challenged with the selected bacterial strains showed overexpression of genes encoding CAT and PAL, but in the case of further infection, CAT transcript concentrations decrease while PAL expression increases. These findings indicate a variable and complex response of plants, which can ensure better protection against pathogen attack and a differentiate priming effect conferred by beneficial bacterial strains.

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METHODS FOR MANAGING THE PEST *TUTA ABSOLUTA* MEYRICK IN TOMATO CROPS IN GREENHOUSE

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Abstract

Chemical and biological methods are widely used for pest control in tomato crops. In order to manage Tuta absoluta infestations, a greenhouse tomato experiment was conducted in 2025 at RDIVFG Vidra, using the hybrids Tolstoi F1 and Kalina F1, planted at a density of 33.500 plants per hectare. The crops were protected with black plastic mulch to reduce evapotranspiration and limit weed development. The experiments were arranged in four variants, each with three replications, and climatic conditions were monitored using a thermo-hygrometer. In the first variant, treatments were applied exclusively with chemicals; in the second variant, chemical products were combined with plant extract-based products; in the third variant, only biological products were applied throughout the entire growing season; whereas the fourth treatment served as an untreated control, with no phytosanitary applications. The effectiveness of the treatment variants ranged from 64.14% to 90.87%. In reported experimental conditions, the best results were obtained with the variant in which chemical treatments were applied.

Key words: biological control, chemical products, plant protection.

INTRODUCTION

Tuta absoluta Meyrick, known as the tomato leafminer, is a destructive moth that prefers to attack tomatoes (*Solanum lycopersicum* L.) but its attack of this insect has also been reported on potatoes (*Solanum tuberosum* L.), tobacco (*Nicotiana tabacum* L.), black nightshade (*Solanum nigrum* L.) (Abbes et al., 2016). The attack is easily noticeable, as the larvae feed on the leaves, creating leaf mines that reduce the plant's photosynthetic capacity. This leads to leaf necrosis and consequently to premature leaf drop or death (Desneux et al., 2010).

Farmers frequently turn to chemical control methods as the initial and main approach to managing *T. absoluta* (Machekano et al., 2020), as these insecticides offer a quick, accessible, and simple solution to control pest populations; however, excessive dependence on them can result in issues such as insecticide resistance, pest resurgence, environmental contamination, and adverse effects on both human health and the ecosystem (Mawcha et al., 2025).

In order to manage pests in an environmentally responsible way, researchers investigate natural compounds as potential sources of insecticidal agents. For centuries, plants with insecticidal properties have been utilized worldwide to control insect populations. Owing to their low toxicity to warm-blooded animals and their high volatility, these natural substances are regarded as viable alternatives to conventional pesticides. Compared to synthetic products, plant-derived insecticides tend to be more eco-friendly and are generally easy for farmers and small-scale industries to process and apply (Ayilara et al., 2023; Rasouli et al., 2025).

The use of biopesticides based on entomopathogenic fungi such as *Beauveria bassiana* represents an important alternative for the sustainable control of *Tuta absoluta*. This fungus infects insect hosts by penetrating the cuticle, proliferating within the body, and ultimately causing death (Vega et al., 2012; Mascarín & Jaronski, 2016).

Under greenhouse and field conditions, *B. bassiana* has shown consistent potential in

reducing pest populations, although its efficacy is generally lower compared to chemical insecticides. However, its advantages, such as environmental safety, low toxicity to non-target organisms, and reduced risk of resistance development, make it a valuable component of sustainable agriculture (Faria & Wraight, 2007; Lacey et al., 2015).

The aim of this study was to analyze the effectiveness of chemical and biological products based on plant extracts for the control of the tomato leaf miner.

MATERIALS AND METHODS

In 2025, under greenhouse conditions, a first-cycle tomato crop was established at RDIVFG Vidra on April 29. The biological material consisted of the hybrids Tolstoi F1 and Kalina F1, with five rows planted for each hybrid.

The tomato crop was established at a density of 33 500 plants per hectare, equivalent to 3.35 plants per m². The planting scheme included a spacing of 0.35 m between plants within the row and 0.50 m between rows, thereby ensuring an optimal balance between plant aeration and efficient space utilization.

The crop was established on soil mulched with black plastic film, a technological measure aimed at reducing evapotranspiration, limiting weed development, and maintaining a uniform soil temperature. Climatic factors were recorded using a CEM DT-172 thermo-hygrometer.

Tolstoi F1 is an indeterminate tomato hybrid exhibiting resistance to *Fusarium oxysporum* f. sp. *lycopersici* races 0 and 1, tomato mosaic virus (ToMV), and verticillium wilt. Kalina F1 is an indeterminate tomato hybrid with a growing period of 100-120 days. The plants are resistant to tomato mosaic virus (ToMV), verticillium wilt, fusarium wilt, and nematodes. The experiments included four treatments, each arranged in three replicates. The greenhouse was divided into four compartments, separated by agryl fabric. The first compartment comprised the chemical treatment experiment, followed by the second compartment containing the integrated chemical + biological treatment, the third compartment with exclusively biological treatments, and the fourth compartment representing the untreated control.

In Treatment 1, throughout the entire growing season, the plant protection scheme consisted exclusively of applications of chemical-based products. In Treatment 2, chemical plant protection products were applied during the early stages of plant development; subsequently, upon the onset of the physiological fruit maturation stage, the treatment program was supplemented and replaced with plant extract-based products. In Treatment 3, during the entire growing period, phytosanitary management was carried out solely through the application of biological products.

The experimental variants for pest control were as follows: V1 - Coragen (chlorantraniliprole 20%) at 175 ml ha⁻¹; V2 - Coragen (chlorantraniliprole 20%) at 175 ml ha⁻¹ + Oleorgan (Neem extract 40%) at 300 ml in 100 L, which means a concentration of 3%;

V3 - Oleorgan (Neem extract 40%) at a concentration of 3%; V4 - Untreated control.

The degree of attack caused by *Tuta absoluta* (tomato leaf miner) was calculated in accordance with the S.O.P./Mar. 2010 standard. Damage assessment was performed on five plants per replicate (20 plants per treatment), and five leaves were selected from each plant (25 leaves per replicate, 100 leaves per treatment). The efficacy of the control products was calculated using Abbott's formula.

During the growing season, observations were conducted regarding the occurrence and progression of pathogen attack (frequency and intensity of infection), based on this, the degree of attack (%) and product efficacy (%) were determined. Yield data were recorded dynamically by treatment and replicate and were subsequently statistically processed using the analysis of variance (ANOVA) method.

RESULTS AND DISCUSSIONS

Following the application of the four treatment variants for the control of *Tuta absoluta* (Figures 1, 2, and 3) in the tomato hybrid Kalina F1, the results obtained highlight clear differences among the control methods employed.

The lowest degree of attack was recorded in the variant treated exclusively with chemical products (V1), where the mean value of the degree of attack (DA) was 1.72%.

This represents a reduction of 11.25% compared to the untreated control, while treatment efficacy reached 86.74% (Table 1). The difference compared to the control was statistically highly significant, confirming the high effectiveness of the chemical treatment in reducing pest populations.



Figure 1. *Tuta absoluta* attack on tomato leaf



Figure 2. *Tuta absoluta* attack on tomato fruit



Figure 3. *Tuta absoluta* - adult

Table 1. Efficacy of control products against *T. absoluta* in the Kalina F1 hybrid

Variant	Mean DA (%)	Difference compared to the untreated control	% Compared to Control	Significance	E (%)
1	1.72	-11.25	13.25	000	86.74
2	2.29	-10.69	17.62	000	82.36
3	4.65	-8.32	35.82	000	64.14
4	12.98	-	100.00	-	-

LD 5% = 0.67; LD 1% = 0.98; LD 0.1% = 1.47

A high efficacy was also observed in the variant combining chemical and biological treatments (V2). In this case, the degree of attack was 2.29%, representing a reduction of 10.69% compared to the control, while the treatment efficacy reached 82.36%. Although slightly lower than V1, these results indicate that integrating biological products into the control scheme significantly contributes to reducing pest attack while promoting a more sustainable approach.

In the variant using only biological products (V3), treatment efficacy decreased to 64.14%, with a recorded degree of attack of 4.65%. While differences compared to the control remain significant, the results indicate that biological treatments, although beneficial, do not achieve the same level of pest control as variants including chemical products. This may be explained by the slower mode of action of biological agents or by the need for more frequent applications to reach maximum efficacy. The untreated control (V4) exhibited the highest degree of attack, with a mean value of 12.98%, confirming the high pest pressure under the experimental conditions of this year.

Table 2. Efficacy of control products against *T. absoluta* in the Tolstoi F1 hybrid

Variant	Mean DA (%)	Difference compared to the untreated control	% Compared to Control	Significance	E (%)
1	1.20	-11.95	9.10	000	90.87
2	2.43	-10.72	18.45	000	81.52
3	4.58	-8.57	34.85	000	65.17
4	13.15	-	100.00	-	-

LD 5% = 0.63; LD 1% = 0.91; LD 0.1% = 1.37

In the Tolstoi F1 tomato crop, the lowest degree of attack was recorded in the variant treated exclusively with chemical products (V1), where the DA was only 1.20%. This represents a reduction of 11.95% compared to the control, and the treatment efficacy reached 90.87%, the highest among all analyzed variants (Table 2). Differences compared to the control were statistically highly significant, confirming that the chemical treatment provided the most effective pest control for this hybrid. The variant receiving a combined chemical + biological treatment (V2) recorded a degree of attack of 2.43%, representing a reduction of 10.72% compared to the untreated control. The

treatment efficacy was 81.52%, demonstrating that integrating biological products into the chemical scheme maintains a high level of control over *Tuta absoluta*. The exclusively biological treatment (V3) achieved an efficacy of 65.17%, with a degree of attack of 4.58%. Although the reduction compared to the control remains statistically significant, the effectiveness of this treatment is lower than that of variants including chemical products. This suggests that biological products, while useful in pest management, require regular applications and may be more sensitive to environmental conditions, providing a moderate level of control over *T. absoluta*.

The untreated control (V4) exhibited the highest degree of attack at 13.15%, reflecting the significant pest pressure in Tolstoi F1.

In the 2025 experiment, for the Kalina F1 tomato crop, Treatment 1 (V1) resulted in an average yield of 3.374 Kg/m², representing a highly significant increase of +2.40 kg/m² compared to the control (348.07%; Table 3), indicating a positive impact on production. Treatment 2 (V2) recorded a yield of 2.868 kg/m², with a difference of +1.90 Kg/m² compared to the control (295.84%). In Treatment 3 (V3), using only biological products, the yield was 1.683 kg/m², with a significant increase of +0.71 kg/m² compared to the control (173.66%).

Thus, treatments involving chemical products, whether applied alone or in combination with biological products, demonstrated significant effectiveness in increasing tomato yield, whereas exclusively biological treatments had a less pronounced effect, and the absence of treatment led to a substantial decrease in yield.

Table 3. Yield obtained in 2025 for the Kalina F1 tomato crop

Variant	Mean (Kg/m ²)	Difference compared to the untreated control	% Compared to Control	Significance
1	3.374	+2.40	348.07	***
2	2.868	+1.90	295.84	***
3	1.683	+0.71	173.66	*
4	0.969	-	100.00	-

LD 5% = 0.56; LD 1% = 0.82; LD 0.1% = 1.24

In the Tolstoi F1 tomato crop, the yield obtained in Treatment 1 (Table 4) was 3.330 kg/m², representing a highly significant increase of

+2.45 kg/m² compared to the control (377.09%). Treatment 2 recorded a yield of 3.038 kg/m², with a highly significant difference of +2.15 kg/m² compared to the control (344.02%). Treatment 3, with biological products, achieved a yield of 2.210 kg/m², showing a significant increase of + 1.33 kg/m² compared to the control (250.28%). The untreated control (V4) had a yield of 0.883 kg/m², confirming the negative impact of pest attack on production in the absence of curative control measures.

Table 4. Yield obtained in 2025 for the Tolstoi F1 tomato crop

Variant	Mean (kg/m ²)	Difference compared to the untreated control	% Compared to Control	Significance
1	3.330	+2.45	377.09	***
2	3.038	+2.15	344.02	***
3	2.210	+1.33	250.28	*
4	0.883	-	100.00	-

LD 5% = 0.98; LD 1% = 1.43; LD 0.1% = 2.15

This study was a comparison between chemical and biological control of the mining moth. The best results were obtained with the chemical variant, but the trend is to eliminate chemicals and try biological control by releasing parasites and predators and using biopesticides.

Microbial biopesticides are generally less damaging to the environment than chemical insecticides, tend to be safer for humans and other vertebrates, and can coexist with other beneficial organisms, such as natural predators of arthropods (Desneux et al., 2022).

Bioinsecticides are pest control agents obtained from living organisms, including bacteria, fungi, and viruses. They are widely used in the management of insect pests and are regarded as environmentally sustainable alternatives to conventional synthetic insecticides (Mawcha et al., 2025). Compared to chemical products, bioinsecticides generally exhibit lower toxicity toward non-target species and demonstrate reduced persistence in the environment. Owing to these advantages, they have attracted considerable interest in their potential role in sustainable agricultural pest management (Fazeli-Nasab et al., 2022; Mawcha et al., 2025). Entomopathogenic agents such as *Bacillus thuringiensis*, *Beauveria bassiana*, spinosad, and neem extract represent environmentally

friendly and efficient solutions for pest control in both high plastic tunnel systems and open-field cultivation (Sridhar et al., 2019; Ali & Muhammad, 2023).

Fungal biocontrol agents, especially *Metarhizium anisopliae* and *Beauveria bassiana*, show strong potential against *Tuta absoluta*. Several *M. anisopliae* strains caused significant mortality, including 37.14% in females, while some *B. bassiana* strains achieved over 90% larval mortality (Shiberu et al., 2018; Basit et al., 2025). Mortality rates of 87%, 57%, and 66% were recorded in second-instar radish leaf miner larvae exposed to *M. anisopliae*, *Verticillium lecanii*, and *B. bassiana*, respectively (Basit et al., 2025). Other fungi, such as *Isaria*, *Lecanicillium*, *Purpureocillium*, *Aspergillus*, and *Clonostachys* spp., have also been used (Basit et al., 2025).

Managing *Tuta absoluta* in tomato crops remains a major challenge for agriculture worldwide. Nevertheless, the use of biotechnological approaches offers promising opportunities for significant advancements.

CONCLUSIONS

The findings of this study confirm that chemical treatments remain the most effective method for controlling *Tuta absoluta* in both Kalina F1 and Tolstoi F1 tomato hybrids, as they ensured the lowest degree of attack and the highest yields. For example, in the Kalina F1 hybrid, the chemical treatment (V1) reduced the degree of attack to 1.72% and achieved an efficacy of 86.74%, while in Tolstoi F1 it reached an even higher efficacy of 90.87% with a degree of attack of only 1.20%. Similarly, these treatments resulted in the highest yields, such as 3.374 kg/m² in Kalina F1 and 3.330 kg/m² in Tolstoi F1. The combined chemical and biological treatment (V2) also showed strong performance, with efficacy values of 82.36% (Kalina F1) and 81.52% (Tolstoi F1), demonstrating that integrating biological products can maintain a high level of control while supporting more sustainable practices. In contrast, the biological-only treatment (V3) provided moderate results, with lower efficacy (around 64–65%) and reduced yields compared to V1 and V2, although still significantly better than the untreated control. The control variants (V4),

which recorded high attack levels (up to 13.15%) and very low yields (as low as 0.883 kg/m²), highlight the severe impact of the pest in the absence of treatment. Overall, although chemical control offers the best results, the integration of biological methods represents a viable and increasingly important strategy for sustainable agriculture.

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EFFECTS OF HIGH TEMPERATURE ON GROWTH CHARACTERISTICS IN TOMATOES

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Abstract

The effects of high temperatures continue to be a major problem for food security. Therefore, the creation of high temperature-tolerant varieties is an essential economic measure in the adaptation of plants to climate change. The paper presents the results of screening some tomato prospective lines based on resistance to high temperatures (41, 43°C) in early ontogenesis. Seedlings grown at 25°C served as the control variant. The analysis of the variability of the resistance trait was carried out based on the length of the embryonic radicle, stem, and entire seedling. As a result of the analysis of the percentage of variance in the source of variation of the traits, it was found that the contributions of genotype, temperature, and the genotype x temperature interaction for radicle length were 13.9, 75.7, 5.1%; stem - 9.3, 82.9, 4.3%, and for seedling - 10.2, 80.2, 5%. So, the variability of the lengths of the embryonic radicle, stem, and seedling depends mostly on temperature. By cluster analysis (k-means) in the 43°C variant, the lines L 504, L 506, L 507 were identified, which formed a separate cluster with the highest values of the evaluated characters. Line 506 exhibited the highest values of growth traits at both levels of stressful temperature, which offers opportunities for its use in breeding programs as a resistance donor.

Key words: tomato, resistance, temperature, variability.

INTRODUCTION

Agriculture is one of the sectors negatively affected by climate change, which is expected to be more pronounced in the coming years (Akhoundnejad, 2020). The agricultural sector has a fragile structure, directly dependent on climatic events. Therefore, studies and measures taken to minimize the possible adverse effects of climate change in developed countries are considered "important economic concepts" (Acar, 2010; Akhoundnejad, 2020). The Republic of Moldova is located in the geographical area where the adverse effects of climate change are inevitable. Therefore, it is extremely important to improve the tolerance of crops to thermal stress. In this regard, the following are of major importance: i) the development of reliable and applicable methods and techniques for identifying tomato genotypes tolerant to stress caused by high temperatures; ii) the creation and implementation of resistant parents in breeding programs for the creation of new varieties resilient to climate change (Mihnea, 2022; Mihnea, 2025). Tomatoes (*Solanum lycopersicum* L.) are the most cultivated vegetable in the world after

potatoes (FAOSTAT, 2020) and the most consumed as a fresh culinary additive or as a processed product, mainly due to the nutritional value associated with the pigments present in the ripe fruits (Chaudury et al., 2019).

Temperature stress (TS) is one of the most important abiotic stresses limiting crop productivity worldwide. In both cool and warm seasons, productivity is reduced due to reduced fruit set. Under the current global warming scenario, temperatures are expected to increase by 2-5°C by the end of the 21st century (Masson-Delmotte, 2021). The global average temperature at the soil surface will cause serious damage to plant growth and development, resulting in a strong reduction in the productivity of many crops (Iizumi, 2018). Moreover, tomatoes are one of the main species that are very susceptible to high temperatures. The optimal temperatures for the development of floral organs, pollen, and fruits vary between 15 and 32°C, and temperatures of 35°C and above this level directly affect vegetative and generative development (Daşgan, 2013; Akhoundnejad, 2018; Akhoundnejad, 2020). Although all plant tissues are severely affected by increased temperatures, reproductive tissues

are the most sensitive, and a temperature increase of a few degrees during the flowering period can cause complete crop loss, as occurs in cereal crops (Hasanuzzaman, 2013). In general, the reproductive period is more sensitive to thermal stress than the vegetative period (Mittler & Blumwald, 2010; Ruan et al., 2010; Zinn et al., 2010). It has been found that under stress conditions, resistant tomato genotypes have the ability to form a much higher number of fruits than sensitive ones (Comlekcioglu et al., 2010).

ST has negative effects on physiological processes in the plant (disruption of photosynthesis, increased respiration), which leads to a decrease in plant productivity (Prasad et al., 2017; Singh et al., 2017). It is accepted that the reduction in the life cycle, associated with the decrease in plant productivity in the case of ST, is caused by its impact on the rate of respiration and photosynthesis (Barnabas, 2008). Another effect of ST is the negative impact on the root system, which provides support for the plant, nutrient absorption, and transport to other organs of the plant (Valdés-López et al., 2016). These phenomena lead to poor root development, disruption of growth stages, pollination, and flowering (Sehgal et al., 2017).

In addition, it has been reported that high temperatures causing floral abortion lead to 80% flower loss in tomato plants, which significantly reduces the number of fruits (Ruan et al., 2010; Rieu et al., 2017). It is worth noting that due to its quantitative nature, resistance depends on a number of factors, including genotypic, environmental, and genotype x environment interactions. High and stable yields of agricultural crops can only be obtained from varieties with high productivity potential and resistance to adverse environmental factors.

The purpose of our research was to evaluate the effect of heat stress on the growth and development traits of tomato plants and identify those with potential use in breeding programs.

MATERIALS AND METHODS

As initial material for the planned research, the following lines were used: L 502, L 503, L 504, L 505, L 506, L 507 L 508; L 601 and L 602 selected from intraspecific hybrid combinations F5, Local form Timisoara x Mary Gratefully,

Desteptarea x Florina, Desteptarea x Pontina, Prestij x Pontina, Pontina x Prestij, Mary Gratefully x Florina, Desteptarea x Florina, in comparison with the control variety Mary Gratefully (MG).

For the analysis of *genetic variability*, *heritability*, and *genetic progress*, the following formulas were applied:

σ^2_g - genotypic variance;

σ^2_{ph} - phenotypic variance;

h^2 - broad heritability coefficient;

PCV - phenotypic coefficient of variation;

GCV - genotypic coefficient of variation;

X - overall mean of the trait;

σ^2_e (variance errors, or VE) = MSE;

GA - genetic advantage;

K - selection differential = 2.06 at 5% selection pressure;

σ_{ph} - overall standard deviation of the trait (Adeniji, 2018; Balkan, 2018).

Cluster analysis of the degree of similarity/dissimilarity of tomato genotypes based on growth and development characters at different temperatures was performed using the iterative dendrogram construction algorithm and the *k*-means centroid method (Kanavi et al., 2020). The obtained data were statistically processed in the STATISTICA 7 software package.

RESULTS AND DISCUSSIONS

Testing the reaction of perspective lines at different temperature levels on tomato growth traits in early ontogenesis demonstrated that the response of plants to the 3 temperatures (25°C - optimal, 41° and 43°C - heat stress) was differentiated - specific to the genotype and trait analyzed.

In the case of radicle length, it was found that under optimal conditions, it varied within the limits of 36.4-58.0 mm (Figure 1A), and at a temperature of 41°C - 23.6-52.8 mm (Figure 1B). The degree of growth inhibition for the studied forms varied in the range of -1.6...-49.8% (compared with optimal conditions). Strong inhibition was found in the Mary Gratefully variety (49.8%) and L 601 (22.58%). Stimulation was observed in the lines L 504 (2.7%), L 505 (4.4%) and L 602 (2.1%), and relatively low inhibition - in the lines L 503 (1.6%) and L 506 (9%).

Under influence of 43°C temperature, significant inhibition of radicle growth was observed in Mary Gratefully (46.5%) and L 601 (46.9%). Weak sensitivity was observed in the L 504 (-5.4%) and L 507 (-5.4%) lines (Figure 1C). As for stem, inhibition of growth at a temperature of 41°C was significant only in the Mary Gratefully variety (-8.4%) and relatively low in L 502 (-1%), and in the other genotypes, stimulation was recorded at 9.5...37.3%. Under the influence of a temperature of 43°C, the inhibition varied within the limits of 9.5...49.4%. The most sensitive proved with be Mary Gratefully (-41.0%) and L 602 (-49.4%), and stimulation (by 10.7%) was recorded in L

504. Increased resistance was recorded in L 507, the decrease in stem length compared with the control was 9.5% (figure 1 B).

In the case of seedling length, it was found that its reduction relative to the control ranged between 2.8-45.7% at 41°C and 6.7-44.7% at 43°C. At the same time, the temperature of 41°C most strongly influenced the Mary Gratefully variety (-45.7%) and the L 601 line (-12.0%), and stimulation was recorded in the L 503, L 504, L 505 and L 602 lines at 2.8; 14.3; 7.7; 17.9%, respectively. Significant inhibition at 43°C was demonstrated by the varieties Mary Gratefully (44.7%), L 502 (31.0%), L 505 (33.6%), L 506 (32.6%), L 601 (33.5%) (Figure 1C).

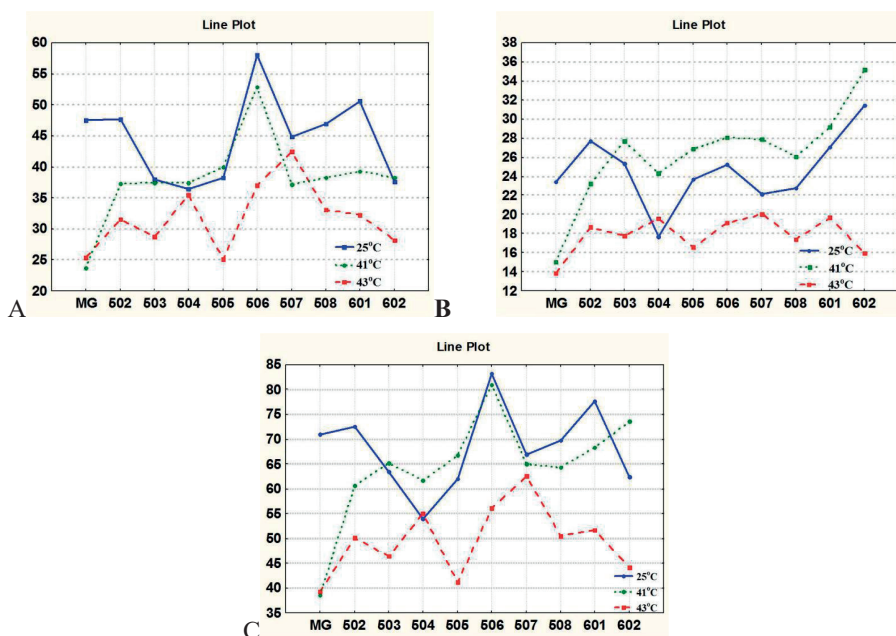


Figure 1. Variability of growth characters: A - radicle length; B - stem length; C - seedling length at different temperature levels

The distribution dendrogram of the perspective lines based on the reaction of the embryonic radicle, the stem, and the seedling demonstrates the existence of similarities, but also differences between the tomato genotypes both in optimal and stress conditions.

The distribution based on Euclidean distances highlighted the formation of distinct clusters within the evaluated genotype set: 2 - under optimal conditions (Figure 2 A) and 3 - heat stress conditions (Figure 2 B, C).

The degree of similarity between tomato genotypes according to the reaction of growth organs to different temperature levels was different. For example, under optimal conditions, the Mary Gratefully variety and lines L 508, L 502; L 503 and L 505, recorded the highest similarity, confirmed by the smallest Euclidean distance for all evaluated characters. The differences between these genotypes were accentuated in the case of stress temperatures, which led to their localization in different clusters.

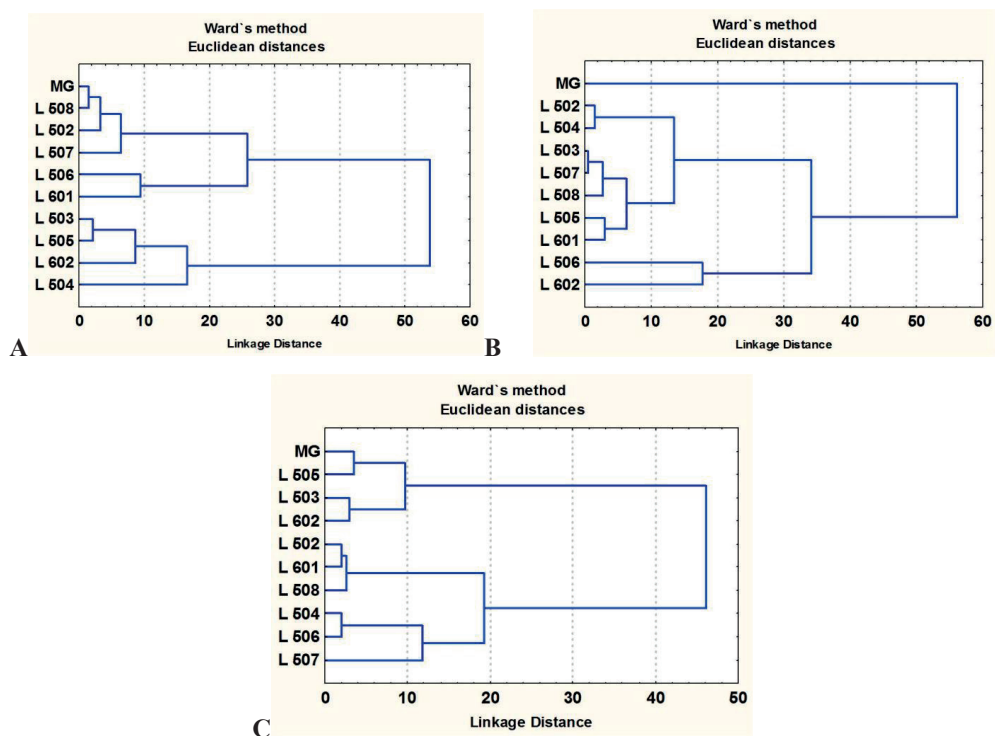


Figure 2. Dendrogram of distribution of tomato genotypes based on growth traits under optimal conditions: A - 25°C and heat stress; B - 41°C, C - 43°C

The calculation of inter- and intracluster distances by the centroid clustering method (k-means) demonstrated that, except for stem length under optimal conditions, the distance between clusters was much greater than within it at all 3 temperatures, which indicates that the tomato lines were successfully distributed into

clusters based on the 3 characters under study. The ratio of intercluster to intracluster distance demonstrated that the highest capacity for differentiating genotypes occurred at 41°C, and stem length exhibited a diminished discriminatory capacity compared to the other 2 characters (Table 1).

Table 1. Analysis of inter- and intracluster variance in the interaction of tomato genotypes with temperature

Variant	Intercluster variance	df	Intracluster variance	df	Report Intercluster variance/ Intracluster variance	F	p
25°C							
Legth of the radicle	405.4760***	2	34.3600	7	11.79	41.30283	0.000133
Legth of the stem	11.4415	2	100.5825	7	0.11	0.39813	0.685867
The seedling length	553.9840***	2	88.2850	7	6.28	21.96233	0.000963
41°C							
Legth of the radicle	426.3322***	2	7.4888	7	56.84	199.2538	0.000001
Legth of the stem	143.6452*	2	93.3988	7	1.54	5.3829	0.038397
The seedling length	956.4800***	2	115.6200	7	8.27	28.9541	0.000412
43°C							
Legth of the radicle	220.3148**	2	44.41417	7	4.96	17.36162	0.001934
Legth of the stem	27.5707**	2	7.79333	7	3.5	12.38203	0.005024
The seedling length	401.8058***	2	60.59418	7	6.63	23.20883	0.000815

*, **, *** - significant differences for p<0.05, 0.01, 0.001.

By classifying the genotypes based on the evaluated characters, it was found that in the 41°C variant, L 506 formed a separate cluster with the highest values of growth characters, and

at 43°C the highest values of the characters radicle, stem, and seedling length were recorded by lines L 504, L 506 and L 507 (Table 2).

Table 2. Descriptive cluster analysis

Cluster	Character	x	Genotype	x	Genotype	x	Genotype
		25°C		41°C		43°C	
1	The length of the radicle, mm	37.5	L 503, L	38.1	L 502,	31.4	L 502, L 503,
	The length of the stem, mm	24.5	504, L 505,	27.6	L 503,	18.4	L 508, L 601
	The length of the seedlings, mm	60.4	L 602	65.7	L 504, L 505, L 507, L 508, L 601, L 602	49.7	
2	The length of the radicle, mm	46.7	Mary Gratefully, L 502, L 507, L 508	52.8	L 506	26.2	Mary Gratefully, L 505, L 602
	The length of the stem, mm	23.2		28.1		15.4	
	The length of the seedlings, mm	70.0		80.9		41.5	
3	The length of the radicle, mm	54.3	L 506, L 601	23.6	Mary Gratefully	38.3	L 504, L 506, L 507
	The length of the stem, mm	26.1		15.0		19.6	
	The length of the seedlings, mm	80.4		38.5		57.9	

They are of interest in the breeding process as possible sources of resistance to stress temperatures.

Statistical processing of experimental data for all genotypes studied through factor analysis allowed the determination of the variability of characters and the degree of influence of each factor (genotype, temperature), as well as their

interaction on the variability of the studied traits (Table 3).

By calculating the percentage contribution to the source of variation of characters, it was found that the contribution of genotype, temperature, and the genotype x temperature interaction in radicle length was 13.9; 75.7; 5.1%; stem - 9.3; 82.9; 4.3% and seedling - 10.2; 80.2; 5.0%.

Table 3. The contribution of genotype and temperature to the growth capacity of tomatoes

Source of variation	Freedom degree	The length of the radicle		The length of the stem		Length of the seedling	
		Mean sum of squares	WSV, %	Mean sum of squares	WSV, %	Mean sum of squares	WSV, %
Genotype	9	219.8*	13.9	66.4*	9.3	368.0*	10.2
Temperature	2	1200.9*	75.7	591.4*	82.9	2892.8*	80.2
Genotype x temperature	18	80.1	5.1	30.8	4.3	179.0	5.0
Random effects	60	84.6	5.3	25.1	3.5	168.3	4.7

*- $p < 0.05$.

Thus, through factor analysis, it was found that the weight of temperature in the source of variation of the length of the radicle, stem, and seedling was the highest, which denotes that the temperature factor is decisive for the evaluated characters. From the data presented, it is observed that in general the role of genotype (9.3-13.9%) and genotype x temperature interactions (4.3-5.1%) were less relevant for the growth of

tomato seedlings. However, based on one of the integral indices - seedling length, we can observe at L 504, L 507, relatively small differences between the 25°C, 41°C, 43°C variants, which denotes their advanced adaptability to limiting temperatures (Figure 1C).

The genotypic (σ^2_g) and phenotypic (σ^2_{ph}) variances were much higher for radicle length and seedling length than for the stem (Table 4).

Table 4. Genetic variability and heritability of tomato growth traits under heat stress at early stages of plant development

Parameter	The length of the radicle	The length of the stem	Length of the seedling
σ^2_g	45.07	13.79	66.57
σ^2_{ph}	129.67	38.85	235.06
h^2	0.35	0.35	0.28
GCV, %	17.54	16.23	13.30
PCV, %	29.82	27.25	25.26
PCV – GCV, %	12.28	11.02	11.96
GAM, %	20.93	20.72	15.05

The difference between PCV and GCV was 11.02-12.28% and reflects the effect of stress temperatures on the response of growth organs. The broad-sense heritability coefficient ($h^2=0.28-0.35$) indicates a weak-medium capacity for the hereditary transmission of the characters under study under high-temperature conditions. However, its association with medium-high values of genetic progress leads to the idea of the involvement of additive factors in the control of growth characters.

CONCLUSIONS

The analysis of tomato growth traits – radicle, stem, and seedling in tomato perspective lines at different temperature levels highlighted the differentiated nature of their reaction to limiting temperatures. In some cases, stress temperatures produced significant inhibition of growth organs.

Through cluster analysis (k-means), 3 lines were identified - L 504, L 506, L 507, which recorded the highest values of radicle, stem, and seedling length at 43°C, which offers opportunities for their use in breeding programs as resistance donors.

Factorial analysis of the genotype x temperature interaction based on testing the reaction of the embryonic radicle, stem, and tomato seedling at three temperature levels demonstrated that, in the source of variation in the characters, the main role belongs to the temperature factor (75.7-82.9%), followed by the genotype (9.3-13.9%) and the tomato x temperature interaction factors (4.3-5.1%). The data indicate that the temperature factor is the most important for the growth and development of tomato plants. However, genotypes were identified - L 504, L 507, in which relatively small differences were recorded in the whole plant between the optimal

variant (25°C) and the variants with thermal stress, which denotes their adaptability to high temperatures.

High temperatures reduce not only the normal biological parameters of growth and development characters but also their hereditary transmission capacity. However, the association of h^2 values of 0.28-0.35 with relatively high indices of genetic progress (15.1-20.9%) reveals real opportunities to create resistant tomato genotypes through individual selection.

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PHENOTYPIC DESCRIPTION OF *VIGNA UNGUICULATA* SUBSP. *SESQUIPEDALIS* ACCESSIONS AT BRGV BUZĂU

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Abstract

Vigna unguiculata subsp. *sesquipedalis* (yardlong bean) is a legume with high agronomic and nutritional potential, yet underutilized in Romania. This study aimed to perform a phenotypic characterization of six distinct clusters (C1-C6), each comprising 30 genetically related lines conserved at the Vegetable Genetic Resources Bank (BRGV) Buzău. Qualitative traits, including growth habit, flower, and pod color, as well as quantitative traits such as pod length, number of seeds per pod, and number of pods per node, were evaluated. Data were analyzed using cluster analysis to assess similarity and differentiation among accessions. The results highlighted one elite, E3, which exhibited superior productivity compared with the others. Pod length for E3 ranged from 16 to 22 cm, with approximately 15 seeds per pod and 2–3 pods per node. Cluster analysis confirmed the distinctiveness of E3 within the studied material. Based on its phenotypic performance, E3 is proposed for official variety registration and patenting. The identified traits recommend E3 as valuable material for breeding programs. Overall, this study provides essential phenotypic information to support the development and sustainable cultivation of yardlong bean in Romania.

Key words: germplasm evaluation; elite selection; agromorphological traits; genetic resources conservation; breeding potential.

INTRODUCTION

Vigna unguiculata (L.) Walp. subsp. *sesquipedalis*, commonly known as yardlong bean, is an important vegetable legume cultivated for its long, tender pods and high nutritional value. The crop is widely grown in tropical and subtropical regions, where it represents an essential source of proteins, vitamins, and dietary fiber. However, despite its agronomic and nutritional importance, it remains underutilized in European agriculture, including Romania.

Phenotypic characterization is a fundamental step in the evaluation and utilization of plant genetic resources. Morphological traits such as growth habit, flower characteristics, pod shape, and color are commonly used descriptors for distinguishing genotypes and assessing their agronomic value. As reported by Saha et al. (2013), “morphological characters play an important role in the identification and selection of superior genotypes”, particularly in vegetable legumes where marketable traits are directly linked to phenotype. Similarly, studies conducted on yardlong bean genotypes showed

that “significant variability exists among genotypes for morphological traits and yield components”, indicating the potential for effective selection based on phenotypic performance (George and Sarada, 2015). Moreover, it has been reported that “domestication of yardlong bean involved significant modifications in pod length and seed-related traits, reflecting strong selection for agronomic performance and consumer preferences” (Kongjaimun et al., 2012). Yield performance in yardlong bean is strongly influenced by several interrelated traits. Previous research has demonstrated that “pod length, number of seeds per pod and number of pods per plant are major yield contributing characters,” and their variation significantly affects productivity (Haque et al., 2015). In addition, “pod length is a quantitatively inherited trait controlled by multiple genetic loci, highlighting its importance as a key target in breeding programs for yield improvement” (Xu et al., 2013). In addition, it has been observed that “considerable variation was recorded among genotypes for pod length and number of pods per node”, emphasizing the

importance of these traits in the evaluation of germplasm collections (Huque et al., 2012). Such variability provides a valuable basis for identifying elite genotypes with superior agronomic performance.

The assessment of genetic resources in advanced generations and diverse environments has further confirmed the importance of phenotypic evaluation. It was reported that “wide variability among genotypes for yield and its components suggests good scope for selection,” especially when traits exhibit consistent expression across conditions (Hasanuzzaman et al., 2014). Moreover, clustering of genotypes based on phenotypic traits has proven effective in identifying distinct groups and superior lines, facilitating the selection of promising material for breeding programs.

In addition to morphological and yield-related traits, seed germination and viability are essential parameters for the conservation and efficient use of plant genetic resources. Germination capacity reflects the ability of seeds to produce normal seedlings under favorable conditions, while viability indicates the presence of living tissues capable of germination. Studies have shown that “germination percentage varies significantly among genotypes and is an important indicator of seed quality” (Islam et al., 2013). Furthermore, it has been emphasized that “seed viability and vigor are influenced by genetic factors as well as storage conditions”, which may lead to differences in germination performance even among closely related lines (Hasanuzzaman et al., 2014).

An important aspect in seed evaluation is the possible discrepancy between viability tests and actual germination. It has been observed that “high viability does not always correspond to high germination due to the presence of dormancy or physiological constraints”, highlighting the need for complementary testing methods in gene bank management. This is particularly relevant for conserved germplasm, where accurate assessment of seed quality is critical for regeneration and long-term preservation.

In the context of biodiversity conservation and sustainable agriculture, the phenotypic characterization of yardlong bean accessions is essential for identifying valuable genetic

material. The Vegetable Genetic Resources Bank (BRGV) Buzău maintains a diverse collection of *Vigna unguiculata* germplasm, which requires systematic evaluation to support its utilization in breeding and cultivation.

Therefore, the present study aims to perform a detailed phenotypic characterization of *Vigna unguiculata* subsp. *sesquipedalis* accessions conserved at BRGV Buzău, with particular emphasis on morphological traits, yield components, and seed germination and viability, in order to identify superior genotypes suitable for future breeding programs and potential variety registration.

MATERIALS AND METHODS

Biological material and experimental site

The biological material analyzed consisted of accessions of *Vigna unguiculata* (L.) Walp. subsp. *sesquipedalis*, preserved at the Plant Genetic Resources Bank (BRGV) Buzău. These were grouped into six clusters (C1-C6), each comprising 30 genetically related lines.

Phenotypic characterization

Phenotypic characterization included both qualitative and quantitative traits and was performed using the international descriptors established by the International Union for the Protection of New Varieties of Plants.

The qualitative traits analyzed focused on morphological aspects such as growth habit, flower color, and pod color, while the quantitative traits included pod length, number of seeds per pod, and number of pods per node. Biometric measurements of the seeds were performed using the WinSEEDLE system, based on high-resolution scanning and image analysis, which allows for precise measurement of seed dimensions and shape, ensuring high accuracy and reproducibility (Figure 1).

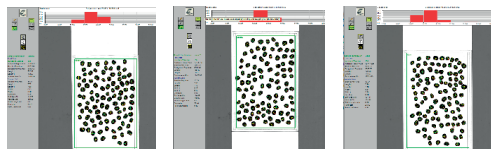


Figure 1. Seed morphometric analysis using the WinSEEDLE system

Germination determination

Given the large size and round shape of the seeds, the accordion-fold method was used, employing absorbent paper measuring 58.9 × 57.5 cm.

Substrate moistening was performed in two stages: initially to approximately 60% of water-holding capacity, followed by a second watering after 24 hours, until the optimal level was reached.

This method was used to prevent rapid water absorption and damage to the cotyledons.

The experiment was conducted in four replicates of 100 seeds each, spaced evenly (approx. 4 cm apart) on the substrate, to allow for normal seedling development and to prevent damage or secondary infections. The samples were placed in transparent plastic bags to prevent water evaporation and then incubated in a germinator at a temperature of 30°C, optimal for thermophilic species. Relative humidity was maintained at approximately 60%, and lighting was provided throughout the test.

The evaluation was conducted in two stages: germinative energy (EG) - at 5 days and facultative germination (FG) - at 10 days.

The seeds were classified into the following categories: normal seedlings - with fully developed structures capable of ensuring normal plant growth; abnormal seedlings - with major defects that prevent further development; fresh ungerminated seeds - viable but which did not germinate under test conditions; hard seeds - impermeable, with no changes during the test; dead seeds - soft, damaged, or infected, with a non-viable embryo (Figure 2).



Figure 2. Seeds on the germination tray; normal seedlings; abnormal seedlings; fresh seeds and hard seeds

To determine viability, 2,3,5-triphenyltetrazolium salts were used in a 1% solution. The principle of the method consists of highlighting living tissues by staining them carmine red through the hydrogenation process, forming a stable compound, formazan. The test consisted of 4 replicates of 100 seeds that were prepared in advance by soaking them in water for 18 hours at a temperature of 25°C. The seeds were then placed in the staining solution so that it completely covered the seeds. The test was incubated for 18 hours at 30°C in the dark. After the incubation period, the degree of staining was checked. The seeds, washed with cold water, were dried and evaluated according to ISTA evaluation criteria. Based on the degree of staining, the seeds were grouped into two categories:

- Viable seeds. These are seeds that are completely stained red or whose essential structures are stained to the extent that they can be considered a viable embryo.
- Non-viable seeds. All seeds that are not fully stained or that exhibit areas of interest with unstained, soft, or necrotic structures.
- Hard seeds. These are seeds that did not stain and did not change size following the procedure. They are considered alive but dormant (Figure 3).

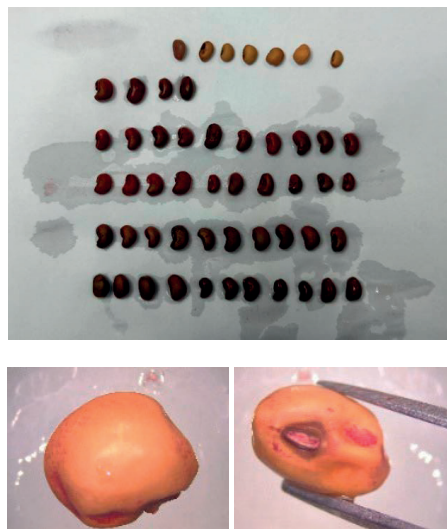


Figure 3. Seeds prior to tissue examination

RESULTS AND DISCUSSIONS

Cluster C1 - The plants are semi-erect climbers (claw-type), with a pyramidal growth habit. The leaves are light green and slightly rough. The terminal leaflet is triangular to circular. The pods are light green, cordate in cross-section, slightly curved or nearly straight, with an acute tip and a smooth or slightly rough surface. The seeds are kidney-shaped, beige in color with a brown ring around the hilum.

Cluster C2 - Climbing plants, pyramidal architecture, without anthocyanin pigmentation. The leaves are light green, with very slight or no roughness. The terminal leaflet has an intermediate shape (triangular-circular). The pods are light green, oval in cross-section, slightly curved, with an acute tip and a smooth surface. The seeds are similar to those of other clusters (kidney-shaped, beige with brown around the hilum).

Cluster C3 - Climbing plants with anthocyanin pigmentation. Medium-green leaves, without obvious roughness. Circular terminal leaflet. The pods are longer and green in color, with an oval cross-section, slightly curved, and a moderately rough surface. This cluster is distinguished by its uniformity and vigor. The seeds are kidney-shaped, with a typical color.

Cluster C4- Climbing plants with anthocyanin pigmentation and a pyramidal growth habit. The leaves are very light in color. The terminal leaflet is circular. The pods are light green, heart-shaped in cross-section, more noticeably curved, and have a concave tip. The surface is smooth. The seeds are similar to those of other clusters.

Cluster C5 - Climbing plants lacking anthocyanin pigmentation. Light green leaves with moderate roughness (most evident among the clusters). The terminal leaflet is circular. The pods are green, heart-shaped in cross-section, nearly straight, with an acute tip and a smooth surface. The seeds are wider in cross-section.

Cluster C6 - Climbing plants with a pyramidal growth habit. Light green leaves, smooth. The terminal leaflet is circular and larger than those in the other clusters. The pods are green, elliptical in cross-section, slightly curved, with a variable tip (acute to truncate). The seeds are wider.

Table 1. Comparative analysis of quantitative traits in *Vigna unguiculata* clusters (means $\pm$ statistical grouping by Duncan test, $p \leq 0.05$)

Trait/cluster	C1	C2	C3	C4	C5	C6
Terminal leaflet: size (cm)	11.5 ^b	10.9 ^c	11.3 ^b	9.9 ^d	10.6 ^c	14.0 ^a
Pod: length (cm)	15.7 ^b	13.3 ^c	17.5 ^a	17.2 ^a	13.3 ^c	-
Pod thickness (cm)	0.9 ^a	0.8 ^b	0.8 ^b	0.8 ^b	0.7 ^c	0.8 ^b
Pod : width (cm)	0.7 ^a	0.7 ^a	0.7 ^a	0.7 ^a	0.7 ^a	0.6 ^b
Seed: weight (g)	0.16 ^c	0.22 ^b	0.20 ^b	0.20 ^b	0.28 ^a	0.21 ^b
Seed: length (cm)	0.7 ^c	1.0 ^b	1.1 ^a	1.1 ^a	1.0 ^b	1.0 ^b
seed/pod	16 ^b	16 ^b	18 ^a	16 ^b	14 ^c	15 ^c
Pod yield (g)	176 ^c	169.2 ^c	228.1 ^a	139.8 ^d	206 ^b	125.1 ^d
Fresh weight of 100 seeds (g)	40 ^a	37 ^c	40 ^a	40 ^a	40.1 ^a	39 ^b

Analysis of quantitative traits reveals significant phenotypic variability among the six clusters of *Vigna unguiculata* subsp. *sesquipedalis*, a finding confirmed by the groupings obtained using Duncan's test ($p \leq 0.05$).

Regarding the length of the terminal leaflet, cluster C6 differed significantly, recording the highest value (14.0 cm) and being distinctly classified in group "a". The other clusters exhibited lower values, distributed across several statistical groups, indicating moderate diversity for this trait.

Pod length is of major agronomic interest, where clusters C3 and C4 formed the upper group ("a"), with values of 17.5 cm and 17.2 cm, respectively. These results suggest high productive potential, as pod length is a trait directly correlated with seed number and total yield.

In the case of pod width and thickness, the variations were smaller. However, cluster C1 stood out with the greatest width (0.9 cm), while cluster C6 had the smallest thickness (0.6 cm), differing significantly from the other clusters.

Individual seed weight showed clear differences between clusters, with cluster C5 being significantly higher (0.28 g, group "a"). This characteristic indicates a biological type oriented toward the formation of large seeds with high commercial potential, but not necessarily correlated with a high number of seeds per pod (Figure 4).



Figure 4. Morphological aspects of *Vigna unguiculata*: plant, leaf, pods and seeds

In terms of seed length, clusters C3 and C4 recorded the highest values (1.1 cm), which were significantly higher than those of the other variants. This trait contributes to the overall size of the seed and may influence consumer preferences.

Analysis of the number of seeds per pod shows that cluster C3 stands out significantly (18 seeds/pod), confirming its efficiency in converting resources into reproductive output. In contrast, cluster C5 had the lowest values (14 seeds/pod), highlighting a trade-off between seed size and number (Figure 5).

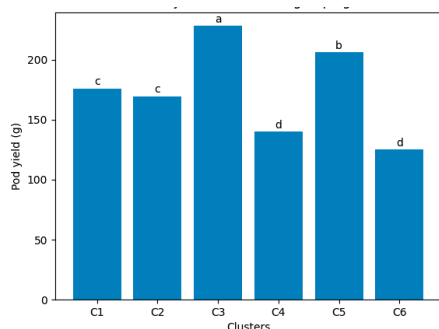


Figure 5. Pod yield with Duncan grouping

The graph highlights significant differences among the clusters of *Vigna unguiculata* subsp. *sesquipedalis*, as confirmed by Duncan's multiple range test ($p \leq 0.05$).

Cluster C3 stands out clearly, falling into the top statistical group ("a"), with the highest pod yield (228.1 g). This performance indicates high

efficiency in resource allocation to reproductive organs and confirms the superior agronomic potential of this cluster.

Cluster C5 occupies the second position (group "b"), with a yield of 206 g, significantly lower than C3 but higher than the other clusters. This result suggests a trade-off between yield components, as it is known that this cluster is characterized by larger seeds but fewer per pod. Clusters C1 and C2, classified in group "c", show intermediate values and do not differ significantly from one another. They reflect relatively stable genetic material, but without maximum performance in terms of yield.

The lowest values are recorded by clusters C4 and C6, classified in group "d", with no significant differences between them. These results indicate reduced productive potential, which limits their direct use for commercial purposes but does not exclude their value in breeding programs.

The Duncan group analysis confirms the presence of significant variability and identifies C3 as the superior genotype, followed by C5, while C4 and C6 represent the lower extremes of performance.

For 100-seed weight (TSW), cluster C3 ranked in the top group ("a") with 7.2 g, suggesting a favorable combination of seed size and density. At the opposite end, cluster C5 recorded the lowest value (5.6 g), being statistically distinct. The most relevant agronomic indicator, pod yield, revealed very significant differences between clusters. Cluster C3 stood out clearly, with a yield of 228.1 g (group "a"), followed by C5 (206 g, group "b"). Clusters C1 and C2 formed an intermediate group ("c"), while C4 and C6 had the lowest values (group "d") (Table 2).

Table 2. Morphometric parameters of *Vigna unguiculata* seeds ($n = 3 \times 100$ seeds)

Parametre	Media $\pm$ SD	CV (%)
Average curved length (mm)	7.66 $\pm$ 0.12	1.62
Average perimeter (mm)	22.36 $\pm$ 0.34	1.53
Aspect ratio	0.889 $\pm$ 0.014	1.59
Seed surface (mm ²)	9094.44 $\pm$ 283.16	3.11
Volume (mm ³)	146.32 $\pm$ 6.44	4.40
Seed length (mm)	7.41 $\pm$ 0.14	1.86
Seed width (mm)	6.08 $\pm$ 0.11	1.83

Morphometric analysis of *Vigna unguiculata* seeds ($n = 3 \times 100$ seeds) reveals a high degree of uniformity in the biological material, as

reflected by the low values of the coefficient of variation (CV%) for all analyzed traits. The mean curved length parameter recorded a value of 7.66 ± 0.12 mm, with a CV of 1.62%, indicating very low variability and high homogeneity in the longitudinal dimension of the seeds. This trend is also supported by the value of the average perimeter (22.36 ± 0.34 mm; CV = 1.53%), suggesting good dimensional consistency along the seed contour. The shape coefficient (0.889 ± 0.014 ; CV = 1.59%) indicates a relatively regular shape, close to elliptical, specific to reniform seeds. Values close to 1 reflect a high degree of roundness and morphological uniformity. Regarding the derived dimensional parameters, the average seed area (9094.44 ± 283.16 mm²) and volume (146.32 ± 6.44 mm³) exhibit slightly higher CV values (3.11% and 4.40%, respectively), which indicates moderate variability. This is to be expected, as these parameters are cumulatively influenced by variations in linear dimensions. Principal dimension analysis shows that seed length (7.41 ± 0.14 mm; CV = 1.86%) is slightly greater than width (6.08 ± 0.11 mm; CV = 1.83%), confirming an elongated shape specific to this species. The length-to-width ratio (L/W) is 0.824 ± 0.008 , with the lowest coefficient of variation (1.00%), indicating very high morphological stability of seed shape. This parameter is essential for characterizing seed type and can be used as a discriminatory indicator in studies of genetic variability.

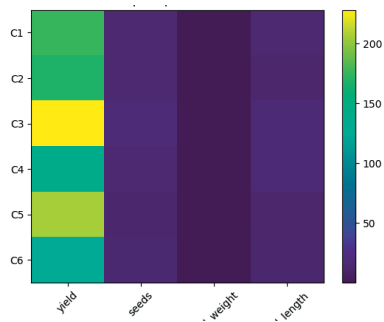


Figure 6. Heatmap of quantitative traits

The heatmap (Figure 6) provides an overview of the variation in quantitative traits across clusters, highlighting clear differences in phenotypic profiles.

Cluster C3 exhibits a distinct pattern, characterized by high values for most traits associated with yield (pod length, number of seeds, total yield), confirming its dominant position also observed in the univariate analysis. The intensity of the colors associated with this cluster indicates a concentration of high values, reflecting a balanced and high-performing agronomic profile. Cluster C5 is distinguished by high values for seed weight but lower values for the number of seeds per pod, highlighting a distinct biological strategy. This dissociation between yield components is visible in the color contrast of the heatmap. Clusters C1 and C2 exhibit a relatively uniform profile, with no obvious extremes, suggesting low variability and phenotypic stability. They occupy an intermediate position within the overall variation. Cluster C4 is characterized by moderate to low values for most traits, while C6 exhibits low values particularly for yield and certain pod dimensions; thus, it grouped in the lower performance range.

Table 3. Germination Test Results

No. of repetitions	GN	GA	FS	HS	DS
1	69	6	3	7	15
2	70	4	1	17	8
3	81	0	2	8	9
4	67	3	5	15	10
M	287	13	11	47	42
%	72	3	3	12	10

*GN-normal germs; GA-abnormal germs; FS-fresh seeds; HS- hard seeds; DS- Dead seeds

The average value of 72% normal germination (GN) (Table 3) indicates acceptable germination capacity for the first year after harvest. However, the 28% gap from maximum potential is explained not so much by a loss of viability as by the presence of dormant forms. The low percentage of abnormal germinants (3%) demonstrates the effectiveness of the two-stage moistening method; by controlling water intake, imbibition stress was minimized, which in legumes frequently causes fractures of the embryonic axis or cotyledons. The most significant observation is the 12% percentage of hard seeds (HS). In seed physiology, this phenomenon is interpreted as a physical barrier imposed by the impermeable seed coat, an evolutionary survival strategy that ensures

staggered germination over time. From a technological standpoint, this 12% threshold suggests that the batch could result in uneven emergence under field conditions, necessitating an increase in the seeding rate to avoid density losses during sowing. The presence of the 3% fresh seeds (FS), which remain turgid but do not initiate the growth process, indicates a form of superficial physiological dormancy. The 10% dead seeds (DS) represent the only irreversible loss in the batch. This finding can be attributed either to mechanical damage during harvesting or to the attack of endogenous pathogens that took advantage of the optimal humidity and temperature conditions (30°C) in the germination chamber. However, the low rate of secondary contamination confirms that spacing the seeds on the bed using the PP method was an appropriate technical measure for isolating infection foci.

Interim conclusion. The results demonstrate that *Vigna unguiculata* maintains an energy-conservation strategy through hard seeds, requiring a careful interpretation of “cultivated value” that includes not only immediate germination but also biochemically confirmed viability reserve (Table 4).

Table 4. Viability test results

Viable seeds		Non-viable seeds		Hard seeds	
75	"bright red" coloration	white	7	18	The seeds show no changes in size or color compared to their original form.
	1/3 of the white root	With a colored root tip			
	<50% of the cotyledon surface is white	Necrotic			
	Light red tint	White growth bud			

The "bright red" coloration confirms intense activity of dehydrogenase enzymes in the embryonic axis.

The Correlation Between Germination and the Viability Test (Tetrazolium)

The 2,3,5-triphenyl tetrazolium salt test provided deeper insight into the biological

potential of the batch. Viability vs. Germination: The percentage of viable seeds (75%) is higher than the average for normal seedlings, demonstrating that some of the seeds that did not germinate in the standard test are, from a metabolic standpoint, alive. In contrast, seeds considered non-viable (with a white growth tip or necrosis on the radicle) explain the appearance of abnormal seedlings in the germination test. Practical implications and laboratory conclusions

The incidence of dead seeds (DS) and fungal attacks (observed as soft/moldy seeds) suggests the need for strict hygiene monitoring in the germination room, even under optimal lighting conditions (Figure 7).

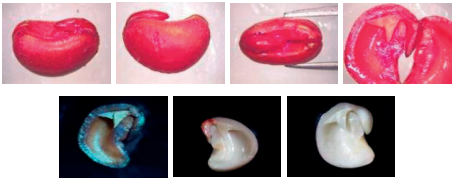


Figure 7. Viable seeds (first row) and non-viable seeds (second row)

CONCLUSIONS

The present study provided a comprehensive phenotypic characterization of six clusters (C1-C6) of *Vigna unguiculata subsp. sesquipedalis* conserved at the Vegetable Genetic Resources Bank (BRGV) Buzău, highlighting their agronomic potential and variability.

The analysis of qualitative traits revealed a relatively uniform architectural pattern, with all clusters exhibiting a climbing growth habit and a pyramidal plant architecture. However, distinct differences were observed in traits such as anthocyanin pigmentation, leaf color intensity, pod shape, and surface texture, confirming the existence of phenotypic diversity within the studied germplasm.

Quantitative trait analysis demonstrated significant variability among clusters, particularly for yield-related characters. Cluster C3 clearly stood out as the most valuable genotype, combining superior pod length, the highest number of seeds per pod, and maximum pod yield. These characteristics indicate a high efficiency in resource allocation toward reproductive structures, making C3 a strong

candidate for breeding programs and potential cultivar development.

Cluster C5 was distinguished by the highest individual seed weight, suggesting a different biological strategy oriented toward seed size rather than seed number. This highlights the presence of a trade-off between yield components, which is essential for targeted breeding objectives depending on market requirements.

The morphometric analysis of seeds revealed a high degree of uniformity, with all coefficients of variation below 5%, indicating a stable and well-conserved genetic material. The low variability in shape descriptors and dimensional parameters supports the reliability of the material for both conservation and breeding purposes.

Germination tests indicated an acceptable germination capacity (72%), while the tetrazolium viability test revealed a higher proportion of viable seeds (75%), suggesting the presence of dormancy mechanisms, particularly physical dormancy expressed through hard seeds (12%). This reflects an adaptive survival strategy but also indicates the need for pre-sowing treatments to ensure uniform field emergence.

Overall, the study confirms the existence of valuable genetic resources within the BRGV Buzău collection and emphasizes the importance of integrated phenotypic and physiological evaluation. Cluster C3, along with the elite line E3, is recommended for further testing, official registration, and inclusion in breeding programs aimed at improving yield and adaptability of yardlong bean in Romania.

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APPLYING MARVIN SEED ANALYZER FOR PHENOTYPIC CHARACTERIZATION OF COMMON BEAN COLLECTION

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Abstract

*The National Crop Genebank of Bulgaria was established in 1984 at the Institute of Plant Genetic Resources in Sadovo (Central South Region). It maintains extensive germplasm collections of local and foreign origin. Landraces constitute a strategic genetic reservoir, requiring systematic characterization and conservation. The study was conducted in the experimental field of the institute including 84 accessions of common bean (*Phaseolus vulgaris* L.). They were analysed through phenology observations and biometric measurements, using the international descriptors and analysed with MARViN VisionLine seed screening. Biological, morphological, and quality traits with averaged data across two replications were assessed. Statistical analyses were performed with Microsoft Excel and SPSS, applying diverse approaches to reveal trait variability. Seed colour and the used morphometric parameters from the analyzer may prove to be highly informative traits that can be used both for morphological classification and for genetic and breeding purposes. Local populations are the golden gene pool and they should not only be studied but also preserved and open access should be guaranteed. The research was carried out under the project IS-PGR-SADOVO (Contract KP-06-H86/9/09.12.2024 of Bulgarian National Science Fund).*

Key words: genebank, *Phaseolus vulgaris*, seed traits, data base, statistical analysis.

INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) is an important protein crop which is cultivated all over the world. In Bulgaria it is one of the valuable and traditional growing pulses. (Stoilova et al., 2014). It occupies a central and almost obligatory place in the local holiday tables. Due to its good taste and healthy qualities, it is mainly used for the preparation of various dishes as a main or additional food product or including it in the diverse diets.

The common bean is an excellent precursor for almost all field crops because it enriches the soil with nitrogen (Ivanova et al., 2019). Legume species are capable of meeting their nitrogen needs through both soil absorption and symbiotic nitrogen fixation, which provides an additional nitrogen source for the plant (Unkovich et al., 2008). In common bean, roughly 60% of nitrogen is obtained from the soil, whereas about 40% is contributed by the symbiotic fixation pathway (Unkovich and Pate, 2000; Peoples et al., 2009).

Different common bean subspecies, such as those with small seeds, tend to germinate and

multiply their seed weight more rapidly than those with large seeds, depending on external factors (Laing et al., 1984). However, at lower temperatures (12°C), large-seeded varieties tend to germinate more rapidly than those with small seeds (Austin and Maclean, 1972). This is consistent with the observation that varieties adapted to cooler climates tend to have larger seeds. The seeds themselves can be from 0.5–2 cm long and exhibit remarkable colour variation across varieties, ranging from red, white, purple, grey, brown, black, a mix of colours, etc. (Smith and Rao, 2021). With the development of technology, it is now possible to more precisely assess genetic diversity. The devices designed for colour spectrophotometric and morphometry are equipped with high-resolution cameras that capture different shades of colour space and offer morphometric measurements of plant materials with high precision.

All *Phaseolus* plant genetic resources, ranging from landraces and ancient varieties to heterogeneous populations are essential for advancing breeding programs, supporting scientific research, and ensuring their preservation in genebanks for future use (Stoilova et al., 2024).

Capturing the full diversity of grain legumes and maintaining high-quality, accessible collections of well-characterized genetic resources are essential requisites for the advancement and improvement of the crop. The comprehensive characterization of gene bank accessions is crucial for unlocking their full potential (Brezeanu et al., 2025).

Situated in Sadovo and part of the Agricultural Academy, the Institute of Plant Genetic Resources “Konstantin Malkov” is committed to the collection, documentation, investigation, conservation, and sustainable use of Bulgaria’s plant genetic resources. The National Seed Genebank, housed within the Institute, ensures long-term preservation of both local and introduced accessions in line with FAO Standards (2014). The Institute expands its ex-situ collections through targeted expeditions, international germplasm exchange, and the storage of breeding materials (Velcheva et al. 2024). Collecting activities aimed at acquiring local varieties and populations are conducted according to FAO Guidelines (2019). The full range of *Phaseolus* germplasm, encompassing landraces, old varieties, and heterogeneous populations are of significant importance for breeding and research activities and for safeguarding genetic resources through ex-situ genebank conservation (Stoilova et al., 2024). By applying statistical analyses, the study aims to explore the diversity within a collection of local and introduced common bean (*Phaseolus vulgaris* L.) accessions and to highlight groups that could be valuable for breeding efforts and practical applications.

MATERIALS AND METHODS

The experiment was conducted in the field of IPGR “K. Malkov” (42.1197° N, 24.8997° E) in the town of Sadovo over two consecutive years, 2024 and 2025. In this study 84 accessions with diverse origin are included. Most of them are of Bulgarian (local) origin and four of them are breeding varieties (Eliksir, Trackiya, Skitiya and Ustrem) created in Dobroudzha Agricultural Institute in General Toshevo, Dobrich Region (Figure 1).

All the variants were sown in rows 2 m long with an inter-row spacing of 0.7 m. A non-standard method for establishing a field experiment with randomized allocation of the variants was used.

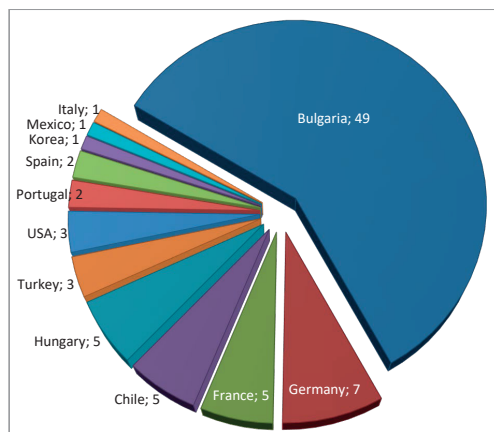


Figure 1. Origin of the accessions included in the study

Key developmental stages of the crop were evaluated and the length of the vegetation period was recorded, based on which the accessions were classified as early, medium-early, and late. The number of days from emergence to mass flowering, the duration of flowering, and the number of days from emergence to bean formation were calculated. A visual characterization is performed for the seed colour. For biometric measurements, a digital scale with an accuracy of 0.01 g was used to determine the mass of 100 seeds and seed mass per plant. A digital clipper with data transfer cable was used for measure the seed dimensions (length, width, and thickness) measured in millimetres (mm). For the characterization and evaluation of the *Phaseolus vulgaris* L. accessions, the IBPGR (1982) and UPOV (2013) descriptors were used.

In 2025 an additional study was conducted and a morphometric analysis of the seeds was performed using a Marvin Seed Analyzer (MARViTECH GmbH, Germany), designed for precise, non-destructive seed measurements. The device uses a high-resolution camera and specialized software for automatic extraction of geometric and colour parameters. Before analysis, the seeds were arranged in a single layer on the standard measuring plate, ensuring uniform illumination and contrast. All measurements were carried out under constant lighting conditions. The morphometric analysis included the following parameters: seed area converted to square millimetres (mm²); seed perimeter in millimetres (mm); mean values of the Red, Green, and Blue channels (RGB); I-

Max mean; mean values in the HSL colour space – H (hue), S (saturation), and L (lightness). Variation analysis was applied where variation is considered low if the coefficient does not exceed 10%, medium when it is between 10-20%, and high (significant) when it exceeds 20% (Dimova and Marinkov, 1999).

Experimental data from the Marvin Seed Analyzer and phenotyping were processed through descriptive, correlation, variation, and cluster analyses which revealed both positive and negative relationships among the studied traits.

Correlation between all traits was determined, using the correlation coefficient “r”. In the correlation tables, the strength of relationships is indicated as follows: weak dependence – $*(0.3 \leq |r| < 0.5)$; medium dependence – $**(0.5 \leq |r| < 0.7)$; strong dependence – $***(|r| \geq 0.7)$.

The hierarchical clustering was performed, using Ward’s method, which minimizes within-cluster variation by merging the clusters that result in the smallest increase in the total sum of squared errors (Ward, 1963).

All data were processed, using the statistical programs Microsoft Excel - Analysis ToolPak and IBM SPSS Statistics.

RESULTS AND DISCUSSIONS

According to the length of the vegetation period, the samples are classified as early, medium-early, and late (Figure 2).

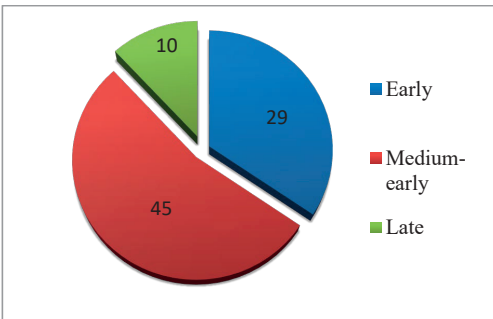


Figure 2. Classification of the accessions according to the number of days to maturity

Phenological data cover four key stages of plant development: length of the vegetation period, mass flowering, bean formation, and flowering duration. Descriptive statistics allow to assess the variability between samples and to

determine the average values and ranges characteristic of the studied forms.

According to the vegetation, the mean duration is 77.54 days with a relatively low standard deviation of 5.37 days. The minimum value is 68 days (69886 - Germany; 771423 - Hungary) and the maximum 90 days (69847 - France; 69859 - Hungary; 84201015 - Germany), outlining a range of 22 days. The median (77 days) and the mode (77 days) coincide, which indicates a symmetrical distribution and the absence of extreme deviations. The variation is low, suggesting that the vegetation period is a stable trait with only minor differences among the genotypes.

Table 1. Descriptive statistic for the duration of the vegetation period and the number of days to flowering for 84 common bean accessions

Vegetation period		Mass flowering	
Mean	77.54	Mean	35.14
SE	0.59	SE	0.37
Med	77.00	Med	34.00
Mo	77.00	Mo	33.00
SD	5.37	SD	3.42
S ²	28.83	S ²	11.69
R	22.00	R	15.00
Min.	68.00	Min.	31.00
Max.	90.00	Max.	46.00
CV%	6.93	CV%	9.73
Count	84.00	Count	84.00

The days to mass flowering show that earliest forms enter mass flowering in the 31st (A7E0137 - Devin; C1E0051 - v. Rogozinovo; C4E0004v. Karavelovo; 91201091 - Hungary; 91201120 - Hungary) and latest at the 46 days (91201088 - Spain) after emergence, which corresponds well with the mean value of 35.14 days. The standard deviation is 3.42 days and the CV% = 9.73 falls within the category of low variability. This indicates that the timing of mass flowering is a relatively stable trait. The differences between genotypes are limited and do not lead to substantial divergence in the flowering phase, even though the range within the studied collection reaches 15 days. In most cases, however, due to sharply rising maximum temperatures at the beginning of summer, this two-week interval may be critical for this stage of crop development.

On table 2 the period of bean formation and the duration of flowering are presented. The mean

value for the period from emergence to bean development is 52.63 days, with a standard deviation of 2.79 days, which represents the lowest variability among all phenological traits. The range is 13 days. The median (52.5 days) and the mode (55 days) are close to the mean, suggesting a normal distribution. The earliest accession in this period is C4E0004 - from Karavelovo and the latest 69847 - France. This is the most stable indicator among all phenological traits. The low variability shows that bean formation is highly uniform across genotypes. However, the trait shows low variation and stability.

The phase of seed growth and flowering are most influenced by the agro-climatic conditions of the environment, as plants in these phases of development consume 60% of the water needed for the entire growing season (Ivanova et al., 2019). The year, growing conditions, and genotype are related and directly affect the realization of good yields.

Table 2. Descriptive statistics for the period of bean formation and the duration of flowering for 84 common bean accessions

Bean formation		Duration of flowering	
Mean	52.63	Mean	22.48
SE	0.30	SE	0.42
Med	52.50	Med	23.00
Mo	55.00	Mo	26.00
SD	2.79	SD	3.81
S ²	7.80	S ²	14.49
R	13.00	R	21.00
Min.	46.00	Min.	14.00
Max.	59.00	Max.	35.00
CV%	5.31	CV%	6.93
Count	84.00	Count	84.00

The mean value for flowering duration is 22.48 days with a standard deviation of 3.81 days and a range of 21 days. The mode (26 days) is higher than the median (23 days). However, the range of values is relatively wide with minimum of 14 (69846 - Germany) and maximum 35 days (cultivar - Trakya). The coefficient of variation remains within the limits of low variability. This indicates, that despite the visible differences, the relative variability in relation to the mean is moderate and the trait is more stable than it appears based on absolute values. A similar pattern was observed for the length of the vegetation period.

Differences in vegetation periods are important for breeding evaluation, as they allow the identification of genotypes with shorter or longer flowering duration, as well as forms that transition earlier or later into reproductive development.

The traits determining productivity included in this study are: 100-seed weight; seed weight per plant; seed length, width, thickness, perimeter, and area. The descriptive statistics are presented in several tables.

The first one (Table 3) includes the traits seed weight per plant and 100-seed weight. The mean seed weight per plant is 8.39 g, with a median of 7.81 g. The lowest value is 4.46 g (A8E0458 - Momchilovgrad), while the maximum reaches 17.61 g (69846 - Germany), resulting in a substantial range of 13.15 g. The coefficient of variation is 31.77%, indicating high variability. This shows that seed weight per plant is a highly variable trait within the studied group.

Table 3. Descriptive statistic of the seed mass from plant (SeedMass) and 100 seeds weight (TSW). The traits are measured, using digital scale with precision 0.01g

SeedMass		TSW	
Mean	8.39	Mean	27.72
SE	0.29	SE	0.73
Med	7.81	Med	27.09
Mo	5.85	Mo	#N/A
SD	2.67	SD	6.66
S ²	7.10	S ²	44.32
R	13.15	R	30.42
Min.	4.46	Min.	15.12
Max.	17.61	Max.	45.54
CV%	31.77	CV%	24.02
Count	84	Count	84

The mean value of TSW is 27.72 g. The minimum is 15.12 g (91201017 - Portugal) and the maximum reaches 45.54 g (cultivar - Skitya), resulting in a large range of 30.42 g. The standard deviation is 24.02%. This is a key indicator of productivity and technological suitability and the high variation highlights the presence of substantial differences. This confirms that the collection is represented by those with lower and those with higher mass per 100 seeds, from which we can conclude the presence of small-seeded and large-seeded genotypes.

In relation to seed size, the statistical indicators, concerning the biometric traits, especially seed length, width, and thickness are presented in table 4. The results show average variation for all three traits and a considerable range among the studied samples.

The descriptive statistics indicate that the mean seed length is 11.51 mm, with a median of 11.50 mm, practically identical to the mean. The minimum value is 8.03 mm (B0E0048 - Dimitrovgrad) and the maximum is 14.77 mm (C1E0049 - Mezek), resulting in a range of 6.74 mm. The standard deviation is 1.38 mm and the CV% = 11.96, indicating a transition from low to average variability.

The mean seed width is 6.47 mm, with a median of 6.46 mm, again nearly identical. The minimum value is 4.84 mm (91201151 - France) and the maximum is 8.11 mm (C0E0320 - v. Selcha), giving a range of 3.26 mm. The standard deviation is 0.76 mm, and the CV% = 11.72 which also corresponds to medium variability.

The mean value for seed thickness is 4.80 mm, with a median of 4.78 mm, very close to the mean. The minimum value is 3.78 mm (91201017 - Portugal) and the maximum is 6.21 mm (cultivar - Skitya), resulting in a range of 2.44 mm. The standard deviation is 0.54 mm, and the CV% = 11.16. Notably, this trait has the lowest coefficient of variation among the seed size parameters examined so far.

The moderate variability in all seed dimensions confirms the presence of diversity among the genotypes.

Table 4. Descriptive statistic of the seed length, width, and thickness, using digital clipper with data transfer cable

SeedLength		SeedWidth		SeedThickness	
Mean	11.51	Mean	6.47	Mean	4.80
SE	0.15	SE	0.08	SE	0.06
Med	11.50	Med	6.46	Med	4.78
Mo	11.24	Mo	6.29	Mo	4.68
SD	1.38	SD	0.76	SD	0.54
S ²	1.89	S ²	0.57	S ²	0.29
R	6.74	R	3.26	R	2.44
Min.	8.03	Min.	4.84	Min.	3.78
Max.	14.77	Max.	8.11	Max.	6.21
CV%	11.96	CV%	11.72	CV%	11.16
Count	84	Count	84	Count	84

The descriptive statistics for the morphometric traits seed area and seed perimeter, using Marvin seed analyzer, are presented in Table 5.

The mean seed area is 86.83 mm², with a slightly higher median (90.73 mm²), suggesting a slight shift toward larger values. The minimum area is 17.59 mm² (C4E0002 - Karavelovo) and the maximum reaches 136.52 mm² (91201170 - Mexico), resulting in a very large range of 118.93 mm². The standard deviation is 27.57 mm² and the CV% = 31.75, indicating high variability. Seed area reflects the overall size of the measured seed surface and such high variability is fully expected, given the substantial differences in TSW.

Table 5. Descriptive statistic of the seed perimeter mm (Perimeter) and seed area mm² (Area) morphometry, using Marvin seed analyzer

Perimeter		Area	
Mean	45.95	Mean	86.83
SE	0.99	SE	3.01
Med	42.90	Med	90.73
Mo	#N/A	Mo	#N/A
SD	9.03	SD	27.57
S ²	81.56	S ²	760.22
R	41.99	R	118.93
Min.	32.71	Min.	17.59
Max.	74.70	Max.	136.52
CV%	19.66	CV%	31.75
Count	84,00	Count	84

The mean seed perimeter is 45.95 mm, with a median of 42.90 mm. The minimum value is 32.71 mm (91201141 - Germany) and the maximum reaches 74.70 mm (C4E0001 - v. Karavelovo), resulting in a significant range of 41.99 mm. The standard deviation is 9.03 mm and the coefficient of variation (CV% = 19.66) places this trait at the boundary between medium and high variability, according to the applied scale. This indicates that the perimeter is more variable than the linear dimensions (length, width, thickness), yet more stable than the TSW, SeedMass and seed area. This intermediate level of variability may reflect differences in seed shape which are not fully captured by individual linear measurements. Based on the traits examined so far, the samples were classified into four cluster groups using cluster analysis, according to Ward's method

(Figure 3), which is widely recommended for multivariate quantitative data and is considered one of the most robust agglomerative algorithms (Murtagh and Legendre, 2023).

A table summarizing the variation of the indicators for each cluster, including the mean values and CV%, was prepared (Table 6). The clustering was performed, using all recorded quantitative traits, and the resulting structure represents the overall multivariate relationships among the samples.

Table 6. The mean values and the variation coefficient (CV%) in the brackets of the vegetation periods and the productivity traits for four cluster groups for 84 common bean accessions

Traits	Cluster 1	Cluster 2	Cluster 3	Cluster 4
Veg.	76.19 (3.70%)	85.59 (4.17%)	73.15 (4.21%)	75.87 (5.37%)
M/F	33.48 (3.52%)	38.53 (8.95%)	33.62 (3.75%)	35.74 (11.94%)
B/F	53.29 (3.73%)	54.24 (4.59%)	49.23 (3.23%)	52.48 (5.63%)
D/F	24.45 (14.66%)	22.94 (17.80%)	19.54 (14.11%)	21.13 (13.56%)
WSP	8.33 (32.79%)	10.26 (32.11%)	7.30 (18.53%)	7.70 (25.38%)
TSW	32.26 (20.89%)	27.00 (13.34%)	29.43 (6.19%)	21.15 (19.88%)
S/L	12.32 (11.69%)	11.44 (8.88%)	11.38 (5.47%)	10.55 (11.43%)
S/W	6.64 (8.34%)	6.60 (8.78%)	7.34 (4.51%)	5.64 (8.37%)
S/T	4.92 (6.86%)	4.67 (6.72%)	5.61 (4.87%)	4.28 (7.79%)
Area	83.36 (38.46%)	85.18 (34.03%)	107.25 (11.75%)	81.18 (26.71%)
Per.	50.77 (19.47%)	47.90 (20.39%)	42.11 (3.49%)	40.16 (12.30%)

Cluster 1 group 31 samples with a medium-early vegetation period (76.19 days; CV% = 3.70). The flowering duration is relatively long (24.45 days), showing moderate variability (CV% = 14.66). In terms of productivity, this cluster is characterized by a relatively high seed weight per plant (8.33 g; CV% = 32.79) and the highest values for TSW (32.26 g; CV% = 20.89). These are productive, medium-early forms with high seed weight, making them suitable for use in breeding programs aimed at developing high-yielding common bean cultivars.

Cluster 2 includes 17 forms with the longest vegetation period of 85.59 days (CV% = 4.17), as well as the longest period to flowering of 38.53 days (CV% = 8.95). Regarding seed weight per plant, the mean value (10.26 g) in this cluster is the highest among all clusters,

suggesting that this may be attributed to genetic differences and that later-maturing forms tend to exhibit higher seed weight per plant.

Cluster 3 groups 13 genotypes characterized by the earliest mass flowering (33.62 days; CV% = 3.75), the shortest flowering duration (19.54 days; CV% = 14.11), and a vegetation period of 73.15 days. The seed perimeter, measured using the Marvin seed analyzer, has a mean value of 42.11 mm and low variability (CV% = 3.49). This cluster also contains the accessions with the largest seed area (107.25 mm²) (also measured with the Marvin analyzer), with the trait showing moderate variability (CV% = 11.75) and represented by very uniform values of all clusters. This cluster includes the samples with the most stable values across all clusters for the following traits: TSW (CV% = 6.18), seed weight per plant (CV% = 18.53), and seed dimensions (length CV% = 5.47; width CV% = 4.51; thickness CV% = 4.87). This is a cluster with very low internal variability in morphometric traits. Additionally, the short vegetation periods highlight these samples as suitable donors for breeding programs, aimed at developing early-maturing common bean varieties.

Cluster 4 groups 23 samples, including forms with mostly medium-early vegetation period (75.87 days; CV% = 5.36) and the lowest seed perimeter values (40.16 mm; CV% = 12.30). The most stable traits within this cluster are the seed dimensions (length CV% = 11.43; width CV% = 8.37; thickness CV% = 7.79).

These results demonstrate that the morphometric characteristics of the seeds play a primary role in differentiating the genotypes, whereas the phenological traits further refine the separation by capturing differences in the duration of the developmental periods.

The Marvin Seed Analyzer data on RGB, HSL and I-Max colour parameters should be considered. The colour analysis separates the genotypes into four distinguishable groups, using Ward's hierarchical clustering method (Figure 4). The resulting clusters show high internal consistency and a clear distinction between light, medium-dark, mottled, and dark seeds. Table 7 presents the mean values and the coefficient of variation for each cluster.

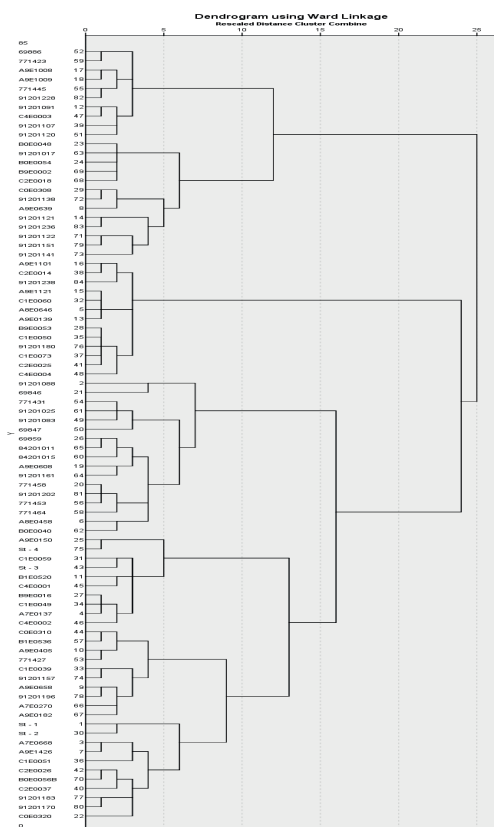


Figure 3. Cluster analysis of the vegetation periods and the productivity traits for 84 common bean accessions

Table 7. The mean values and the variation coefficient (CV%) in the brackets of the I-Max, RGB and HSL colour parameters

Traits	Cluster 1	Cluster 2	Cluster 3	Cluster 4
I-Max	246.11 (1.92%)	226.76 (2.63%)	218.31 (2.36%)	218.87 (3.18%)
Red	229.02 (3.24%)	222.71 (1.25%)	151.40 (19.37%)	72.10 (53.81%)
Green	218.87 (4.78%)	213.89 (1.51%)	110.89 (21.61%)	59.27 (60.94%)
Blue	185.77 (6.71%)	181.93 (3.22%)	84.46 (23.39%)	57.57 (52.75%)
Hue	47.35 (7.81%)	47.77 (4.93%)	29.25 (38.36%)	237.99 (25.17%)
Saturation	0.5798 (10.41%)	0.4475 (9.46%)	0.3633 (16.34%)	0.2118 (75.00%)
Lightness	0.8141 (4.71%)	0.7940 (1.90%)	0.4613 (20.18%)	0.2500 (52.09%)

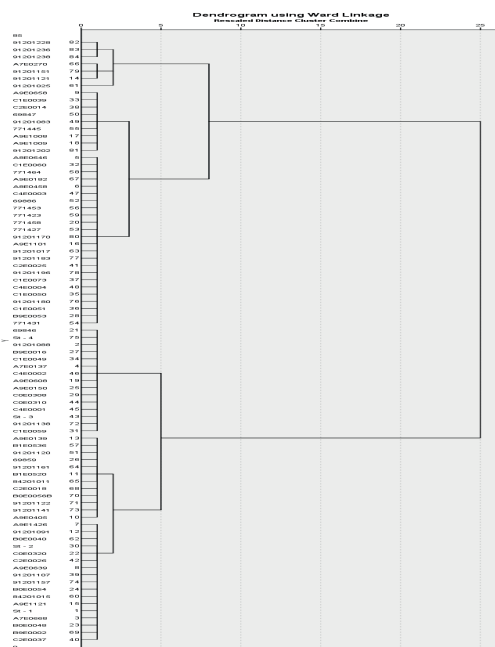


Figure 4. Cluster analysis (Wards linkage) of I-Max, RGB and HSL color parameters

Cluster 1 groups 29 samples. It is characterized by the highest I-Max values (246.11; CV% = 1.92). The RGB components are also high - Red 229.02 (CV% = 3.24), Green 218.87 (CV% = 4.78), and Blue 185.77 (CV% = 6.71). Lightness is high (0.8141; CV% = 4.71), confirming that the seeds in this cluster are the lightest among all groups. The colour types represented in this cluster, based on comparison with the visual assessment of the seeds, include: white seeds (Figure 5 - C2E0026 - Sveta Petka); white seeds with slightly darker shades or the presence of spots (Figure 6 - 91201122 - Germany); and white seeds with a secondary colour concentrated around the hilum (Figure 7 - C2E0018 - Velingrad).

Cluster 2 groups 14 accessions and includes seeds with relatively high brightness, although lower than that observed in Cluster 1. The I-Max value is 226.76 (CV% = 2.63), indicating stable but moderate intensity. The RGB values are high and highly consistent: Red 222.71 (CV% = 1.25), Green 213.89 (CV% = 1.51), and Blue 181.93 (CV% = 3.22). The Hue value is 47.77 (CV% = 4.93), almost identical to that of Cluster 1, suggesting a similar colour tone. Lightness is 0.7940 (CV% = 1.90), indicating light-coloured seeds, though not as bright as those in Cluster 1.



Figure 5. Accession C2E0026 - Sveta Petka



Figure 6. Accession 91201122 - Germany



Figure 7. Accession C2E0018 - Vrelingrad

All samples are characterized by white coloration, but they can be described as: pure white, white with a slightly grayish tint, or white with lower saturation. This cluster represents white seeds with very high colour stability and minimal variability.

Cluster 3 is represented by 33 genotypes and is the most diverse in terms of colour characteristics. It includes the widest range of colors, based on the visual assessment - brown (Figure 8 - A9E1009 - Dragoshinovo), pinto (Figure 9 - C4E0004 - v. Karavelovo), beige (Figure 10 - 771427 - Chile), gray (Figure 11 - C2E0025 - Velingrad), brown-white on half of the seed (Figure 12 - 771445 - France), etc. The

high variability of the RGB and Hue parameters confirms that this is the cluster with the greatest internal colour diversity.



Figure 8. Accession A9E1009 - Dragoshinovo



Figure 9. Accession C4E0004 - v. Karavelovo

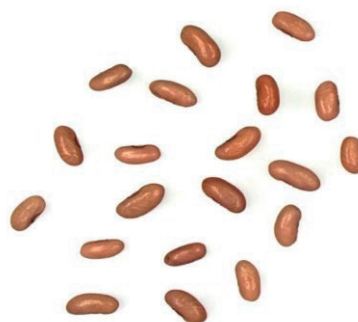


Figure 10. Accession 771427 - Chile

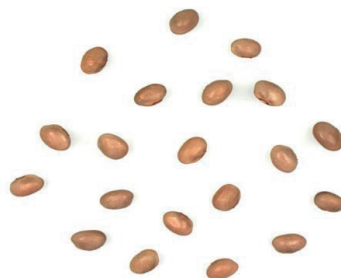


Figure 11. Accession C2E0025 - Velingrad



Figure 12. Accession 771445 - France

Cluster 4 is represented by 7 samples with the darkest seed coloration. The I-Max value is 218.87 (CV% = 3.18), similar to that of Cluster 3. The RGB values are substantially lower: Red 72.10 (CV% = 53.81), Green 59.27 (CV% = 60.94), and Blue 57.57 (CV% = 52.75). Hue is exceptionally high at 237.99 (CV% = 25.17). Saturation is the lowest among all clusters (0.2118; CV% = 75.00), indicating an almost complete lack of color saturation. Lightness (0.2500; CV% = 52.09) is the lowest value across all clusters. This cluster includes black (Figure 13 - 91201151 - France), red (Figure 14 - 91201025 - Portugal), black-white on half of the seed (Figure 15 - 91201151 - France), and accession which include different color of seeds (Figure 16 - A7E0270 - v. Mikrevo).



Figure 13. Accession 91201238 – Türkiye

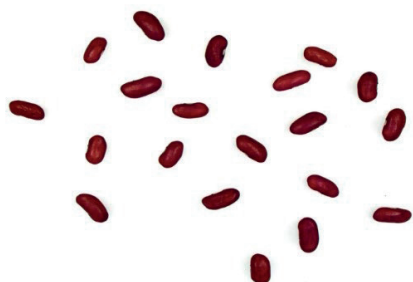


Figure 14. Accession 91201925 – Portugal



Figure 15. Accession 91201151 - France



Figure 16. Accession A7E0270 - Mikrevo

The colour parameters measured, using the Marvin Seed Analyzer, enabled a reliable and precise grouping of the samples into four clearly differentiated clusters. These clusters reflect the actual visual differences among the genotypes and may provide valuable information for breeding, variety identification, and phenotypic characterization. Seed colour may prove to be a highly informative trait that can be used both for morphological classification and for genetic and breeding purposes.

The correlation analysis (Table 8) reveals a clear distinction between the different groups of all measured traits and parameters. The phenological indicators show predominantly weak relationships, highlighting the relative independence of the individual developmental stages, with the exception of the strong correlation between the beginning of mass flowering and the duration of the vegetation period. Colour parameters (RGB and HSL) demonstrate the strongest relationships in the matrix, which is due to the mathematical relationship between the two-colour models, and not to biological differences. The biometric traits show both medium and weak associations: seed width and thickness are medium correlated, reflecting the stability of seed shape, whereas seed length and width show a weak relationship. The TSW is medium correlated with seed perimeter, confirming that the total seed size is a key factor for productivity.

Table 8. Correlation analysis of all 18 traits

	Veg.	M/F	B/F	D/F	I-Max	Red	Green	Blue	Hue	S.	L.	Area	P.	S/L	S/W	S/T	S/M
Veg.	1																
M/F	0.587**	1															
B/F	0.498*	0.498*	1														
D/F	0.286	-0.015	0.332*	1													
I-Max	-0.063	0.101	-0.026	-0.099	1												
Red	0.026	-0.011	0.052	0.008	0.184	1											
Green	0.054	0.015	0.086	0.066	0.158	0.966***	1										
Blue	0.066	0.016	0.093	0.085	0.132	0.935***	0.991***	1									
Hue	0.065	0.061	0.028	0.119	-0.115	-0.519	-0.382	-0.295	1								
S.	0.076	0.023	0.070	0.096	0.029	0.796***	0.769***	0.746***	-0.382	1							
L.	0.048	0.003	0.076	0.049	0.160	0.983***	0.996***	0.984***	-0.415	0.784	1						
Area	0.096	0.098	-0.058	0.219	-0.146	-0.108	-0.104	-0.102	0.076	0.121	-0.107	1					
P.	-0.246	-0.263	-0.279	-0.122	0.012	0.080	0.075	0.079	-0.057	-0.020	0.079	-0.403	1				
S/L	-0.116	-0.176	0.094	0.128	-0.123	-0.053	-0.033	-0.021	0.063	-0.093	-0.037	0.119	-0.148	1			
S/W	-0.035	-0.024	-0.168	0.033	0.194	0.080	0.023	0.021	-0.180	-0.068	0.049	0.089	0.045	0.334*	1		
S/T	-0.282	-0.218	-0.353	-0.046	0.053	0.052	0.015	0.026	-0.190	-0.075	0.037	0.114	0.165	0.105	0.679**	1	
S/M	-0.151	-0.099	-0.064	-0.046	0.068	-0.019	-0.053	-0.057	-0.007	-0.008	-0.040	-0.032	0.216	-0.209	0.024	0.110	1
TSW	-0.130	-0.128	-0.222	0.027	0.266	0.079	0.086	0.081	-0.021	0.014	0.080	0.169	0.576**	-0.078	0.136	0.198	0.208

CONCLUSIONS

The seed perimeter is valuable trait that reflects differences in seed shape and complements linear measurements as an integrative morphometric indicator. It may reflect differences in seed shape which are not fully captured by individual linear measurements.

The 100-seed weight and seed area exhibit high variability, which identifies them as selection-relevant and highly polymorphic traits.

Cluster analyses performed on 84 common bean genotypes revealed a clearly structured phenotypic separation into four groups, differing in their morphological, biometric, phenological, and colour characteristics. The resulting clusters demonstrate both internal homogeneity and distinct differences between groups, highlighting the high level of phenotypic diversity within the studied collection.

Seed colour and the morphometric parameters from Marvin seed analyzer may prove to be highly informative traits that can be used both for morphological classification and for genetic and breeding purposes.

To identify promising groups, the study should be continued in the following experimental years. The model of common bean seed analysing, could be applied to other crops, stored in the genebank.

ACKNOWLEDGEMENTS

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THE ROLE OF ANTIOXIDANT ENZYMES IN SALINITY STRESS TOLERANCE OF BITTER CUCUMBER (*MOMORDICA CHARANTIA* L.)

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Abstract

Momordica charantia, a tropical and subtropical species belonging to the family Cucurbitaceae, is valued both for its nutritional importance and its medicinal properties. Salinity stress represents a major factor limiting agricultural productivity by affecting processes such as photosynthesis, plant growth, and development. Catalase and peroxidase are the principal antioxidant enzymes involved in the degradation of excess accumulated hydrogen peroxide. The aim of the present study was to assess the salinity stress tolerance of five bitter cucumber genotypes (Brâncuși, Rodeo, Line 3, Line 4, and Line 1) by determining their enzymatic activity. The results showed that genotype Line 3 exhibited the highest enzymatic activity (over 50%) under salinity stress conditions (100 mM and 200 mM NaCl), indicating superior antioxidant capacity and increased tolerance to saline conditions. These findings were statistically validated using Pearson's correlation analysis.

Key words: salinity stress, catalase, peroxidase, *Momordica charantia*.

INTRODUCTION

Momordica charantia is a climbing plant species belonging to the genus *Momordica*, family Cucurbitaceae, native to regions of Asia and Africa (Wang et al., 2017). Bitter cucumber has a long history of use in traditional medicine and is particularly consumed for its antidiabetic properties, which have earned it the designation of “plant insulin” (Chanda et al., 2019). In addition to its medicinal role, this species also possesses significant nutritional value. It is rich in vitamins, especially A, C, E, and the B-complex group, and provides essential minerals such as potassium, zinc, and iron. Furthermore, it contains valuable bioactive compounds, including polyphenols, flavonoids, and carotenoids (Kumar & Bhowmik, 2010; Gayathry & John, 2022; Sheikhalipour et al., 2023).

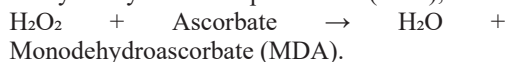
In the context of rapid global population growth, projected to exceed 9 billion by 2050, and the increasing demand for food security, abiotic stress represents a major constraint on agricultural production, significantly affecting crop growth, development, and yield (Oshunsanya et al., 2019; Moulick et al., 2024; Kopecká et al., 2023).

Salinity is one of the principal abiotic factors threatening the future of agricultural crops. This type of stress is characterized by elevated concentrations of mineral salts accumulated in water and soil, which disrupt essential physiological and biochemical processes in plants, thereby limiting productivity and ecosystem sustainability (Meloni et al., 2004; Ahmad & Prasad, 2013; Schubert, 2011).

High salinity induces osmotic stress, reducing the plant's ability to absorb water and mineral nutrients. Mark Tester Munns described this as the first phase of salinity effects, which is immediately perceived by plants (Munns, 1993). The second phase of salt stress occurs with the excessive accumulation of sodium and chloride ions in plant organs. Ionic stress is accompanied by oxidative stress, defined by the excessive accumulation of reactive oxygen species (ROS), such as the superoxide radical (O_2^-), hydroxyl radical ($OH^\bullet$), and hydrogen peroxide (H_2O_2). This elevated accumulation is referred to as an “oxidative burst” (Sharma et al., 2012). ROS alter molecular and cellular components by oxidizing proteins, carbohydrates, lipids, enzymes, and even DNA, ultimately leading to

programmed cell death (Huang et al., 2019; Kesawat et al., 2023).

Plants respond to oxidative stress by increasing the levels of enzymatic and non-enzymatic antioxidants, which act synergistically to neutralize reactive oxygen species and protect cells against oxidative damage (Zheng et al., 2025). The primary enzymatic mechanisms for H₂O₂ scavenging in plants involve catalase and peroxidase. However, their modes of action differ: catalase directly converts H₂O₂ into H₂O and ½O₂ and is the main enzyme involved in detoxification (Ann et al., 2020). When catalase balance is altered in plants, compensatory mechanisms such as peroxidase activation are triggered. Peroxidase activity requires the regeneration system of ascorbate and glutathione. The first reaction of this cycle, catalyzed by ascorbate peroxidase (APX), is:



Glutathione peroxidase (GPX) also detoxifies H₂O₂ to H₂O but directly uses glutathione as a reducing agent (Sofa et al., 2015).

The aim of the present study was to identify *Momordica charantia* genotypes resistant to salinity stress by investigating their enzymatic profiles.

MATERIALS AND METHODS

Materials

Leaves of *Momordica charantia*, KH₂PO₄, Na₂HPO₄, NaOH, C₇H₈O₂ (guaiacol), and H₂O₂ were used in the present study.

Experimental Design

Five genotypes of *Momordica charantia* were used, including two Romanian cultivars (Rodeo and Brâncuși) and three experimental lines (Line 1, Line 3, and Line 4). The experiment was conducted in the greenhouse of the Research Institute for Agriculture and Environment, affiliated with the “Ion Ionescu de la Brad” Iași University of Life Sciences, as well as in the vegetable-growing tunnels of the Horticulture discipline located at the Vasile Adamache Experimental Farm.

Seeds of uniform size and appearance were sown in greenhouse trays. After seedling establishment, the plants were transplanted into the tunnel system. The plants were divided into the following variants: control (no salt

treatment), V1 (plants treated with 100 mM NaCl), and V2 (plants treated with 200 mM NaCl).

A total of three saline treatments were applied at the following BBCH phenological stages: 201 BBCH (corresponding to the appearance of the first lateral shoot), 501 BBCH (appearance of the first flower), and 701 BBCH (appearance of the first fruit). Leaves used for enzymatic analyses were harvested seven days after the application of the final saline treatment.

Catalase Activity

Catalase enzymatic activity was determined using the protocol described by Hugo Aebi (1984), with minor modifications. A 30% hydrogen peroxide solution was diluted in phosphate buffer (pH = 7.0) until the absorbance reached 0.55.

A quantity of 0.2 g of fresh plant material was homogenized in a mortar with 3 mL of buffer solution and subsequently centrifuged for 15 minutes at 10,000 rpm.

The reference sample consisted of 2.9 mL of buffer solution mixed with 0.1 mL of supernatant obtained from the plant extract. The reaction mixture for analysis contained 2.9 mL of hydrogen peroxide solution (prepared in buffer) and 0.1 mL of supernatant.

Ten absorbance readings were recorded at intervals of 15 seconds.

One unit (U) is defined as the amount of enzyme required to degrade 1 μmol of hydrogen peroxide per minute. Catalase activity was determined by monitoring the decrease in absorbance at 240 nm over time. By plotting absorbance (A) versus time (min), the reaction rate (slope) was calculated and subsequently used in the equation for determining specific activity:

$$\text{U/g FW} = (\text{slope} \times V_r) / (\varepsilon \times m)$$

where:

- slope - reaction rate,
- V_r - total reaction volume,
- ε - molar extinction coefficient of hydrogen peroxide,
- m - mass of fresh plant material used for analysis.

Peroxidase Activity

Peroxidase activity was determined spectrophotometrically by monitoring the oxidation of guaiacol in the presence of hydrogen peroxide, measuring the increase in absorbance at 470 nm, and calculating the reaction rate to determine the

specific activity. The protocol and calculation method used for peroxidase determination were identical to those described for catalase.

RESULTS AND DISCUSSIONS

High salinity significantly disrupts photosynthesis and respiration processes, leading to the formation of the superoxide radical ($O_2^{\bullet-}$). Superoxide dismutase catalyzes its dismutation into hydrogen peroxide (H_2O_2), which is subsequently decomposed, primarily by catalase, into H_2O and O_2 (Hasanuzzaman et al., 2021). According to the scientific literature, tolerant genotypes exhibit elevated enzymatic activity even under normal environmental conditions, enabling them to respond rapidly when salinity triggers oxidative stress (Xiao and Zhou, 2023; Ghiasy & Majid, 2025).

Among the studied *Momordica charantia* genotypes, the highest catalase activity under control conditions was recorded in genotype Line 3 (9.82 U/g FW), suggesting a potentially high tolerance of this experimental line to stress. In contrast, the lowest catalase activity was observed in genotype Line 4 (1.41 U/g FW).

Moderate salinity (100 mM NaCl) induced a significant increase in catalase activity in all studied genotypes, with the most pronounced difference compared with the untreated control observed in Line 3 (28% increase relative to the control). The smallest increase was recorded in Line 4, where no statistically significant difference was observed. The 200 mM NaCl treatment elicited a differential response among the bitter cucumber genotypes. Increases in catalase activity relative to the control variant were recorded in Line 1 (14%) and Line 3 (58%). In contrast, the Brâncuși, Rodeo, and Line 4 genotypes exhibited decreases in enzymatic activity compared with both the V1 treatment and the untreated control (Figure 1). Under severe salinity stress conditions, catalase activity declines in sensitive plants due to excessive ROS accumulation, direct enzymatic inhibition caused by ionic toxicity, and accelerated protein degradation. This response indicates that the plant's antioxidant defense capacity has been exceeded (Sofa et al., 2015).

In contrast to catalase, the *Momordica charantia* genotypes exhibited a stronger peroxidase activity, which may be explained by their

prolonged exposure to abiotic stress conditions. Under control conditions, the highest enzymatic activity was recorded in Line 3 (13.37 U/g FW), followed by Line 1 (9.34 U/g FW). The remaining studied genotypes showed lower peroxidase activity, highlighting increased sensitivity.

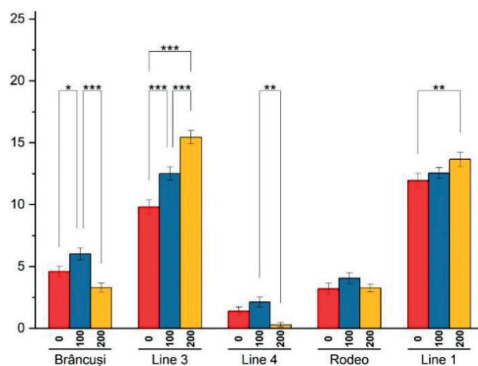


Figure 1. Effect of salinity stress on catalase activity expressed as U/g FW in the studied bitter cucumber genotypes, seven days after the application of the final saline treatment. Error bars represent the standard deviation, and asterisks indicate statistical significance according to Tukey's post hoc test as follows: * – $p \leq 0.05$; ** – $p \leq 0.01$; *** – $p \leq 0.001$; $n = 3$

Treatment with 100 mM NaCl led to an intensification of enzymatic activity in all studied genotypes, with a maximum observed in Line 3 (23.47 U/g FW), representing a 76% increase compared to the control. A significant increase in peroxidase activity reflects the plant's rapid response to salinity-induced stress conditions (Wu et al., 2022).

Application of the 200 mM NaCl treatment further enhanced enzymatic activity compared to both the untreated control and the 100 mM NaCl treatment. Relative to the control, the highest enzymatic activity was recorded in Line 1 (98% increase), followed by Line 3 (92%). Regarding the variation between stress treatments, highly statistically significant differences were observed only in experimental Lines 1 and 3, further emphasizing their rapid response to unfavorable environmental conditions (Figure 2).

Significant increases in peroxidase activity under salinity stress have also been reported by Neto and colleagues (2005). The authors investigated enzymatic activity in both salt-tolerant and salt-sensitive maize cultivars and

observed an accelerated increase in peroxidase activity in salt-tolerant cultivars following the onset of salinity stress (Neto et al., 2006).

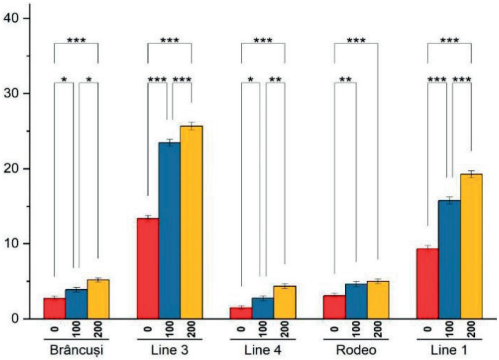


Figure 2. Effect of salinity stress on peroxidase activity expressed as U/g FW in the studied bitter cucumber genotypes, seven days after the application of the final saline treatment. Error bars represent the standard deviation, and asterisks indicate statistical significance according to Tukey's post hoc test as follows: * – $p \leq 0.05$; ** – $p \leq 0.01$; *** – $p \leq 0.001$; $n = 3$

In Figure 3, the Pearson correlation coefficient was calculated for catalase and peroxidase analyses performed on the leaves of the bitter cucumber genotypes. For the Pearson correlation analysis, specific abbreviations were used as follows: B, R, L1, L3, and L4 represent

the studied genotypes Brâncuși, Rodeo, Line 1, Line 3, and Line 4, respectively. These were combined with the notations M, V1, and V2, corresponding to the control, 100 mM NaCl treatment, and 200 mM NaCl treatment. The abbreviations C and P refer to catalase and peroxidase, respectively.

The correlation matrix displays a color gradient ranging from light yellow, indicating a strong negative correlation, to violet, representing the absence of correlation, and up to black, indicating a strong positive correlation.

Analysis of the Pearson correlation matrix revealed strong positive relationships in all studied genotypes under the 100 mM NaCl treatment, demonstrating a favorable response of the genotypes to moderate abiotic stress conditions. In plants treated with 200 mM NaCl, strong negative correlations between the activities of the two studied enzymes were observed in the Brâncuși, Line 4, and Rodeo genotypes. This was associated with a decrease in catalase activity under severe stress, highlighting their sensitivity to harsh environmental conditions.

In contrast, Line 3 and Line 1 maintained strong positive correlations under severe salinity, emphasizing their enhanced tolerance to salt stress.

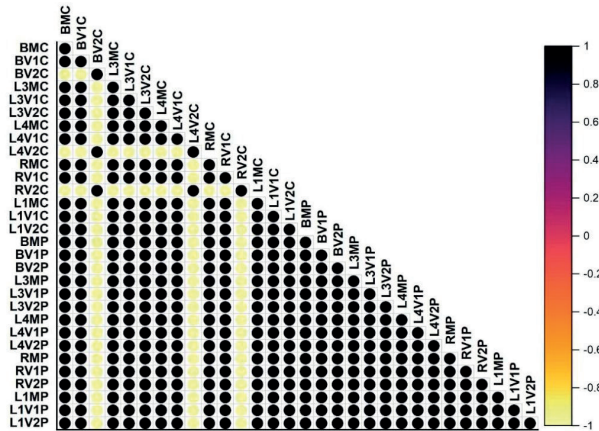


Figure 3. Pearson correlation results for catalase and peroxidase determinations following the application of the final saline treatment in the studied bitter cucumber genotypes

CONCLUSIONS

Hydrogen peroxide (H₂O₂) is one of the primary reactive oxygen species (ROS) produced in

excess under high salinity conditions. To protect against cellular oxidative damage, plants enhance their enzymatic metabolism by increasing the levels of catalase and peroxidase.

Comparative analysis revealed that Lines 1 and 3 exhibited the highest catalase and peroxidase activities under salinity stress, indicating enhanced tolerance and the ability to maintain an efficient enzymatic response under severe oxidative stress.

These findings open new research perspectives, as the identified salt-tolerant genotypes can serve as a starting point for further studies on the mechanisms underlying salinity resistance.

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PRETREATMENT OF SEEDS WITH *TRICHODERMA* STRAINS IMPROVES FORL RESISTANCE AND INDUCES THE EXPRESSION OF CHITINASE GENE IN TOMATO PLANTS

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Abstract

Some *Trichoderma* isolates are known to produce a variety of hydrolytic enzymes (e.g. chitinase, β 1-3 glucanase, and β 1-6 glucanase) that are involved in plant disease resistance through complementary modes of action. The antagonistic activity of six *Trichoderma* strains against *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL) was evaluated alongside their chitinase production. The expression of the defense-related chitinase 26 gene in tomato plants was evaluated by RT-PCR analysis. The results show that the *Trichoderma* spp. isolates under investigation exhibited elevated chitinase activity, with one *T. asperellum* and two *T. longibrachiatum* strains showing particularly high levels. All *Trichoderma* isolates reduced the mycelial growth of FORL, the efficacy of this reduction varying depending on the *Trichoderma* strain. A positive correlation was observed between the antagonistic activity of *Trichoderma* isolates against FORL and their production of chitinases. Regarding the impact of various treatments on chitinase 26 expression in tomato plants, several treatments enhanced chitinase 26 expression, while one treatment suppressed this response. These findings indicate that *Trichoderma* isolates can have biocontrol potential and that they can elicit different effects on tomato defense activation.

Key words: gene expression, *Fusarium oxysporum* f. sp. *radicales-socialistes*, chitineuse, plant disease.

INTRODUCTION

Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *lycopersici*, is one of the most severe diseases affecting tomato (*Solanum lycopersicum* L.) cultivation worldwide. It leads to significant yield losses in both greenhouse and field production systems (Haque et al., 2023). This soil-borne pathogen invades plant roots and disrupts the transport of water and nutrients, resulting in wilting and plant death. Frequent and intensive application of chemical fungicides for disease management raises concerns about human health and environmental safety due to the persistence of toxic residues and the development of pathogen resistance (Guzmán-Guzmán et al., 2025).

Beneficial microorganisms, such as *Trichoderma* spp., have emerged as a sustainable alternative for biological disease

control in agriculture. *Trichoderma* species are a ubiquitous type of soil fungi that have a well-documented and significant role in plant health, including pathogen suppression, growth promotion, and modulation of plant immune responses (Guzmán-Guzmán et al., 2025; Basak et al., 2026). Recent studies have shown that *Trichoderma* contributes to sustainable crop production through two mechanisms: direct antagonism of pathogens and enhancement of host defence mechanisms (Haque et al., 2023). Among the mechanisms employed by *Trichoderma* spp., mycoparasitism plays a pivotal role in the suppression of fungal pathogens. Mycoparasitism involves direct interaction with the pathogen and the secretion of hydrolytic enzymes, such as chitinases and glucanases, that degrade fungal cell walls (Sharma et al., 2025). These enzymes contribute to pathogen lysis and nutrient acquisition,

thereby limiting disease development (Basak et al., 2026).

Chitinases are key components of the *Trichoderma* chitinolytic system and are capable of hydrolysing chitin, a major structural component of fungal cell walls, into oligomers. The activity and expression of these enzymes vary among *Trichoderma* strains, resulting in differential antagonistic capacity and biocontrol efficiency (Akanksha et al., 2025). Chitinases produced by *Trichoderma* spp. have been implicated in the suppression of several soil-borne pathogens. Furthermore, it has been shown that the production of chitinases is influenced by environmental cues and substrate availability (Haque et al., 2023).

Numerous studies have been conducted to evaluate the expression of chitinases and β -1,3-glucanases enzymes produced by various strains of *Trichoderma* spp. as part of their antagonistic mechanism involved in disease resistance in different crops (Lorito et al., 1998; Kubicek et al., 2001; Viterbo et al., 2002; Gajera et al., 2012; Gajera et al., 2013; Khatri et al., 2017). *Trichoderma* endochitinase encoded by the *ech42* gene appears to be more effective in controlling fungi than chitinases found in plants (Harman et al., 1993; Lorito et al., 1998). The inhibition of spore germination and hyphal elongation seem to be key mechanisms in this process. Matarese et al. (2012) conducted a study to evaluate the transcription of genes encoding chitinolytic enzymes that have been associated with mycoparasitism. They observed an upregulation of all genes tested, indicating the involvement of these chitinolytic enzymes produced by *Trichoderma* in the interaction with *Fusarium graminearum* and *Fusarium culmorum*.

Qualhato et al. (2013) reported that a positive correlation exists between the amounts of secreted cell-wall-degrading enzymes by *Trichoderma* strains and their ability to control plant pathogenic fungi. The results obtained by El-Komy et al. (2022), showed a significant positive correlation between the antagonistic capacity of *T. Asperellum* isolates against *FORL* growth and the production of chitinase and β -1,3-glucanase.

In this study, the production of chitinase enzymes and defence-related gene expression (*chitinase 26*) in six *Trichoderma* spp. isolates

was examined, and their antagonistic performance against *Fusarium oxysporum* f. sp. *radicis-lycopersici* in tomato plants under greenhouse conditions was evaluated.

MATERIALS AND METHODS

Fungal strains and culture conditions

Trichoderma spp. and *Fusarium oxysporum* f. sp. *radicis-lycopersici* isolates used in the present study were obtained from the culture collection of the Research and Development Institute for Plant Protection, Bucharest. Six *Trichoderma* strains were used in this study: *Trichoderma asperellum* Td50, *Trichoderma asperellum* Td85, *Trichoderma asperellum* al12, *Trichoderma longibrachiatum* Tk 14, *Trichoderma longibrachiatum* Tk20, *Trichoderma asperellum* Tk25.

The synthetic medium used for culturing *Trichoderma* spp. strains consisted of the following components (g/L): $\text{MgSO}_4 \times 7 \text{H}_2\text{O}$ (0.2); K_2HPO_4 (0.9); KCl (0.2); NH_4NO_3 (1.0); FeSO_4 (0.002); MnSO_4 (0.002); ZnSO_4 (0.002); and colloidal chitin (10,0). All components were supplied by Sigma-Aldrich (USA). A spore suspension of each *Trichoderma* strain (1×10^7 spores/ml) was inoculated into 100 ml of the synthetic liquid medium. To this, 1% colloidal chitin, prepared according to the method of Berger and Reynolds (1958), was added. The cultures were then incubated at 30 °C for six days with agitation at 150 rpm. After filtering the culture medium, it was centrifuged at 5000 rpm for 10 minutes. The resulting supernatant was then tested for enzyme activity or stored at -20°C until analysis.

Chitinase activity assay

Chitinase activity was quantified using the spectrophotometric method described by Boller and Mauch (1988) by measuring the reducing sugars released from colloidal chitin.

The reaction mixture consisted of 1 ml of culture filtrate and 1 ml of 1% colloidal chitin suspended in 0.2 M sodium phosphate buffer (pH 6.5) and was incubated at 50°C for 60 minutes. The reaction was stopped by adding 1 ml of 1% NaOH solution and boiling the mixture for 5 minutes. The resulting mixture was then centrifuged at 4000 rpm for 10 minutes. One millilitre of the supernatant was mixed with one

millilitre of 3,5-dinitrosalicylic acid, and the resulting mixture was incubated for five minutes in a boiling water bath. The mixture was then cooled, after which the optical density was measured at 540 nm.

The amount of NAG (N-acetylglucosamine) released was determined using a standard calibration curve. Chitinase activity was expressed as μmol N-acetylglucosamine released per ml per minute (μmol NAG/ ml $\times$ min). Enzyme activity was measured at 48, 96, and 120 h of culture incubation.

Plant material, fungal treatment, and pathogen inoculation

Tomato seeds (*Solanum lycopersicum* L., cultivar Buzău 1600), obtained from the Vegetable Research and Development Station Buzău (SCDL Buzău), were sown in 300 g of soil sterilised at 121°C for 15 minutes in an autoclave (Tuttnauer BV, Netherlands).

The soil was inoculated at sowing with *Trichoderma* spore suspensions, as well as *Fusarium oxysporum* f. sp. *radicis-lycopersici* ZUM 2407, depending on the treatment. Untreated plants, grown in the absence of both pre-treatment with fungi and pathogen, served as controls.

Trichoderma spp. spore suspensions were prepared by scraping conidia from the culture surface after the addition of 20 ml of sterile distilled water. Spore concentration was determined using a hemocytometer (Marienfeld Superior, Germany) and adjusted to 1×10^8 spores/ml. A volume of 50 ml of *Trichoderma* suspension was applied per pot for treatment.

For *Fusarium oxysporum* f. sp. *radicis-lycopersici* ZUM 2407 a spore suspension of 5×10^6 spores/ml was prepared, and 20 ml was applied per pot.

Plants were grown for 35 days in a greenhouse (22–24°C, under a natural photoperiodic alternation of 12 h). Each treatment consisted of three biological replicates, with each replicate represented by one pot.

RNA extraction

To extract the RNA, 100 mg of plant material (root tissue) was immersed in liquid nitrogen and ground. The resulting powder was then used to isolate RNA using the SV Total RNA Isolation System kit (Promega, USA), following

the manufacturer's instructions. The entire RNA isolation process was carried out under sterile conditions to prevent contamination and degradation during handling.

The concentration and purity of the RNA were assessed using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, USA).

RT-qPCR analysis

Total RNA was transcribed into complementary DNA (cDNA) and quantitative real-time PCR (RT-qPCR) was performed using SYBR Green chemistry.

Primer sequences for the target gene (chitinase 26) and the reference gene (elongation factor 1- α , EF1 α) were obtained from Ene et al. (2013) and synthesised by Generi Biotech (Czech Republic). Sequences (5'–3') were:

- Chitinase 26:
F: CGAAACTAATGGTGGTAGTGC;
R: TCGCAACTAAATCAGGGTTG
- EF1 α :
F: AGCCCAAGAGGCCATCAGACAA;
R: GAGTGCCTCCTGGAGAGCTTCG

Primers targeting the reference ribosomal protein L2 gene were also tested but excluded due to non-specific amplification.

RT-qPCR reactions were performed using the GoTaq® 1-Step RT-qPCR System (Promega, USA) on an Eppendorf Mastercycler® ep realplex system. The amplification programme consisted of an initial reverse transcription step (50°C for 15 min), followed by a hot-start activation step (95°C for 10 min) and 40 cycles of denaturation at 95°C for 15s, annealing at 60°C for 1 min, and extension at 72°C for 30s. Amplification specificity was verified at the end of each RT-qPCR run by melt curve analysis. Only reactions displaying a single melting peak were included in the analysis. Each reaction was conducted with three technical replicates for each of the three biological replicates.

Relative gene expression levels were calculated using the $2^{-\Delta\Delta\text{Ct}}$ method as described by Livak and Schmittgen (2001), with untreated plants used as the control condition. Ct values were processed and analysed using Microsoft Excel (Microsoft).

RESULTS AND DISCUSSIONS

In the present study, all six *Trichoderma* strains demonstrated chitinase activity when cultured on medium with colloidal chitin, confirming that the chitinolytic system is a conserved trait within this genus. However, substantial differences in enzyme levels and temporal dynamics were observed among the strains, highlighting the importance of strain-specific characterization for agricultural applications. As shown in Table 1, the chitinase activity measured for the six *Trichoderma* spp. strains examined in this study varies depending on the strain and species.

Table 1. Variation in chitinase activity over 120 hours

Strain	Chitinase activity ($\mu\text{mol N-acetyl-glucosamine/ ml} \times \text{min}$)		
	48 h	96 h	120 h
Td50	53.7	115.3	100.0
Td85	66.5	141.3	135.1
al12	85.6	150.2	345.2
Tk14	78.3	157.8	300.6
Tk20	82.1	160.1	287.8
Tk25	51.6	70.1	65.4

Two temporal activity patterns were observed among the strains. Chitinase activity increased progressively over time for strains Td85, al12, Tk20, and Tk25, reaching the highest levels at 120 hours. In contrast, for strains Td50, Td85, and Tk25, maximal chitinase activity was observed at 96 hours, followed by a slight decrease at 120 hours. Despite these differences in temporal profiles, all strains demonstrated chitinolytic activity throughout the incubation period. Similar variability in chitinase production among *Trichoderma* isolates has been reported previously and is considered a key factor influencing antagonistic efficiency against soil-borne pathogens (Harman et al., 2004; Parmar et al., 2015).

Strains al12, Tk14, and Tk20 exhibited the highest levels of chitinase activity after prolonged incubation, suggesting a significant capacity for the degradation of chitin-containing fungal cell walls. Among the strains studied, the following stand out, demonstrating the highest levels of activity:

- al12 with a maximum chitinase activity of 345.2 $\mu\text{mol NAG/ml} \times \text{min}$;

- Tk14 with an activity of 300.6 $\mu\text{mol NAG / ml} \times \text{min}$, and
- Tk20 with an activity of 287.8 $\mu\text{mol NAG / ml} \times \text{min}$.

High chitinase activity has been directly associated with mycoparasitism mechanism and suppression of *Fusarium* spp. in the rhizosphere (Viterbo et al., 2002; Woo et al., 2014). The decline in enzyme activity observed in some strains at later incubation stages may be attributed to substrate depletion or regulatory feedback mechanisms, as previously described for extracellular hydrolytic enzymes produced by *Trichoderma* spp. (Vinale et al., 2008).

Effect of fungal pre-treatment and pathogen challenge on chitinase 26 expression

The relative expression of the chitinase 26 gene was assessed in tomato plants that had been pre-treated with *Trichoderma* strains in the presence and absence of FORL (Figure 1).

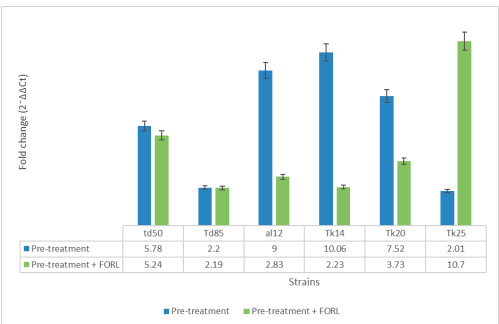


Figure 1. Relative expression of the chitinase 26 gene in tomato plants pre-treated with *Trichoderma* strains in the presence and absence of *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL)

The pre-treatment of tomato plants with *Trichoderma* resulted in increased expression of *chitinase 26* even in the absence of the pathogen, indicating an induction effect of plant defence responses.

Defence through priming with beneficial fungi is a well-documented mechanism associated with induced systemic resistance (ISR), enabling plants to respond more rapidly and efficiently upon attack by pathogens (Conrath et al., 2006; Pieterse et al., 2014).

From an agricultural perspective, this primed state is particularly relevant, as it supports the

preventive use of beneficial microorganisms in integrated disease management strategies.

Following inoculation with *Fusarium oxysporum* f. sp. *radicis-lycopersici*, tomato plants' defence responses varied depending on the *Trichoderma* strain applied, reflecting the complexity of plant-beneficial pathogen interactions.

Thus, in the presence of the pathogen, chitinase 26 expression was differentially modulated in a strain-dependent manner, with some treatments resulting in reduced transcript levels compared to pre-treatment alone.

Notably, strain Tk25 induced a strong increase in *chitinase 26* expression in response to pathogen challenge, despite displaying comparatively low *in vitro* chitinase activity.

This observation suggests that biocontrol efficacy is not solely dependent on enzymatic activity, but may also involve additional mechanisms such as efficient root colonization, competition for nutrients, or modulation of plant signalling pathways (Vinale et al., 2008; Woo et al., 2014).

It was observed that soil treatment of Tk14, Tk20 and al12 strains resulted in the greatest disease inhibition when compared with the control, and that there was an increase in growth parameters of tomato plants as compared with other treatments (Table 2).

Table 2. Effect of *Trichoderma* treatments on growth parameters of tomato plants

Treatment	No. of leaves	Root length (cm)	Plant height (cm)	Root fresh weight (g)
Td50	7.0	13.0	26.5	4.39
Td50 + FORL	6.3	9.7	24.7	4.10
Td85	7.9	14.3	28.3	5.45
Td85 + FORL	7.5	10.2	26.8	5.00
al12	8.0	14.5	29.8	6.65
al12 + FORL	7.9	9.8	27.2	5.50
Tk14	6.0	14.0	25.3	4.70
Tk14 + FORL	5.6	9.2	23.0	4.40
Tk20	7.0	13.8	25.9	5.00
Tk20 + FORL	6.5	9.5	23.5	4.80
Tk25	5.8	11.8	24.4	4.00
Tk25 + FORL	5.0	8.0	22.5	3.20
FORL	3.9	8.0	16.8	2.90
Control	4.7	10.5	21.8	3.90

The present study confirms the results obtained by Uddin et al. (2011), who reported that *T. harzianum* significantly reduced the damping-

off of chili in comparison with the control and enhanced seed germination.

Similar results were reported by Moka et al. (2021) wherein the different strains of *Trichoderma* showed significant efficacy in controlling the damping-off of onion caused by *Fusarium oxysporum* f.sp. *cepae*. This effect was attributed to the substantial production of chitinase enzymes by these strains.

Overall, our findings demonstrate that effective biocontrol by *Trichoderma* involves multiple mechanisms, including direct enzymatic antagonism and indirect activation of plant defence responses, which are strain-dependent. Therefore, the integration of the enzymatic profile with plant response analyses represents a valuable approach for the selection of *Trichoderma* strains with high potential for sustainable disease control in tomato cultivation.

CONCLUSIONS

The study confirms that *Trichoderma* strains differ significantly in terms of their chitinase activity and their capacity to stimulate defence responses in tomato plants.

Strains al12, Tk14, and Tk20, characterized by high chitinase activity and associated with robust plant defence induction, emerge as promising candidates for biological control strategies against *Fusarium oxysporum* f. sp. *radicis-lycopersici*.

Trichoderma pre-treatment has been shown to effectively prime tomato plants for enhanced defence, suggesting its practical application in integrated disease management programs.

Strain-specific differences in plant responses highlight the necessity for a targeted selection of *Trichoderma* isolates based on multiple functional traits in addition to enzyme activity alone.

These findings support the implementation of selected *Trichoderma* strains as environmentally friendly biocontrol agents in sustainable tomato production systems.

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STUDY OF THE VARIABILITY OF MORPHOLOGICAL AND PRODUCTION CHARACTERS IN SOME *VICIA FABA* L. GENOTYPES

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Abstract

Vicia faba L. is an annual species of the Fabaceae family, cultivated for the value of its seeds rich in vegetable proteins and for its contribution to improving and maintaining soil fertility by fixing atmospheric nitrogen. The study aims to analyze the variability of some morphological and productivity characters of an assortment of twelve faba bean genotypes. The following traits were analyzed: plant height, number of branches, minimum height of the first pod, pod length, pod width, number of pods per plant, number of seeds per pod, weight of 100 seeds, and total weight of pods per plant. Significant differences were observed between genotypes for the studied characters. From a productive point of view, genotypes G2, G3, G6, G7 and G10 were noted, whose production ranged between 41.33-54.17 g/pl. These genotypes can be recommended for cultivation in the South-West area of Romania and considered valuable and useful for future breeding programs.

Key words: agro-morphological traits, faba bean, phenotypic variability.

INTRODUCTION

Broad beans (*Vicia faba* L.), native to the Mediterranean and Central Asia, are an important vegetable crop that can be used as food for human consumption and as animal feed (Yu et al., 2023; Stavropoulos et al., 2024). Broad beans are cultivated in the continental area from northern Europe to Ethiopia and in all countries on the Mediterranean coast. China, Ethiopia, Morocco, France, Italy, Tunisia, and Spain are among the countries with the largest cultivated areas (Akgun and Canci, 2023). Faba bean (*Vicia faba*) is one of the oldest vegetables in the world and is the fourth most important winter legume after chickpea, dried peas, and lentils (Barcanu et al., 2023). World production of faba bean is divided into two categories: fresh beans and dried beans. Fresh faba beans are mainly grown in Algeria (32,315 t in 2021), and dried faba beans are mainly produced in China (804,320 t in 2021). In the legumes category, globally, *Vicia faba* production ranks sixth, after common bean (*Phaseolus vulgaris* L.), chickpea (*Cicer arietinum* L.), field pea (*Pisum sativum* L.), cowpea (*Vigna unguiculata* L.) and lentils (*Lens culinaris* Medik.) (FAOSTAT, 2024).

Legumes are crucial for sustainable agriculture and provide essential plant-based proteins for both human and animal diets (Sokolović et al., 2025). *Vicia faba* seeds are rich in protein, essential amino acids, energy, and mineral compounds and have particularly high levels of unsaturated fatty acids (Meng et al., 2021). They also contain antinutritional factors such as vicine, convicine, and tannin. For a balanced and healthy diet, faba bean varieties should be improved for high seed yield with higher essential amino acid content and low antinutritional factors (Akgun and Canci, 2023). The dried seeds also have the potential to be used as a replacement for soybean meal and/or cereal seeds in animal diets to support milk, meat, and/or egg production (Meng et al., 2021). Legume production, including faba beans, is crucial to mitigate the growing challenges in agricultural production, such as global food stress, land degradation, and excessive use of chemicals (Yu et al., 2023). Faba beans are recommended in crop rotations to enrich the soil with natural nitrogen or used as green manure, because the vegetative part is vigorous and the roots form nodules with nitrogen-fixing bacteria. Due to its adaptability to climate conditions, it is considered a viable agronomic

alternative to cereals, with a potential for free nitrogen fixation of up to 300 kg N ha⁻¹ (Singh et al., 2013).

Vicia faba is an annual, cool-season legume (Meng et al., 2021) with good adaptability to soil and climate conditions (Singh et al., 2013; Papastylianou et al., 2021). However, production results can be influenced by numerous environmental and technological factors (Soare et al., 2024). In general, crop production is also influenced by soil quality, such as texture, porosity, soil reaction (pH), CaCO₃ or nutrient content, as well as its interaction with climatic factors (Grecu and Pavel, 2016; Balan and Popescu, 2024;).

This annual species is not demanding on heat, but has high requirements for humidity during germination, flowering, and pod formation. It is frost-resistant; the more resistant varieties tolerate temperatures of -5°C. It cannot withstand excessive heat during flowering (Singh et al., 2013). The optimal growth and development temperatures are 20-22°C, and those higher than 25°C cause stagnation of development. Regarding the soil, it requires a medium texture, with a pH between neutral and alkaline (pH of 6.5 to 8.0) (Rajan et al., 2012; Vîntoru et al., 2019). Therefore, it is important to use stable genotypes, well adapted to the crop areas, which, together with good management practices, contribute to increasing production and cultivated areas (Papastylianou et al., 2021). It is important to implement *Vicia faba* crop management programs to maintain the genetic diversity of the species, primarily of local populations, at the best possible level (Avramidou et al., 2023).

Classification of *Vicia faba* populations using morphological and agronomic traits is imperative for their effective exploitation in breeding, conservation, and management (Terzopoulos and Bebeli, 2008). Recently, various molecular markers have been successfully used to characterize the genetic diversity of faba bean genotypes (Wang et al., 2012). *Vicia faba* L. crop often faces drought during its growth and development, so that soil moisture deficit limits its production (Khan et al., 2010; Bonea et al., 2022). In this regard, Memari et al. (2022) suggest investigating the effect of drought stress using stress tolerance indices for the selection of genotypes tolerant to this factor.

Plant breeding has significantly contributed to obtaining a large number of varieties with desired traits, and as a result, it is important to analyze some traits considered useful (yield potential, abiotic stress resistance) when choosing faba bean varieties (Yüce et al., 2023). In recent decades, in Romania, the *Vicia faba* crop has been underutilized, with decreasing areas. According to FAOSTAT, in Romania, in 2020 the harvested area of faba beans was less than 12,000 ha, with a production of only 11,250 tons (Barcanu et al., 2022). In 2024, the area cultivated in Romania with dry beans decreased drastically to 6,690 ha, the same trend having the production per hectare of 934 kg/ha (fao.org, 2026). The decline in areas and production is related to susceptibility to biotic and abiotic stresses and the instability of production.

The reduction in areas may also be due to the change in cropping systems in many areas where it was cultivated in the past (Vörösváry et al., 2011; Țigriș et al., 2020).

Plant genetic resources or germplasm are fundamental sources for plant improvement, and the assessment of genetic diversity among germplasm accessions is useful for facilitating a more efficient use of plant genetic resources (Karaköy et al., 2014). The use of genetic diversity is very important for the efficient selection of superior genotypes for all crops (Iacob and Ciulca, 2024). An important strategy for increasing yield and quality is choosing the most suitable cultivars for various environmental conditions (Bonea et al., 2026). In addition to qualitative improvement, abiotic stress management is becoming a major challenge in the research of this species (Torres et al., 2011).

In order to promote the cultivation of *Vicia faba* in Romania, studies were carried out on the behavior of some genotypes under the environmental conditions of different areas: in the East, Horaicu et al. (2023), in the West, in the Timiș Plain Țigriș et al. (2020), in central Romania Barcanu et al. (2022), highlighting the most productive ones. Recently, however, numerous foreign genotypes have arrived in Romania, whose behavior under local climatic conditions is unknown (Horaciu et al., 2024). In this regard, it was studied the behavior of some *Vicia faba* genotypes from local populations and

from abroad, in the climatic conditions of South-West Romania.

MATERIALS AND METHODS

The experiment was set up in the Didactic Field at the Didactica Station, University of Craiova, in 2023 and 2024, on a reddish-brown preluvosol in the south-west of Romania. A randomized complete block design with 3 replications was used. Sowing was carried out in the third decade of March; the spacing between rows was 0.75 m and between plants it was 0.20 m. During the vegetation period, standard grain cultivation practices were applied. For this study, seeds were collected from producers from different regions of Oltenia (Romania) or from sources brought from outside Romania (Table 1). Of the 10 genotypes, Genotype 6 and Genotype 10 come from Italy, and the other 8 come from local populations

cultivated in Romania (G1-Mătășari, G2-Bălești, Genotype 3, G4-Urdari, G5- Vâlcea 2, G7-Tetoiu, G8-Colonesti, G9-Băresti). For the study, the morphological characteristics were monitored: plant height, number of shoots and height of insertion of the first pod, and productivity characteristics: number of pods/plant, seeds/plant, mass of 100 seeds (g) and grain production (kg/ha). The average climatic data for the vegetation period (April - September) are presented in Table 1. The recorded precipitation was higher in the first year of cultivation (2023) than in the second year (2024). Regarding the temperatures during the vegetation period, the plants were subjected to higher temperatures in the second year than in the first year. In 2023, the minimum temperature was 0.3°C in April and the maximum was +37.4°C in August, while in 2024, the minimum temperature was +2.0°C in April and the maximum was +39.6°C in July.

Table 1. Temperatura, total rainfall, and the relative humidity of the air during the experiment *

Month	Temperature (°C)						The relative humidity of the air (%)		Rainfall (mm)	
	minimum		maximum		medium					
	2023	2024	2023	2024	2023	2024	2023	2024	2023	2024
April	+0.3	+2.0	+21.4	+31.4	+10.3	+15.2	74	59	43	33
May	+6.9	+7.9	+26.7	+27.7	+15.9	+16.8	73	66	53	32
June	+11.2	+13.5	+32.9	+36.3	+20.3	+25.4	75	53	116	47
July	+11.5	+14	+36.5	+39.6	+25.1	+27.4	62	48	57	18
August	+12.7	+16.2	+37.4	+38.2	+25.5	+27.0	54	43	28	10
September	+11.9	+7.4	+32.8	+34.4	+21.6	+20.3	56	55	21	30

*[https://m.rp5.ru/Vremea_%C3%AEn_Craiova_\(aeroport\)](https://m.rp5.ru/Vremea_%C3%AEn_Craiova_(aeroport))

Statistics

For each analysis, three to five measurements were performed, calculating the mean, standard deviation (SD) and coefficient of variability (CV %). Production results were statistically analyzed using three-way analysis of variance (ANOVA), MINITAB 22.2.2 Statistical Software (2025), and differences between means were evaluated by Tukey's test (p < 0.05).

RESULTS AND DISCUSSIONS

In this study, data on the variability of the main morphological characters in the considered grain genotypes are presented in Table 2. Thus, the measurements made at harvest regarding the plant height character indicate an amplitude of variation from 84.83 cm and 105.17 cm, the highest value was at Genotype 4 and the lowest

at Genotype 1. Regarding the value of the coefficient of variation, this was ≤ 8.62%, which indicates a low variability of this character. Vörösváry et al. (2011), in a study carried out in different ecological conditions of Transylvania (Romania), on grain germplasm, reported a variation in plant height from 43.9 cm to 62.9 cm, and Horaicu et al. (2023) reported for the eastern area of Romania, variation from 58.33 cm to 86.33 cm. This variability in plant height may be due to genotypes or agro-ecological zones. Regarding the number of stems/plant, a number between 3-4 stems/plant is found, and the coefficient of variation presents values indicating medium to high variability. Horaicu et al. (2023) reported a variation from 2.2 to 2.73 stems per plant.

The height of the first pod insertion (cm), determined from the ground to the first pod, indicates a good capacity of the genotype for mechanized harvesting and the reduction of production losses. Thus, among the 10 genotypes studied, those with the highest first pod insertion, over 14 cm, were Genotype 9, 3, 4, 6, 7 and 8. The coefficient of variability recorded values from 7.23% to 19.97%, indicating a low to medium variability.

Several authors have analyzed the variation of the main agronomic traits in some grain genotypes cultivated in different regions of Romania, finding differences between them in

terms of morphological and agronomic traits, and identifying, based on this, genetic resources with valuable potential for the selection and improvement of this crop can be identified (Vörösváry et al., 2011; Barcanu et al., 2023).

In the present study, the number of pods/plant showed a range of variation from 11 pods/plant (Genotype 5) to 18 pods/plant (Genotype 10), and the coefficient of variation ranged from 7.23% to 17.72%, indicating a low to medium variability. Horaciu et al. (2024), for the genotypes studied in eastern Romania reported range of variation was from 4.87 to 10.6 pods/plant.

Table 2. Variability of morphological production characters in *Vicia faba* genotypes

Genotypes	Plant height (cm)		Stems/plant (no)		Height to first pod (cm)	
	Mean $\pm$ SD	CV (%)	Mean $\pm$ SD	CV (%)	Mean $\pm$ SD	CV (%)
Genotype 1	84.83 $\pm$ 5.46	6.43	3.67 $\pm$ 0.82	22.27	12.33 $\pm$ 1.63	13.24
Genotype 2	97.17 $\pm$ 6.91	7.11	3.30 $\pm$ 0.82	24.49	12.83 $\pm$ 2.56	19.97
Genotype 3	94.33 $\pm$ 5.75	6.10	2.83 $\pm$ 0.75	26.57	14.50 $\pm$ 1.38	9.51
Genotype 4	105.17 $\pm$ 7.19	6.84	2.60 $\pm$ 0.52	19.36	16.50 $\pm$ 2.35	14.21
G5-Valcea 2	96.50 $\pm$ 4.46	4.62	4.00 $\pm$ 0.89	22.36	10.83 $\pm$ 1.17	10.79
Genotype 6	104.67 $\pm$ 4.50	4.30	4.33 $\pm$ 0.52	11.92	17.17 $\pm$ 0.98	5.73
Genotype 7	93.17 $\pm$ 8.04	8.62	3.33 $\pm$ 1.03	30.98	18.67 $\pm$ 1.75	9.38
Genotype 8	99.50 $\pm$ 5.72	5.75	4.17 $\pm$ 0.41	9.80	16.33 $\pm$ 1.51	9.22
Genotype 9	104.83 $\pm$ 5.31	5.06	2.67 $\pm$ 0.52	19.36	14.50 $\pm$ 1.05	7.23
Genotype 10	96.67 $\pm$ 6.65	6.88	3.83 $\pm$ 0.75	19.64	13.00 $\pm$ 1.26	9.73

Seed weight/plant (g), an important indicator of productivity, ranged from 25.17 g/plant (Genotype 8) to 54.17 g/plant (Genotype 6), and the coefficient of variation ranged from 2.16 % to 13.61 %, indicating low to medium variability. Mass of 100 dry seeds (g) showed a difference between the genotypes studied. Thus, the amplitude of variation ranged from 97.83 g

to 129.17 g, and the coefficient of variation ranged from 2.27 % to 8.41%, indicating low variability (Table 3). Other authors reported that in central Romania, the 100-seed mass changed between 50-79.8 g (Vörösváry et al., 2011). Also, in Turkey, Yüce et al. (2022) reported values on 100-grain weight ranging between 48 and 214 g.

Table 3. Variability of production traits in *Vicia faba* genotypes

Genotypes	No. of pods/plant		Seeds/plant (g)		Mass of 100 dry seeds (g)	
	Mean $\pm$ SD	CV (%)	Mean $\pm$ SD	CV (%)	Mean $\pm$ SD	CV (%)
Genotype 1	16.33 $\pm$ 2.16	13.23	36.00 $\pm$ 4.90	13.61	102.83 $\pm$ 4.07	3.96
Genotype 2	13.67 $\pm$ 2.42	17.72	41.33 $\pm$ 1.21	2.93	114.50 $\pm$ 9.09	7.94
Genotype 3	16.67 $\pm$ 2.42	14.53	43.33 $\pm$ 2.42	5.59	110.00 $\pm$ 9.21	8.37
Genotype 4	14.17 $\pm$ 1.72	12.16	28.67 $\pm$ 3.83	13.36	98.67 $\pm$ 5.96	6.04
Genotype 5	11.00 $\pm$ 1.79	16.26	26.50 $\pm$ 2.17	8.18	118.33 $\pm$ 3.27	2.76
Genotype 6	16.17 $\pm$ 1.17	7.23	54.17 $\pm$ 1.17	2.16	129.17 $\pm$ 2.93	2.27
Genotype 7	15.50 $\pm$ 2.07	13.38	42.00 $\pm$ 2.97	7.06	115.83 $\pm$ 1.72	1.49
Genotype 8	8.33 $\pm$ 1.37	16.40	25.17 $\pm$ 2.93	11.63	97.83 $\pm$ 8.23	8.41
Genotype 9	14.50 $\pm$ 1.87	12.90	28.83 $\pm$ 2.32	8.03	98.67 $\pm$ 7.87	7.97
Genotype 10	18.00 $\pm$ 1.41	7.86	41.67 $\pm$ 1.63	3.92	110.17 $\pm$ 7.47	6.78

The results obtained regarding the yield of the studied genotypes are presented in Figure 1. Thus, the production of faba bean showed significant differences between genotypes; the highest production was obtained in Genotype 2, at 3378.7 kg/ha, followed by Genotypes 10 (3277 kg), 7 (3272 kg/ha), 3 (3269 kg/ha) and 1 (3010 kg/ha). It is observed that there are significant differences between the studied genotypes ($p < 0.05$). The lowest production was obtained in Genotype 8, at 1957 kg/ha. Other authors have reported that, under the conditions of the NE of the country, production depending on the genotype ranges between 2792.30 kg/ha

and 4179.82 kg/ha (Horaciu et al., 2024), and that, under the conditions of the Timiș Plain, production was higher in grain crops, ranging between 2994 kg/ha, without nitrogen fertilization and 4876 kg/ha with nitrogen fertilization (Țigriș et al., 2020). This variability in production is influenced by the genotype, the cultivation area, and the technology applied. It can be noted that the species grows in different climatic conditions, ensuring good productivity. By Barilli et al. (2025) grain yield can be positively influenced by rainfall and negatively affected by high temperatures.



Means that do not share a letter are significantly different according to the Tukey test ($p < 0.05$).

Figure 1. Dry seed production (kg/ha) of the studied genotypes

CONCLUSIONS

For *Vicia faba* cultivation, it is important to manage abiotic stress and to estimate its behavior in different cultivation areas in order to choose favorable genotypes. Under the conditions of South-West Romania, the studied faba bean genotypes showed good production stability across the cultivation years, recording productions ranging between 3010 kg/ha and 3378.7 kg/ha. These genotypes collected from different climatic zones, can be new genetic resources with valuable potential for breeding programs. In our country, there is low biodiversity, with a small assortment of cultivated varieties. More frequently, some local populations are found in traditional culture, but characterized by a wide fluctuation in production. Considering the adaptation potential, the lower temperature requirements,

and the agrotechnical and food importance of this species, the genotypes with high production will be studied further, and those with good adaptability for this area of the country will be recommended.

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WHITE CABBAGE CULTURE IN ROMANIA - STATUS, CHALLENGES, TRENDS AND OPPORTUNITIES

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Abstract

White cabbage (Brassica oleracea var. capitata) is a significant crop among the Brassicaceae family cultivated in Romania, with implications for local livelihoods and the regional economy. The study aims to comprehensively analyse the situation of this crop in Romania through important statistical indicators, such as cultivated areas, total production, and average price over a 10-year period (2015-2024). Analysing these indicators, it can be appreciated that the areas cultivated with white cabbage in Romania were larger in the first part of the interval, with a constant downward trend, from 48.718 ha in 2015 to 36.662 ha in 2024, decreasing by 24.6% over this period. Total production exhibits the same downward trend, reflected by values starting from 1.077.978 t in 2015, reaching 565.532 t in 2024 (47.5% decrease). Regarding the average price of early white cabbage and autumn in Romania, it had an increasing trend: in 2015 recorded a value of 1.33 lei/kg, reflecting an increase of 129% and reached 3.05 lei/kg in 2024 and respectively, from 1.83 lei/kg to 3.42 lei/kg, meaning an increase of 86.88%. To increase production, it is necessary to improve cultivation technology and promote the development of the sector.

Key words: *Brassica oleracea, cultivated area, production, growth rate.*

INTRODUCTION

Cabbage (*Brassica oleracea* var. *capitata*) is a species of the *Brassicaceae* family and is an important crop worldwide, valued for its nutritional and economic value. It is a biennial, herbaceous, dicotyledonous species that forms short stems with a rich mass of leaves around a central bud with closed growth in the first year, and forms seed heads in the second year. This species is a rich source of vitamins and essential minerals, such as iron, calcium, magnesium, potassium, sodium, and phosphorus, vitamin C, fiber, and antioxidants (Šamec et al., 2017; Soare et al., 2017; Nawaz et al., 2018; Băbeanu et al., 2020; Bytyçi et al., 2025). Consuming nutritious foods rich in antioxidants increases the body's resistance to free radicals and, therefore, helps protect against chronic diseases (Băbeanu et al., 2017; Ștefan and Ona, 2020), the potential health benefits depend on several factors, including the technology applied, including genotype (Soare et al., 2016).

The main cabbage producing countries worldwide are China, with an area of 998,448 ha, followed by India, with 433,000 ha, resulting in a global production of cabbage and brassicas

in China of approximately 36 million tons and 10 million tons in India in 2023. In the European Union, the main white cabbage producing countries are Poland and Germany, Romania and the Netherlands (Soare, 2025). Poland reported a production of 666.2 thousand tons in 2023, and Germany 660.6 thousand tons (Faostat, 2025). At the global level, China and India represent some of the largest consumer markets (Statista, 2025).

Maintaining stable crop yields is crucial for sustainable agriculture (Hou and Chang, 2025). Cabbage production faces significant challenges due to climate change, disease and pest attacks, or soil salinity (Prajapati et al., 2024). The success of the crop depends on a series of correctly applied cultural practices. The hybrids used in the crop have different requirements for climatic conditions. Obtaining cabbage hybrids with tolerance to biotic and abiotic stress is crucial to increase the productive capacity of this crop.

White cabbage cultivation is practiced seasonally, in three periods: early crop, summer crop, and autumn crop. For this, cultivating the same hybrid regardless of the season, throughout the year, should be avoided.

Cultivation objectives should take into account the destination of production: for fresh consumption, pickling, storage, or processing (Cervenski et al., 2022).

Crop improvement relies on new breeding techniques such as tissue culture, somatic hybridization, molecular-assisted breeding, genetic engineering, RNAi, genome editing, methods that have the potential to address the challenges of improving crop production and abiotic stress tolerance (Jabeen et al., 2024). In addition, optimal agroecological conditions, access to inputs, strong market demand, and the presence of multifunctional agricultural cooperatives can be favorable environments for increasing production and commercialization of head cabbage (Ayalew et al., 2025).

In Romania, as in many other countries, white cabbage is one of the most important vegetable crop species (Stoleru et al., 2012). It is cultivated in the field (early, summer, and autumn crops) and in protected areas. By cultivating this species in protected areas, production is obtained three weeks earlier than in the early crop practiced in the field, when the production is marketed at advantageous prices. Environmental conditions, such as temperature, have a significant influence on production. Along with water, nutrients are an essential factor in obtaining high production, since cabbage is considered a species with high specific consumption. The use of microbial sources in nutrition is an alternative to conventional agricultural methods; by incorporating them, resistance to biotic stress increases, and plant production and quality improve (Al-Khayri and Khan, 2024; Iancu et al., 2024).

The economic importance of this species is linked to the high profitability of the crop due to the large volume of production and consumption. This study followed the evolution of the white cabbage crop in Romania, in terms of cultivated areas, total production, and average price, during the period 2015-2024.

MATERIALS AND METHODS

To carry out the research, data were collected from the National Institute of Statistics for the period 2015-2024, regarding the following indicators: cultivated areas, total production,

average monthly consumption of cabbage and cauliflower/person, quantity purchased by a household (cabbage and cauliflower) and the average selling price of white cabbage grown in Romania. The data were processed in Excel, calculating descriptive statistics, such as mean, standard deviation, coefficient of variation, and annual growth rate.

The standard deviation indicates how much the values are dispersed from the mean. The coded indicators used in the presentation of the descriptive statistics are: σ = standard deviation; x = values of the data series over a number of years; n = number of years considered.

Coefficient of variation: $C = \sigma / \bar{X} * 100$, with the following interpretation: between 0-10% indicates a higher degree of homogeneity of the series; between 10-20% is medium variation; over 20% - high variation.

Annual growth rate indicates the annual increase of the analysed indicator: $R (\%) = (\text{Ending Value} \div \text{Beginning Value}) ^ { (1 \div \text{Number of Periods}) } - 1$.

RESULTS AND DISCUSSIONS

White cabbage is a species that presents high ecological plasticity, being adapted to environmental conditions with a temperate climate and cultivated in Romania throughout the country. Table 1 presents statistical data on the cultivated areas and production of white cabbage in Romania during the period 2015-2024. Thus, the cultivated area during this period ranged between 36,557 ha in 2022 and 48,718 ha in 2015. Larger areas cultivated with white cabbage were recorded in the first part of the studied interval, but in the last years of the analysed series the indicator had a downward trend. The same trend was followed by total production, with the recorded values being higher in the first part of the interval, starting from 1,077,978 t in 2015 and reaching 565,532 t in 2024, showing a significant decrease in production compared to 2015. Regarding the productivity of white cabbage in our country during the period 2015-2024, it is observed that it was oscillating, with a downward trend in the latter part of the interval, starting from 22,126 kg/ha in 2015, reaching 24,324 kg/ha in 2020 and decreasing to 15,425 kg/ha in 2024.

Table 1. Cultivated areas, total production, and yield per unit area of cabbage crops in Romania

Indicators	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024
Area (ha)	48,718	46,248	46,200	47,330	47,153	37,626	37,966	36,557	36,720	36,662
Production (tons)	1,077,978	992,398	1,026,575	1,065,537	985,842	915,246	919,961	689,704	643,316	565,532
Yield (kg/ha)	22,126	21,458	22,220	22,512	20,907	24,324	24,231	18,866	17,519	15,425

Source: INS Tempo, accessed 29.11.2025

Table 2 presents the descriptive analysis of the areas and production of cabbage in the period 2015-2024. Thus, the average cultivated area was 42,118 ha, and the standard deviation was 5,344.20. The coefficient of variation (%) recorded a value of 12.6%, indicating an average variability from one year to another. The average total production was 1,077,978 kg/ha, and the standard deviation was 186,257.48 and the coefficient of variation was 20.9%, indicating average variability. Compared with 2015, cultivated areas and total production decreased by 24.7%, respectively 47.5%, in 2024. Annual growth rate (%) was -2.8% in terms of areas cultivated with white cabbage and -6.24% in terms of total production obtained. It can be appreciated that the negative trend in the case of the indicator regarding production in the period 2015-2024, in Romania was conditioned first of all by the level of cultivated areas, which is decreasing. Also, the impact of climate change has influenced cabbage production in recent years, as precipitation and atmospheric humidity have been low, factors to which white cabbage has high requirements.

Another important factor in the stability of production and prices is the organization of agricultural production through producer organizations, cooperatives, or through interprofessional organizations (Alboiu et al., 2022), but many private producers in Romania hesitate to join such organizations for various reasons. Also, growing conditions and genotype are the predominant factors in obtaining high yields (Dinu et al., 2024).

The lower levels of area and production of white cabbage cultivated in Romania have led the Ministry of Agriculture and Rural Development (MADR) to introduce financial support for producers to support the production of vegetables cultivated in protected areas, including white cabbage, in the period 2025-2027 (madr.ro, 2025). The financial support for de minimis aid is 1.5 euros/sqm, equivalent to 1,500 euros/1,000 sq m, for a cumulative area of at least 1,000 sq m. In Romania, vegetable production should increase to better cover the needs of the domestic market and support exports to the EU market (Popescu, 2013).

Table 2. Descriptive statistics regarding the area and total production of cabbages in Romania in the period 2015-2024

Indicator	2024/2015 (%)	MIN.	MAX	Average	Standard deviation	Coefficient of variation (%)	Annual growth rate (%)
Area (ha)	-24.7	36557	48718	42118	5344.20	12.6	-2.8
Production(tons)	-47.5	565532	1077978	888208.9	186257.48	20.9	- 6.24

Source: INS Tempo, accessed 29.11.2025

Table 3 presents the average monthly consumption of cabbage and cauliflower/person and the quantity purchased by a household in Romania over the last 10 years. Both the average monthly consumption of cabbage and cauliflower/person and the quantity purchased by a household (cabbage and cauliflower) have recorded continuous fluctuations during this period, pointing to a similar pattern. Thus, the average monthly consumption of cabbage and cauliflower/person was between 0.781 kg in

2015 and 0.924 kg in 2023, indicating an oscillating trend, with a slight increase starting in 2021 and then a slight decrease in 2024 (Table 3). This aspect may be influenced by the volatility of market prices, which were higher during this period. Regarding the quantity purchased by a household (cabbage and cauliflower), the values ranged between 0.91 kg in 2015 and 1.043 kg in 2023, the values fluctuating from one year to another (Table 3).

Table 3. Average monthly consumption of cabbage and cauliflower/person and quantity purchased by a household

Year	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024
Average monthly consumption/person (cabbage and cauliflower) - kg	0.781	0.807	0.857	0.823	0.806	0.836	0.855	0.874	0.924	0.9
Quantity purchased by a household (cabbage and cauliflower) - kg	0.91	0.94	1.042	0.945	0.928	1.027	1.017	0.978	1.043	0.981

Source: INS tempo, accessed 29.11.2025

The descriptive analysis of these indicators regarding the consumption and quantity of cabbage purchased is presented in Table 4. Thus, the mean of the indicator regarding average monthly consumption/person (cabbage and cauliflower) was 0.8463 kg, with 0.044 standard deviation, while the coefficient of variation was 5.2%, indicating low variability from one year to another, which presents a relatively stable behaviour in the food preferences of the population regarding cabbage consumption. Regarding the quantity purchased

by a household (cabbage and cauliflower), the average value recorded the value of 0.9811 kg with 0.049 standard deviation, and the coefficient of variation was 5.01%, which indicates low variability from one year to another.

However, compared with 2015, the average monthly consumption of cabbage and cauliflower/person and the quantity purchased by a household increased by 15.23% and 7.8%, respectively, in 2024. The annual growth rate was 1.42% and 0.75%, respectively.

Table 4. Descriptive statistics regarding consumption of cabbages in Romania the period 2015-2024

Indicator	2024/2015 (%)	Min.	Max.	Average	Standard deviation	Coefficient of variation (%)	Annual growth rate (%)
Average monthly consumption/person (cabbage and cauliflower) - kg	15.23	0.781	0.924	0.8463	0.044	5.2	1.42
Quantity purchased by a household (cabbage and cauliflower) - kg	7.8	0.91	1.043	0.9811	0.049	5.01	0.75

The average price/year for early white cabbage registered an oscillating trend, with the minimum value in 2016, at 1.24 lei/kg, and the maximum value in 2024, at 3.05 lei/kg (Table 5). The average price per year for autumn white cabbage also registered an oscillating trend, with a variation amplitude from 1.35 lei/kg in 2017 to 3.42 lei/kg in 2024. This price instability

prevents farmers from obtaining a stable income; therefore, and for this it is necessary to forecast the price for the harvest season so that they can make timely adjustments regarding the cultivated areas.

The selling price, in general, is influenced by supply and demand, as well as by the sales period.

Table 5. Selling price of white cabbage in Romania, 2015-2024

Indicator	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024
Average price/year early white cabbage (lei/kg)	1.33	1.24	1.47	2.69	2	1.93	2.6	2.91	2.88	3.05
Average price/year autumn white cabbage (lei/kg)	1.83	1.41	1.35	1.84	1.75	1.66	2.22	2.31	2.47	3.42

Source: INS Tempo, accessed 29.11.2025

Table 6 presents the descriptive statistics analysis regarding prices for cabbages in Romania in the period 2015-2024. The average price/year for early white cabbage (lei/kg) was 2.21 lei/kg, with 0.70 standard deviation, while the coefficient of variation was 31.16%, indicating very high variability from one year to another. The average price/year for autumn

white cabbage was 2.02 lei/kg, with 0.61 standard deviation, and the coefficient of variation was 30.1%, indicating very high variability from one year to another. Compared to 2015, in 2024, the average price/year for early white cabbage and the average price/year for autumn white cabbage increased by 129% and 86.88%, respectively.

Table 6. Descriptive statistics regarding prices for cabbages in Romania in the period 2015-2024

Indicator	2024/2015 (%)	Min.	Max.	Average	Standard deviation	Coefficient of variation (%)	Annual growth rate (%)
Average price/year early white cabbage (lei/kg)	129	1.24	3.05	2.21	0.70	31.6	8.6
Average price/year autumn white cabbage (lei/kg)	86.88	1.35	3.42	2.02	0.61	30.1	6.4

Annual growth rate (%) regarding the average price for both early cabbage and autumn cabbage was 8.6% and 6.4% respectively. This growth rate may be influenced by higher costs of agricultural inputs and rising energy prices. According to Milin et al. (2025), prices are influenced by seasonality, supply reduction, and changes in consumer preferences.

CONCLUSIONS

In Romania, white cabbage is one of the most cultivated vegetable species. Romania is an important producer in the European Union, according to FAO. During the period 2015-2024, the evolution of cabbage cultivation in Romania, in terms of cultivated areas and total production, has shown a downward trend, while the average selling price has shown an upward trend. The reasons for the decrease in areas and production can be both economic, such as fluctuations in selling prices, higher costs of inputs and energy, etc., and technological, namely: climate change, the attack of diseases and pests, or the use of inadequate technology. To increase production and cultivated areas of white cabbage, it is necessary to choose hybrids with good adaptability to temperature variations, resistant to heat, with easy access to irrigation, farmer training programs on the selection of fertilizers for the development of sustainable and innovative technologies, and the development of strategies to mitigate production risks.

A possible way to mitigate these problems would be to increase the level of information available to farmers regarding supply and demand and market trends, increase the level of association in cooperatives or other organizations to strengthen links with the market, conclude firm contracts, and provide predictability regarding cultivated areas and production costs.

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EVALUATION OF UNSTABLE PHENOTYPIC COMMON BEAN SEEDS: A CASE STUDY

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Abstract

In a normal crop, beans are self-pollinating. The aim of this study was to present the effects of cross-pollination within a bean germplasm collection (over 450 accessions) under climatic conditions from the south-eastern part of Romania. This paper presents the study of L 47 AA climbing bean accession seeds sown, and there were taken into study nine new biotypes harvested. The seeds represented the control variant for the analysis of the harvested biotypes. The seeds were evaluated in what it concerns quantitative characteristics (100 seeds weight, length, width, and thickness) and qualitative characteristics (shape, primary and secondary seed color, shape of the secondary color, intensity and color of the hilum ring). The mean weight of 100 seeds was 42.52 g (with a minimum value of 31.75 g and a maximum value of 55.92 g). At biotype 5 the maximum values of the quantitative characteristics were recorded (weight - 55.92 g, length - 14.93 mm, and thickness - 6.38 mm). A similar coloration to the control was found in biotype 9 (primary color brown, secondary color black in streaks), but white or cream colors were also observed.

Key words: seeds, *Phaseolus vulgaris* L., germplasm collection, qualitative characteristics, quantitative characteristics.

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is one of the most widely consumed legumes globally, valued for its high protein content, dietary fiber, and essential micronutrients like iron and zinc. As a staple food in many regions, particularly Africa, Latin America, and Asia, it is crucial for improving food security and addressing malnutrition (Fayaz, 2025).

In Romania, there are favorable conditions for common bean production, as well as a great tradition in cultivation (Ruști and Munteanu, 2008). Collecting and conserving biodiversity, in what it concerns bean, represents one of the main activity objectives for the Vegetable Research and Development Station (V.R.D.S.) Buzău.

Thus, in 2010 started the collection of local bean population from the main vegetable-growing areas of Romania. The bean germplasm collection of V.R.D.S. Buzău contains over 450 accessions.

Similar studies were conducted in Romania by Rădulescu, 1940; Munteanu, 1985; Stan et al., 1993,1995; Leonte et al., 2004; Riviș and Nedelea, 2008; Giurcă and Murariu, 2009; Danci et al., 2010 and Madoșa et al., 2010, 2011.

Normally, in Romania, the bean (*Phaseolus vulgaris* L.) is considered a self-pollinated plant. Ali et al. 2020 also share the same view. Olaru, 1982 and Ciofu et al., 2003 agree with Ali et al., 2020, but they do not exclude cross-pollination. In a normal crop, Bliss, 1980 and Ruști and Munteanu, 2008 estimate that bean is highly self-pollinated, with only 0.2-0.5% cross-pollination. In Puerto Rico, Vakili (1976), quoted by Bliss (1980), reports that carpenter bees (*Xylocopa brasiliatorum* L.) were responsible for levels as high as 15 to 20% cross-pollination.

In what it concerns some accessions, over time, the phenotypic instability phenomenon has been more intensive because of some particularities concerning flower (Figure 1) morphology (Debouck and Hidalgo, 1986).

Studying flower characteristics in accessions that presented a high variability, it was observed that the stigma and anthers were no longer protected by the keel.

Under the pressure of different insects (especially bees and thrips) the stigma get out of the keel and makes contact with the full of pollen bees' feet from another flower, or it detaches and leaves the stigma and the anthers free (Figure 2).

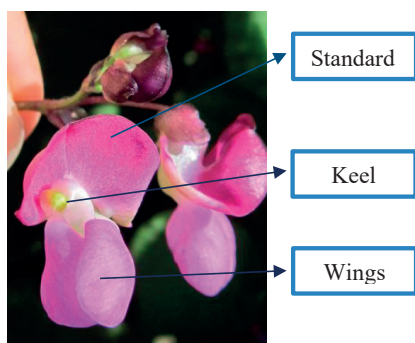


Figure 1. Bean flower morphology (about Teodorescu, 2016)

The turning over inside the keel is prevented by the brush of hairs near the stigma and also because of the style curvature.

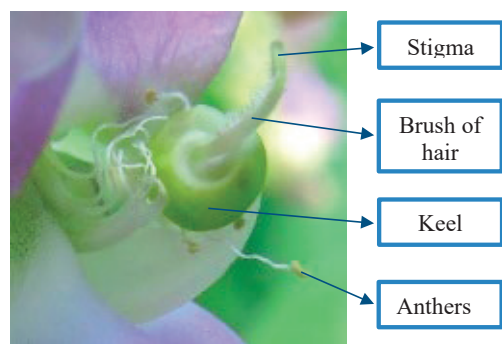


Figure 2. Stigma and anthers were no longer protected by the keel (about Teodorescu, 2016)

At the proveniences that present such flower morphology, pollination is allogamy and can be made by insects - entomophily and by wind - anemophily (Teodorescu, 2016).

The aim of this paper was to present the effects of cross-pollination within a bean germplasm collection under climatic conditions from South - eastern part of Romania.

MATERIALS AND METHODS

The study was carried out in V. R. D. S. Buzău, Romania (45°9'N and 26°49'E) in 2020.

The biological material (climbing accessions) was cultivated according to the common production technology recommended for this area by the specialty literature (Munteanu et al., 1989; Ciofu et al., 2003; Ruști and Munteanu, 2008).

Therefore, the experimental variants were the following:

Mt - 47 AA	5 - 47 AAI
1 - 47 AAA	6 - 47 AAJ
2 - 47 AAB	7 - 47 AAK
3 - 47 AAE	8 - 47 AAL
4 - 47 AAF	9 - 47 AAM

This germplasm collection was cultivated in green - house covered with polyethylene. The greenhouse's ventilation space permitted access of insects.

The determination of plants, pods, and seeds characteristics was made according to the U.P.O.V. guideline (2015), the C.P.V.O. protocol (2009) and the color scale (Genchev and Kiryakov, 2005). For characterization, there were used 10 seeds from each accession, according to the *Handbook on evaluation of Phaseolus germplasm* (De la Cuadra et al., 2001).

Length determination was made in mm, measured in parallel with the hilum; width was measured from the hilum to the opposite side, and thickness was measured perpendicular to width in cross section (Figure 3).

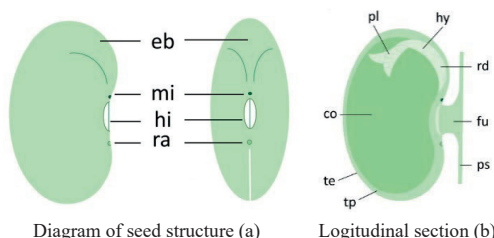


Figure 3. Seed Structure of a Dicotyledon (a): eb – embryo bulge; mi – micropyle; hi – hilum; ra – raphe; and (b): pl – plumule; hy – hypocotyl; rd – radicle; co – cotyledon; fu – funicle; ps – pod; te – testa; tp – testa perenchyma (about Chen et al. 2021)

A more detailed overview of the plumule–radicle complex includes the epicotyl and primary leaves (Debouck and Hidalgo, 1986). Rădulescu (1940) quoted by Olaru (1982) pays particular attention to the coloring area around the hilum, otherwise, the hilum is always white. The edge surrounding the hilum may have a different coloration, with various intensities (absent, weak, medium, and strong) than the rest of the testa, and it is also interrupted at the micropyle and caruncle.

Caruncle (raphe) represents a heart – shaped lift with the tip facing the end of the seed. The stripe of the caruncle represents a narrow band that

leaves from the top of the caruncle, it extends to the end of the seed – were it ends like a swallow's tail, and it has a variable length. (Figure 4).

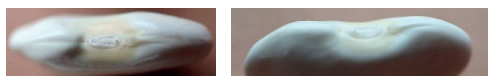


Figure 4. Aspects of the embryo bulge, micropyle, hilum, caruncle, and the stripe of the caruncle (about Teodorescu, 2024)

According to the Descriptor for *Phaseolus vulgaris* (IBPGR, 1982), van Schoonhoven A. and Pastor - Corrales (1994) and Debouck D. (2009) 100 seed mass were randomly chosen for measuring seed mass (small - less than 25 g, medium - 25 g to 40 g, and large - more than 40 g). In order to determine seed weight there was used an electronic balance reading accuracy of 0.0001 g (Partner WAS220/x).

The experimental design was a randomized block design with 3 replicates for each variant. The seeds represented the control variant for the analysis of the harvested biotypes. In order to analyze the results, analysis of variance and multiple comparison methods, using Duncan's test, were used (Harter, 1960; Săulescu and Săulescu, 1966).

There was used coefficient of variation (CV) because it allows the direct comparison of data set variation (Ireland, 2010). The coefficient of variation in a single sample with observations is defined as $CV = s/m$, where m is the mean and s is the standard deviation (Forkman, 2009). Variability assessment according to the CV values (Munteanu and Fălticeanu, 2008; Giurcă and Murariu, 2009) was made as follows: low variability ($CV < 10\%$), mean ($CV = 10-20\%$) and high ($CV > 20\%$).

RESULTS AND DISCUSSIONS

Most of the accessions cultivated presented a high degree of instability. The appearance of a large number of biotypes represents the result of the presence of numerous insects in the crop (Figure 5).

From the first phases of vegetation, it was observed differences in the color of the hypocotyl (Figure 6, a).

Prior to flowering, three biotypes were observed in the culture (Figure 6, b): a green shoot that

later produced white flowers, a shoot with strong anthocyanin coloration that resulted in violet flowers, and a shoot with intermediate coloration.



Figure 5. Insects present in the crop and flowers after their visit (Teodorescu, 2024)

This paper presents quantitative (100 seed weight, length, thickness, and width) and qualitative (shape, seed color, veining, and hilum ring – intensity and color) characteristics of seeds harvested from 9 biotypes in comparison with seeds sown.

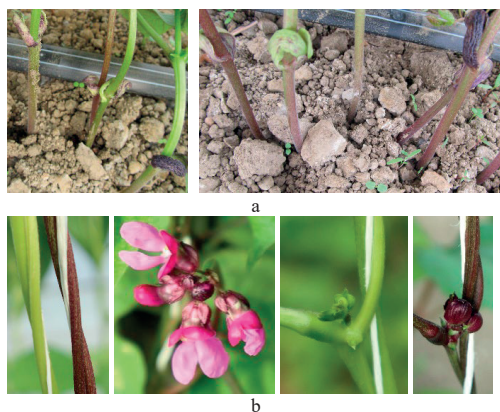


Figure 6. Aspects from culture: a. Different hypocotyl colorations and b. Differences between shoots and flower buds

A medium mass (between 25 and 40 g) had four variants ($V_8 - 31.75$ g, $V_1 - 34.05$ g, $V_2 - 34.47$ g and $V_7 - 36.32$ g), and the other six variants, including the control variant ($V_5 - 55.92$ g, $Mt - 53.93$ g, $V_3 - 46.68$ g, $V_9 - 44.59$ g, $V_4 - 44.27$ g and $V_6 - 43.18$ g) had a large mass - greater than 40 g (Table 1).

Table 1. Main quantitative characteristics of the seeds

Variants	100 seeds mass (g)	Seeds dimensions (mm)			Length/Width	Width/Thickness
		Length	Width	Thickness		
Mt	53.93 a*	14.15 ab	8.46 a	5.98 ab	1.67	1.42
V ₁	34.05 c	14.20 ab	7.43 b	4.06 d	1.91	1.83
V ₂	34.47 c	13.92 bc	6.88 c	4.99 c	2.02	1.38
V ₃	46.68 b	13.38 c	8.28 a	5.91 ab	1.62	1.40
V ₄	44.27 b	12.21 d	8.18 a	6.20 a	1.49	1.32
V ₅	55.92 a	14.93 a	8.12 a	6.38 a	1.84	1.27
V ₆	43.18 b	12.25 d	8.42 a	5.91 ab	1.46	1.42
V ₇	36.32 c	14.27 ab	7.37 b	5.05 c	1.94	1.46
V ₈	31.75 c	13.19 c	6.47 c	5.62 b	2.04	1.15
V ₉	44.59 b	12.45 d	8.26 a	6.38 a	1.51	1.29
Mean	42.52	13.50	7.79	5.65	1.75	1.39
Stand. var.	8.34	0.95	0.70	0.74	0.23	0.18
CV %	19.61	7.06	9.03	13.15	12.95	12.79
Max.	55.92	14.93	8.46	6.38	2.04	1.83
Min.	31.75	12.21	6.47	4.06	1.46	1.15

LSD 5% = 5.2191

LSD 5% = 0.7293

LSD 5% = 0.4243

LSD 5% = 0.4742

LSD 1% = 7.1576

LSD 1% = 1.0002

LSD 1% = 0.5818

LSD 1% = 0.6503

LSD 0.1% = 9.7423

LSD 0.1% = 1.3614

LSD 0.1% = 0.7920

LSD 0.1% = 0.8851

*Different letters between variants denote significant differences (Duncan's test, $p < 0.05$).

The coefficient of variability was medium for two parameters (weight - 19.61% and thickness - 13.15%) and low for the other two parameters studied (length - 7.06% and width - 9.03%).

Seed length ranged between 12.21 mm (V₄) and 14.93 mm (V₅), with no significant differences recorded among the first four values (V₈ - 14.27 mm, V₁ - 14.20 mm, Mt - 14.15 mm and V₂ - 13.92 mm).

The highest width value was observed in the control variant (8.46 mm), while significant

differences were noted in four of the variants (V₁ - 7.43 mm, V₇ - 7.37 mm, V₂ - 6.88 mm and V₈ - 6.47 mm).

The average thickness was 5.65 mm, with a maximum value of 6.38 mm (V₅) and a minimum of 4.06 mm (V₁); significant differences were recorded compared to the last two variants (V₂ - 4.99 mm and V₇ - 5.05 mm).

The same elliptic shape as the control variant (Table 2) was found in V₅ and three biotypes had a circular to elliptic shape (V₄, V₆ and V₉).

Table 2. Main qualitative characteristics of the seeds

Variants	Seeds shape ¹	Color			Hilum ring	
		Main	Secondar	Shape of secondary	Intensity	Color
Mt	elliptic	brown	black	in streaks very fine patches	strong	black
V ₁	kidney s ²	white	—	—	absent	—
V ₂	kidney m	red	—	—	absent	—
V ₃	kidney w	beige	gray brown	very fine patches in fine patches	medium	beige
V ₄	circular to elliptic	reddish brown	brown	very fine patches	weak	reddish brown
V ₅	elliptic	reddish brown	brown	in fine patches	absent	—
V ₆	circular to elliptic	L brown	black	in streaks in patches	weak	reddish brown
V ₇	kidney w	L brown	black	in streaks in patches	medium	black
V ₈	kidney s	brown	black	in streaks very fine patches	strong	black
V ₉	circular to elliptic	brown	black	in streaks very fine patches	strong	black

¹Shape of median longitudinal section L=light D=dark²Degree of curvature for kidney only: weak (w), medium (m) and strong (s)

According to U.P.O.V. guideline (2015), the C.P.V.O. protocol (2009) and also to the color

scale (Genchev and Kiryakov, 2005), for accessions with kidney – shaped seed shape, it

is recommended to evaluate also the degree of curvature (weak - V₃, V₇, medium - V₂ and strong - V₁, V₉).

The main color brown was found in the control variant, V₈ and V₉, light brown in V₆ and V₇,

reddish brown in V₄ and V₅, red in V₂, beige in V₃ and white in V₁.

The secondary (Figure 7) color black was found in five variants (Mt, V₆ V₇, V₈ and V₉) and brown in two variants (V₄ and V₅).

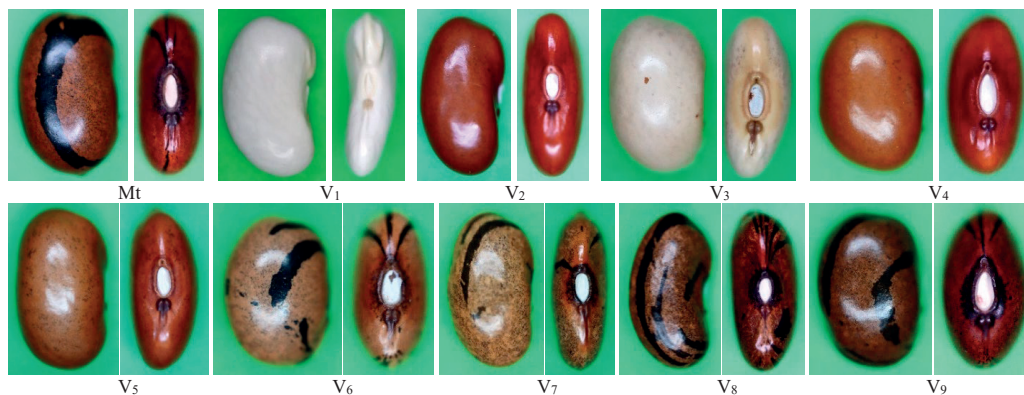


Figure 7. Aspects of qualitative characteristics of the seeds

A similar coloration to the control variant was found in V₉ (main color brown; secondary color black).

Only one color was found in V₁ and V₂. No secondary color was observed in V₁ and V₂, while V₃ exhibited two secondary colors (gray and brown).

The hilar ring is absent in three variants (V₁, V₂, and V₅). The caruncle of V₁ is off-white, and the vascularization is moderate.

At V₄ and V₆, the hilar ring is weak and reddish-brown in color.

A medium intensity of the hilar ring is found in V₃ and V₇. V₃ stands out due to the size and color of the caruncle (darker than the rest of the seed). The control variant, V₈ and V₉, are characterized by high intensity and a black color.

CONCLUSIONS

In what it concerns weight, the maximum values were registered at V₅ following the control variants.

Various intensities of anthocyanin coloration of the hypocotyl show that the sown seeds were uniform only from a phenotypic point of view. Under these conditions, the percentage of cross-pollination determined by insects cannot be specified.

A complete phenotypic characterization of bean seeds must include their quantitative and qualitative evaluation.

All biotypes will be cultivated to determine the characteristics of the plant. Only those that meet the breeding objectives will be kept and introduced into the germplasm collection.

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LANDRACE ELECTRONIC CATALOGUE – *EX SITU/ON FARM* CONSERVATION, DOCUMENTATION AND MANAGEMENT

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Abstract

Although the majority of agricultural production in Europe currently depends on formally registered, genetically uniform cultivars, landraces are still cultivated in home gardens. A comprehensive inventory of on-farm-maintained local varieties is currently lacking in Europe, resulting in limited and dispersed data regarding their cultivation locations and associated crops. The creation of such a catalogue is of considerable importance for promoting actions aimed at enhancing the conservation and utilization of landraces. The results showed that the highest number of landrace cultivation sites in Bulgaria are located in semi-mountainous rural areas and are represented mainly by horticultural species. During the period 2024-2026, progress has been made in creating the database of ex situ conserved landraces through activities conducted under the DIGIVALPGR project, funded by the Bulgarian Science Fund (Contract KP-06-Slovakia/9/13.08.2024). It includes information on over 100 local accessions, adding value to landraces in socio-cultural, environmental, and economic contexts.

Key words: plant diversity, genebank, digitalization, valorization, European collaboration.

INTRODUCTION

Global climate change and its adverse impact on agricultural production and biodiversity are creating serious socio-economic challenges and increasing the risks associated with natural disasters such as droughts and floods. These processes threaten natural resources and highlight the need to explore plant diversity in order to ensure food security and sustainable agricultural systems (FAO, 2022).

The global trend of decreasing agrobiodiversity poses a serious threat to the sustainability of agricultural production. Reduced genetic diversity negatively affects plant resistance to diseases and pests and limits the adaptive capacity of crops under climate change conditions, thereby affecting global food security (Raza et al., 2019).

Plant genetic resources represent the diversity of crops and their wild relatives, including local populations and forms, as well as old, traditional, and improved varieties. Their sustainable conservation for present and future generations is a key objective of the European Strategy for Plant Genetic Resources (ECPGR, 2021).

Landraces and traditional home gardens are increasingly recognized as important heritage

values and hotspots of biodiversity conservation. Studies show that traditional home gardens can preserve crop diversity for more than a century through the maintenance of local seed systems and farmers' knowledge (Jani et al., 2015; Antofie, 2024; Antofie et al., 2024; Lun, 2026).

To feed more than 9 billion people by 2050, agricultural production must significantly increase while ensuring environmental sustainability. Landraces possess valuable adaptive traits developed through long-term cultivation under specific environmental conditions. Their genetic diversity provides important resources for crop breeding and for the adaptation of agriculture to climate change (Kumar, 2026; Fowler, 2026).

Pedrali et al. (2026) highlighted the prospects for the valorization of *Phaseolus* spp. landraces in the mountain areas of Northern Italy. A rich diversity of common bean local varieties - an important traditional grain legume crop in Bulgaria, has also been reported. This diversity is expressed through various biological traits and is explained by the different geographical origins of the accessions (Neshev, 2025).

The risk of losing plant genetic diversity is increasing. Therefore, researchers are exploring

different seed technologies for the conservation and reproduction of horticultural crops (Shallal et al., 2020; Nicolae et al., 2023).

The management of *ex situ* collections includes activities such as collection, evaluation, documentation, and long-term storage of plant genetic resources. As a National Coordinator in Bulgaria, the Institute of Plant Genetic Resources - Sadovo maintains an information system documenting accessions preserved in the National Genebank.

However, information on landraces conserved *ex situ* and maintained *on farm* is often fragmented and dispersed. Digital tools and open access catalogues are essential for improving documentation, management, and utilization of plant genetic resources (Raggi et al., 2024; Maxted et al., 2025).

Therefore, the aim of this study is to develop and present an electronic catalogue of Bulgarian landraces documenting the enrichment of *ex situ* collections in the National Genebank in Sadovo and supporting their conservation, management, and sustainable utilization.

MATERIALS AND METHODS

The Information and Documentation Center at the IPGR-Sadovo was established in 1982. It is responsible for the digitalization of plant genetic resources entering the National Genebank. All accessions are registered in an electronic database according to the approved international FAO/Bioversity descriptors (2017; 2022).

Passport information includes the catalogue number, taxonomic description, accession name, date of registration in the collection, biological status, donor of the accession, ecological and geographical origin, and description.

During the period 2024-2026, collecting missions in rural areas of Bulgaria were conducted within the DIGIVALPGR project following the methodology of the European Cooperative Programme for Plant Genetic Resources (ECPGR, 2017). The seed accessions are donated by local farmers and conserved in the National Genebank (Figure 1). A total of 20 localities and 18 households were investigated. Local farmers were interviewed using a project questionnaire.



Figure 1. Collected landraces for enrichment of the Bulgarian genebank collection (February, 2026)

Open access to plant genetic resources is ensured through the participation of the National Inventory in international databases. The European Cooperative Programme for Plant Genetic Resources (ECPGR) requires a unified structure for building genebank databases to facilitate participation in the European Search Catalogue (EURISCO). In addition, the European Integrated Genebank System (AEGIS) provides access to conserved unique landraces.

RESULTS AND DISCUSSIONS

The climatic characteristics of Bulgaria differ from those typical of many continental European countries, as frequent and unpredictable natural anomalies are increasingly observed. In response to climate change, breeding programs are directed toward the development of new varieties that are well adapted to the specific agro-ecological conditions of the country. In this context, the importance of plant genetic resources, particularly landraces, is increasing, as they represent an invaluable source of useful genes for breeding programs.

Since 1982, a total of 11,539 landraces has been collected through various expeditions and research projects and conserved *ex situ* in the genebank in Sadovo. The collection includes local varieties and populations originating from home gardens and small farms, as well as crop wild relatives collected from their natural habitats.

The main characteristics of the digital registry for documentation of plant genetic resources in Bulgaria are presented in Table 1.

Table 1. Main features of the digital registry of landraces and database structure

Information resource	Database/electronic catalogue
Objective	Conservation and management of plant genetic resources in Bulgaria
Information format	GeneBank System
Content description	Documentation of accessions registered in the National Collection and stored in the National Genebank in Sadovo
Database structure	FAO/Bioversity International Descriptors
Data type	Passport, evaluation and characterization characteristics
Data language	Bulgarian, English, Latin (taxonomic descriptions)
Data update	Periodically
Collection period	1982-2026
Basis for collecting the information	National Biodiversity Conservation Strategy; Convention on Biological Diversity; International Treaty on Plant Genetic Resources for Food and Agriculture
Method of collecting and updating information	Database with information on landraces, received from expeditions; on-farm maintenance of old and forgotten varieties in different geographical regions of the country; marked and surveyed localities for <i>in situ</i> monitoring of crop wild relatives in the habitat area
Purpose of the information	Management of <i>ex situ</i> genebank collections; breeding programmes; international relations – FAO, Bioversity International/ ECPGR; transfer data to international networks – EURISCO, AEGIS, GENESYS, FAO WIEWS; partners including other genebanks, institutes, universities, and botanical gardens; all users
Dissemination and open access	https://genbank.uni-plovdiv.net/ https://eurisco.ipk-gatersleben.de/ https://www.ecpgr.org/aegis
Exchange of seeds	Bulgarian genebank is a partner in the global network for the free germplasm; the Standard Material Transfer Agreement is adapted (SMTA)
Users of the electronic catalogue	Breeders, researchers, authorities, organic producers, farmers, ecologists, botanists, etc.

One of the main directions of the expedition activities is the collection of cultivated forms of field and horticultural crops. In home gardens, traditional varieties of tomato, pepper, cucumber, pumpkin, melon, watermelon, onion, leafy vegetables, and potato have been identified. These genotypes are well adapted to specific agro-ecological conditions and possess valuable traits such as early maturity, resistance to biotic and abiotic stresses, and a high content of biologically active compounds.

For cereal and grain legume crops, particular attention is given to the collection of primitive wheat forms and old populations of maize, as well as traditional varieties of common bean, cowpea, chickpea, lentil, and other legumes.

Of particular interest is the species diversity of several poorly distributed or almost forgotten spice and medicinal plants, which are currently

being rediscovered and increasingly used for dietary and health-promoting purposes, as well as in alternative therapeutic practices for the prevention and treatment of various diseases.

However, increasing urbanization, the expansion of transport infrastructure, and other environmental pressures pose serious threats to a large number of crop wild relatives and other wild species belonging to different botanical families. Their conservation is important not only for maintaining biodiversity but also because they have valuable traits that could be utilized in crop improvement programs using innovative breeding approaches, as molecular and genomic techniques. The collected germplasm includes sources of high protein content, fertility enhancers, resistance to fungal, bacterial, and viral diseases, and high adaptive capacity in the context of global warming and climate change.

Cooperation and information exchange between researchers working on plant genetic resources and plant breeders play a key role in optimizing activities aimed at the sustainable use of genebank collections. Through the introduction of new varieties resulting from targeted breeding programs, as well as through the reintroduction of traditional and old varieties in agricultural holdings, the benefits of conservation activities ultimately reach their end users – the farmers, including organic producers.

In connection with the implementation of the activities within the DIGIVALPGR bilateral project, an inventory of rural areas in Bulgaria was conducted, accompanied by expeditions aimed at collecting landraces. As a result, the electronic register was enriched with 55 landraces collected from Bulgaria, 2 old Bulgarian cultivars, 3 traditional species originating from the Romanian flora, and 67 accessions introduced from the project partner – Slovakian genebank. In total, 127 new accessions belonging to 28 crop species were registered in the electronic catalogue, including: *Apium graveolens* – 1 acc.; *Avena sativa* – 2 acc.; *Calendula officinalis* – 1 acc.; *Capsicum annuum* – 14 acc.; *Cicer arietinum* – 4 acc.; *Citrullus lanatus* – 4 acc.; *Cucumis melo* – 2 acc.; *Cucurbita* sp. – 13 acc.; *Daucus carota* – 1 acc.; *Geranium* sp. – 1 acc.; *Hordeum vulgare* – 2 acc.; *Lactuca sativa* – 2 acc.; *Majorana hortensis* – 1 acc.; *Mentha* sp. – 1 acc.; *Origanum vulgare* – 1 acc.; *Phaseolus vulgaris* – 38 acc.; *Phaseolus coccineus* – 3 acc.;

Pisum sativum – 10 acc.; *Salvia variegata* – 1 acc.; *Secale cereale* – 1 acc.; *Sideritis syriaca* – 1 acc.; *Solanum lycopersicum* – 5 acc.; *Thymus vulgaris* – 1 acc.; *Triticum aestivum* – 1 acc.; *Triticosecale* – 7 acc.; *Vicia faba* – 1 acc.; *Vigna unguiculata* – 3 acc.; *Zea mays* – 2 acc.; *Zinnia elegans* – 1 acc.

Traditional local varieties cultivated in home gardens in Bulgaria are presented in Table 2. Information was collected using a specially developed questionnaire administered to farmers maintaining local varieties of plant genetic resources in Bulgaria.

The survey revealed that in the home gardens of the visited regions in Southern Bulgaria - Pazardzhik, Plovdiv, Smolyan, Madan, Haskovo, Yambol, Blagoevgrad, and Petrich - a large number of vegetables, grain legumes, medicinal plants, and spice species are still widely cultivated. In contrast, in villages located in Northern Bulgaria (in the regions of Sofia, Pleven, and Vratsa), old varieties of maize and common bean, and a rich local diversity of Cucurbit crops, were identified. The results indicate that the highest number of landrace cultivation sites in Bulgaria are located in semi-mountainous rural areas.

Similar data were reported by Antofie (2024) and Antofie et al. (2024), based on an investigation of 120 households in the hilly and mountainous areas of the Romanian Carpathians. The studies found that a rich diversity of traditional crop species is still maintained by local Romanian farmers, with some varieties preserved for more than 100 years.

In most cases, heirloom seeds are used, which have been preserved within families for generations. The long-term conservation of these traditional varieties is closely related to their continued use in local food systems, as well as to the cultural traditions, customs, and festivals of local communities. This unique agricultural heritage enables each village and region to maintain distinctive characteristics and specific taste qualities in its traditional agricultural production.

The structure of the farms from which seeds were collected varies depending on the specific characteristics of the region. In general, these farms are small-scale and primarily oriented toward meeting the needs of individual households.

Table 2. Local varieties used in traditional home gardens in Bulgaria

Taxonomic description of crops	Villages/regions	Number of accessions, local name
<i>Zea mays</i>	Lakatnik, Sofiya	1 old variety (100 years)
	Dolno Belevo, Haskovo	1 acc. Local corn
<i>Phaseolus vulgaris</i>	Bratzigovo, Pazardzhik	1 accession, Prevren
	Sarnitza, Pazardzhik	2 acc. – Ranza, Batev
	Sadovo, Plovdiv	1 acc. Local black
	Ochindol, Vratza	2 acc. – Local black and Ochindolski green (100 years)
	Lakatnik, Sofiya	1 acc. Local white
	Smilyan, Smolyan	1 accession, Sharen bob
	Metcho korito, Pazardzhik	1 acc. Local (30 years)
	Osenovlag, Sofia	2 acc. – Sharen and Pritkan bob
	Visokite, Madan	7 local varieties
	Stoykite, Smolyan	2 acc. Local Rodopski bob
	Varbovo, Smolyan	1 acc. Local bean
	Dolno Belevo, Haskovo	1 acc. Local white
<i>Phaseolus coccineus</i>	Sarnitza, Pazardzhik	1 traditional large variety, Pehlivan
	Visokite, Madan	1 accession, Vasulvitsa, pink with black spots
	Stoykite, Smolyan	1 local accession, brown with black spots
<i>Vigna unguiculata</i>	General Inzovo, Yambol	1 accession, local brown
	Kluch, Petrich	1 old white variety
	Dolno Belevo, Haskovo	1 accession, Bob Roglyo
<i>Capsicum annum</i>	Dolno Belevo, Haskovo	1 acc., Local Kapia (traditional)
<i>Solanum lycopersicum</i>	Bratzigovo, Pazardzhik	2 local pink varieties
	General Inzovo, Yambol	2 old varieties (100 years) – Rozalina (pink) and Red Generalinzovski
	Dolno Belevo, Haskovo	1 acc. – “Royal tomato” from the garden of King Boris III, grown for 80 years
<i>Cucurbita maxima</i>	Levishte, Sofiya	1 local variety, White pumpkin
<i>Cucurbita moschata</i>	Sarnitza, Pazardzhik	1 acc. Violin type
	Levishte, Sofiya	1 acc. Violin type
	Radinovo, Plovdiv	1 acc. Violin type
	Sinitovo, Pazardzhik	1 acc. Violin type
	Dolno Belevo, Haskovo	1 acc. Violin type
<i>Cucurbita pepo</i>	Nikudin, Blagoevgrad	2 accessions, Pork pumpkin type
<i>Cucurbita pepo</i> var. <i>giromontina</i>	Sinitovo, Pazardzhik	1 local accession
<i>Cucumis melo</i>	Nikudin, Blagoevgrad	1 acc. Honey type
	Komarevo, Vratza	1 acc. Dessert type
<i>Citrullus lanatus</i>	Krivodol, Plevan	2 local varieties
	Komarevo, Vratza	1 acc. Local large

*Traditional gardening is located outside the urban area.

Alongside the cultivation of a wide range of local species, farmers often apply ecological cultivation practices and pay particular attention to the selection and preservation of high-quality seeds in order to maintain these traditional varieties. The enrichment of the collection and the establishment of an organized database with passport descriptors for each accession have created favourable conditions for mapping and zoning of the local gene pool. This also provides a solid basis for expanding future expedition activities in specific regions of the country that are rich in valuable plant genetic resources. In many of these areas, informal rural breeding practices are observed, mainly focused on traits such as taste quality, fruit size, and resistance to diseases.

Data were collected using a questionnaire applied through direct interviews with local authorities and household representatives in rural communities. The survey aimed to identify local varieties, document traditional agricultural knowledge, and evaluate opportunities for supporting food security through the conservation and use of local genetic resources. The results identified four major motivational factors for the valorization of landraces, the reintroduction of traditional varieties into farms, and their on-farm conservation: (1) access to reliable and structured information; (2) appropriate segmentation of potential customers, including opportunities related to agrotourism; (3) identification of the optimal size of the target market, such as local markets and rural festivals; and (4) access to consulting services and cooperation with public institutions, including research institutes. In addition, universities play an important role through agricultural education and knowledge transfer.

In recent years, market demand for local agricultural products has increased significantly. This trend highlights the need to develop supportive tools for farmers aimed at facilitating the use of local genetic resources. All surveyed agricultural producers indicated that, if they had greater certainty regarding the marketing and sale of their production, they would increase the cultivation of local varieties due to their higher market value.

Farmers expressed a positive assessment of public institutions, such as research institutes,

universities, and the National Agricultural Advisory Services, particularly with regard to the provision of information. However, local farmers also emphasized the lack of coordination among different institutions within the agricultural sector, which often leads to delayed, fragmented, or inconsistent information when support measures for farmers are implemented.

The economic and social environment also plays a crucial role in shaping farmers' motivation to cultivate plant species originating from local genetic resources. Labor shortages in agriculture, combined with limited mechanization, represent additional challenges for agricultural producers.

Furthermore, limited access to markets, uncertainty regarding the sale of agricultural products, and insufficient storage facilities—especially among small-scale farmers who are more likely to cultivate local varieties—significantly reduce farmers' motivation to utilize local genetic resources.

According to the surveyed farmers, the most important factor for the valorization of local plant genetic resources is the identification of an optimal target market for their products. The second most important factor is rapid access to structured information on the characteristics and advantages of local plant species. The third key factor is effective cooperation with public institutions. These results highlight the importance of building strong relationships with farmers to promote the sustainable use of local genetic resources and to increase the income of small-scale producers. The conservation of traditional varieties is crucial not only for sustainable agricultural development but also for environmental protection and adaptation to climate change.

The developed electronic catalogue provides rapid access to structured information on plant genetic resources. Through the innovative digital platform, farmers who cultivate and preserve local genetic resources can improve their awareness and knowledge of the characteristics and benefits of these varieties. This contributes to increased profitability, improved farm management, enhanced market access, and, importantly, to the preservation of traditional local products and environmental sustainability.

CONCLUSIONS

Public funding for both fundamental and applied scientific research in European countries in the field of conservation and documentation of plant genetic resources, at national and international levels, aims to mitigate biodiversity loss and enhance its valorization in order to ensure food security under the conditions of climate change. In the context of increasing digitalization, an open-access electronic platform has been developed for mapping the local gene pool in Bulgaria, addressing this strategic priority.

The *ex situ* collection of landraces has been established through direct seed donations from farmers and represents specific populations originating from particular localities that have been traditionally cultivated for local consumption.

The highest level of diversity among old varieties and local forms is observed in horticultural crops.

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EVALUATION OF THE PRODUCTION POTENTIAL IN THE COMPARATIVE CROP OF THE DANUBIUS GARLIC VARIETY REGISTERED AT B.R.G.V. BUZĂU

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Abstract

Garlic is representative of the Buzău vegetable-growing basin, known especially to local populations from Râmnicu Sărat, a micro-region with a historical tradition of garlic cultivation. The specialists at BRGV Buzău have given particular importance to this species, including it in intensive conservation and breeding programs. Research began with the creation of a valuable germplasm base composed of more than 30 distinct genotypes collected from across Romania. These cultivars underwent intensive breeding work, resulting in new registered creations: Benone, followed by the Râmniceanul variety, and, most recently, Danubius.

Between 2023 and 2025, the Danubius variety was evaluated in comparative cultivation with the Benone variety, and Danubius recorded a higher production level than the reference variety Benone. The average bulb weight for Danubius was 36 g, while for Benone it was 25 g. The number of cloves per bulb was 9 for Danubius and 13 for Benone. The average weight of a single clove was 5.2 g for Danubius and 3.9 g for Benone. Research will continue with the conservation and enrichment of the germplasm collection and the creation of new varieties.

Key words: clone, breeding, genetic resources, germplasm, conservation.

INTRODUCTION

Garlic (*Allium sativum*) is emblematic of the vegetable growing basin of Buzău, widely renowned for its local populations from Râmnicu-Sărat and for a micro-area with a long historical tradition in garlic cultivation. Due to its exceptional qualities, Râmnicu-Sărat garlic has gradually become a locally recognized brand both nationally and internationally, distinguished by its taste, refined aroma, and high post-harvest storage capacity, especially when preserved using traditional methods in the form of braided strings (Vîntor et al., 2019). The storage capacity of garlic is influenced both by the genetic characteristics of the variety and by post-harvest handling and storage conditions (Belo et al., 2024; El-Mesery et al., 2025).

Local growers from Râmnicu-Sărat have observed that garlic stores better if it is not trimmed after harvest (i.e., if the stem and leaves are not removed). Initially, it was stored in bundles, but over time, in order to better seal the

bulb neck and prevent dehydration of the cloves, locals developed braided strings for long-term storage. These braided structures allow good aeration, reduce oxidation by enabling air penetration into the outer tunics, are easy to market, and allow gradual consumption, bulb by bulb, without affecting the remaining ones (Harris, 2026; Pusik & Pusik, 2021; Pusik & Pusik, 2023).

Due to its outstanding taste qualities, garlic is a key ingredient in several traditional food products from the Buzău area, such as Pleșcoi sausages, babic, ghiudem, and pastrami. The importance of garlic in human nutrition is globally recognized, being valued both for its organoleptic properties and for its bioactive compounds with beneficial health effects (Ciofu et al., 2004; Munteanu, 2003).

Garlic cultivation has predominantly been practiced in small household systems due to its specific soil requirements, as *Allium sativum* prefers light, sandy, well-drained soils that do not favor the persistence and development of

soil nematodes, the most aggressive pests of this crop. Compliance with these soil conditions limits the development of pathogens and specific pests (Ciofu et al., 2004; Roman et al., 2012; Scholz et al., 2025). Additionally, garlic crops can be affected by numerous pathogens and factors that reduce both yield quality and storage capacity (Li et al., 2025).

Massive imports starting in the 1990s led to a collapse in market prices, a significant reduction in cultivated areas, a sharp increase in imports, and even the risk of loss of local genetic resources (Ilie et al., 2020).

To counteract this phenomenon, specialists from the Vegetable Genetic Resources Bank (BRGV) Buzău initiated research aimed at preserving clones known in both scientific literature and practice as “local populations of Râmnicu-Sărat garlic.” Following extensive studies, several varieties were developed and officially registered, the first being Benone, followed by Râmniceanu, and later Danubius, which is the subject of this study. Research efforts were further stimulated by the Romanian Ministry of Agriculture and Rural Development (MADR), which in 2019 implemented the support scheme “minimis aid for garlic production” (Government Decision 108/2019), aiming to revive garlic cultivation in Romania using local genetic resources.

MATERIALS AND METHODS

Research on the Danubius variety began in 2012 with the acquisition of base genetic material. Numerous expeditions were organized for the exploration and collection of genetic resources across the country, with emphasis on traditional areas such as Râmnicu-Sărat, Copălău, Cenad, and Dărăști. These activities resulted in the establishment of a valuable germplasm collection comprising over 80 cultivars with distinct phenotypic traits.

These were subjected to intensive breeding work, and line L24 demonstrated clear superiority, leading to the development, through clonal selection, of the Danubius variety, which was officially registered in the National and European Catalogues in 2025.

To evaluate its production potential, the Danubius cultivar was grown for three years in comparative trials alongside the control variety

Benone, widely cultivated under the “Garlic Program” funded by MADR (Figure 1).

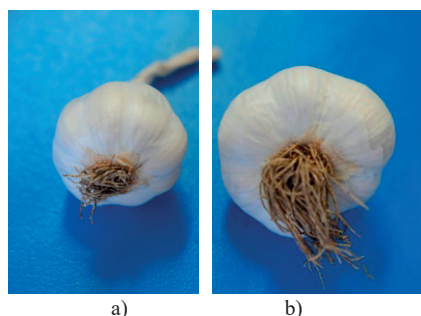


Figure 1. The two varieties studied: a) Benone - the control cultivar; b) Danubius - the subject variety

The research was conducted during the period 2023-2025 in the experimental field of BRGV Buzău. The genetic material used in the study originated from BRGV Buzău, with certified seed as the biological starting material. The cultivation technology applied was specific to intensive garlic production, with a high degree of mechanization throughout all stages, from soil preparation to harvest.

To accurately determine the optimal planting time, two experimental variants were established: V1 - autumn planting and V2 - spring planting.

Throughout the growing season, phenological, biometric, and laboratory observations were carried out, highlighting differences between varieties, experimental variants, and growing years. Soil preparation, planting time, crop maintenance, and harvesting practices were identical for both varieties under study.

RESULTS AND DISCUSSIONS

During the period 2023-2025, the Danubius variety was evaluated in comparative trials with the Benone variety, both developed by BRGV Buzău researchers.

The research began with land preparation, carried out in several stages. Deep ploughing was performed at a depth exceeding 30 cm to ensure proper soil loosening and good water drainage. This was followed by seedbed preparation through soil fragmentation using a cultivator, and for the autumn variant (V1), additional rotary tilling was applied to achieve a well-loosened, fine seedbed.

Subsequently, the soil was shaped into raised beds using a bed-forming machine (MMS) coupled with a low-power tractor (50 HP) designed for vegetable production, with a track width of 1.4 m. Raised beds with a top width of 94 cm were formed (Figure 2).

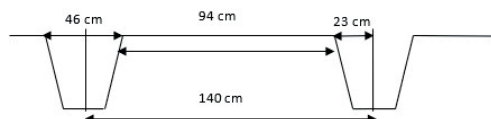


Figure 2. Land modelling scheme

For both experimental variants (V1 and V2), bed shaping was performed in autumn. However, for the spring crop (V2), after the use of the cultivator, no additional rotary tillage was applied, and bed shaping was carried out directly.

Planting of cloves for the autumn crop (V1) was performed on the same date for both Danubius and Benone varieties and varied over the three years between October 20 and November 10.

For the spring crop (V2), planting took place between March 20 and April 10.

Planting depth differed between variants. For the autumn crop (V1), cloves were planted deeper, at 4-6 cm, to ensure better soil contact, prevent exposure caused by strong winds in the area, and avoid displacement by birds (e.g., crows), which can significantly damage the crop. For the spring variant (V2), planting depth was shallower, at 3-5 cm, benefiting from improved soil structure due to natural freeze-thaw cycles during winter.

For both experimental variants (V1 and V2), the crop was established with four rows per bed, arranged in two bands, with 20 cm between rows, 30 cm between bands, and 8-10 cm between plants within the row, according to the planting scheme (Figure 3).

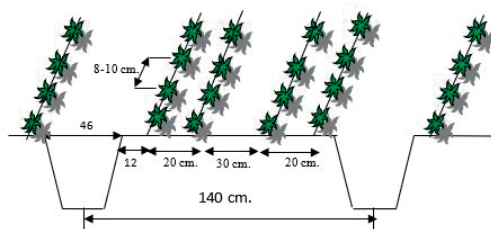


Figure 3. Planting scheme

Further studies were conducted at BRGV Buzău regarding the optimization of the planting scheme, with emphasis on row spacing, plant spacing within the row, planting depth, and the optimal seed rate per unit area.

The research resulted in the development of the optimal planting scheme presented in this paper. It is well known that *Allium sativum* allows the use of various technological approaches for crop establishment; however, in traditional household systems, where maintenance operations are predominantly manual, six rows per bed are typically used, with a much higher plant density per unit area (Figure 4).

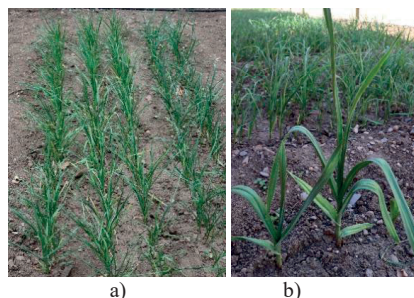


Figure 4. Comparative crops: a) Benone - control variety; b) Danubius cultivar

The land shaping and crop establishment scheme adopted by BRGV Buzău specialists allows for almost complete mechanization of the crop, considering that the most costly maintenance operations are manual weeding and hoeing. In this case, for weed control and soil loosening, the cultivator was equipped with appropriate active working tools to minimize manual intervention. Thus, in the furrow, behind the support wheel and depth control unit, a large V-shaped blade (duckfoot type) was mounted, followed by an adjustable ridger to restore the furrows. Between rows, chisel-type tools were used, while between bands, medium sized V shaped tools were employed.

All mechanical operations for crop establishment, maintenance, and harvesting were carried out using the same tractor, with the same track width, strictly following the wheel tracks established during soil shaping.

The active tools were carefully adjusted to avoid damaging the plants by cutting the root system, while ensuring effective weed control and proper soil loosening. During the growing season, two to three mechanical hoeings and one

to two minimal manual interventions were performed to remove weeds emerging between plants within the row.

The seed rate ranged between 450-500 kg/ha. Experimental results showed that for the Danubius variety, in order to ensure a plant density comparable to the control variety Benone, the seed rate must be increased by 40-50 kg/ha due to the larger clove size.

Throughout both the growing season and the seed storage period, the health status of the comparative crops was carefully monitored, with no significant observations regarding differential behavior of the varieties in response to pathogens.

In 2024, during the growing season, a mild infestation of the stem and bulb nematode (*Ditylenchus dipsaci*) was observed in both variants and varieties, which slowed growth and bulb development, followed by slightly accelerated maturation. This attack was also supported by favorable climatic conditions, specifically the absence of soil frost during winter and higher than normal temperatures both in winter and during the growing season.

In 2025, the onion and garlic fly (*Delia antiqua*) appeared in the area, causing a mild attack and affecting part of the bulbs in the soil. During the storage period, diseases caused by blue mold (*Penicillium* spp.), dry rot (*Fusarium* spp.), white rot (*Sclerotium cepivorum*), gray rot (*Botrytis allii*), and other specific pathogens were carefully monitored. No significant losses were recorded due to the application of preventive treatments.

Experience from previous years indicates that most pathogens originate in the field during the growing season and become evident during storage. During treatment applications, an approved adjuvant was added to the spray solution to improve adhesion, due to the leaf surface being heavily covered with wax (pruina).

Production results showed significant differences between varieties and years, as well as slight variations between V1 (autumn planting) and V2 (spring planting). The main production data for the three-year study period for V1 are presented in Figure 5.

The highest yields in all three years were recorded for the new variety Danubius, with a maximum in 2025 (17.45 t/ha) and a minimum

in 2024 (12.2 t/ha). It should be noted that 2024 was unfavorable for garlic cultivation in the Buzău vegetable basin due to climatic conditions, namely heat stress and prolonged drought. Statistical analysis using Duncan's test indicated significant superiority in all three years.

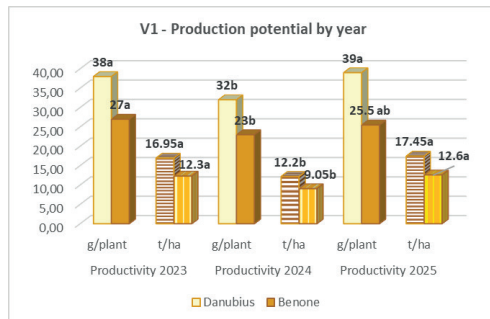


Figure 5. Graphic representation of the production capacity of the autumn crop variant (V1)

The average yield of the Danubius variety during 2023-2025 was 15.53 t/ha, higher than that of the control variety Benone (11.31 t/ha) during the same period. The Benone variety recorded a maximum yield in 2025 (12.6 t/ha) and a minimum in 2024 (9.05 t/ha).

Variant V2 of establishing the crop in spring recorded the following results, which are presented in Figure 6.

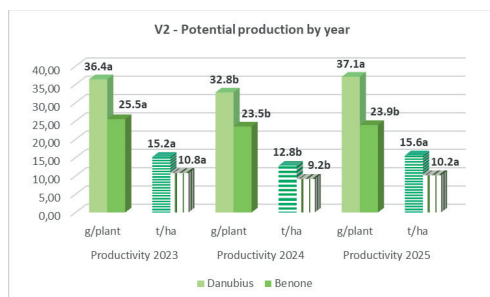


Figure 6. Graphic representation of the production capacity of the spring crop variant (V2)

For both varieties, slightly lower yield values were recorded compared with the autumn variant. However, the Danubius variety consistently outperformed the control variety Benone, with a maximum yield of 15.6 t/ha in 2025, while Benone recorded significantly lower values (10.2 t/ha). The lowest yields were observed in 2024: 12.8 t/ha for Danubius and 9.2 t/ha for Benone.

Over the three-year study period, the average yield was 14.53 t/ha for Danubius and 10.06 t/ha for Benone. Duncan’s test confirmed statistically significant superiority in all years. In Figure 7, the difference in the quantitative characteristics: size and number of garlic cloves, between the two studied varieties is evident: at Danubius, it was observed fewer garlic cloves, but they were larger in size and weight compared with the control variety Benone, which had a greater number of garlic cloves that were smaller in size and weight.

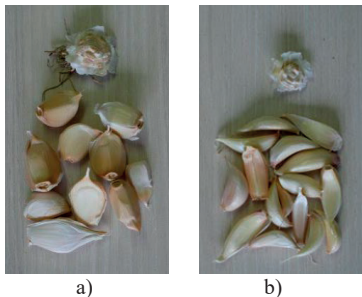


Figure 7. Phenotypic differences regarding the number and size of garlic bulbs: a) Danubius; b) Benone - control cultivar

In this regard, quantitative characteristics and biomorphological observations were carried out during the period 2023-2025 and the results obtained can be found in Table 1.

Table 1. Quantitative characteristics of bulbs at commercial maturity

Variety	2023			2024			2025		
	N G C	MGC (g)	NO TL	N GC	M GC (g)	NO TL	N GC	M GC (g)	NO TL
Danubius	9	4.2	6	8	4	5	8	4.8 7	5
Benone	13	2.0 7	7	14	1.6 4	8	12	2.1 2	6

Notes: NGC = number of garlic cloves; MGC = garlic clove mass; NOTL = number of outer tunic leaves.

The number of cloves per bulb and their average weight varied depending on the variety and year, highlighting morphological differences and adaptation to environmental conditions. The Danubius variety showed a lower number of cloves per bulb, with a maximum of 9 cloves in 2023 and 2025. The average clove weight was 4.2 g in 2023 and 4.37 g in 2025.

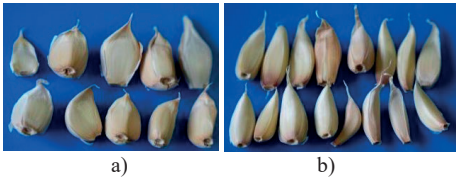


Figure 8. Details on the 2024 garlic bulb crop: a) Danubius; b) Benone - control cultivar

In contrast, the control variety Benone showed a higher number of cloves, with a maximum of 14 in 2024 and a minimum of 12 in 2025, while the average clove weight ranged from 1.64 g (2024) to 2.12 g (2025) (Figure 8).

CONCLUSIONS

The Danubius variety recorded superior yield values compared to the control in both experimental variants (V1 - autumn planting and V2 - spring planting) and in all three study years, demonstrating clearly superior genetic potential in terms of productivity and quality. Danubius was characterized by stability and the formation of large cloves, while the control variety Benone showed higher variability and a bulb structure consisting of a larger number of smaller cloves. The results confirm that crop establishment can be successfully performed both in autumn and spring, provided that cultivation technology is respected, particularly planting depth (deeper in autumn and shallower in spring). The bulb quality of the Danubius variety is superior to that of the control due to two key characteristics: clove size and uniformity, making it more preferred by consumers. However, it is necessary to increase the seed rate by more than 50 kg/ha to ensure optimal plant density.

Both varieties, Danubius and the control Benone, tolerate various technological approaches, especially regarding row spacing, plant spacing, and planting time. Due to this technological flexibility, they can be successfully cultivated from small-scale household systems to highly mechanized intensive systems, with particular attention to ensuring sufficient nutritional space, with at least 5 cm between plants within the row.

Feedback from growers who received promotional seed material of the Danubius variety from BRGV has been encouraging, with a positive market response, recommending the variety for large-scale production.

In 2026, following the obtained results, there has been a significant increase in requests from authorized genetic resource multipliers for the Danubius variety.

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FLORICULTURE,
ORNAMENTAL PLANTS,
DESIGN AND
LANDSCAPE
ARCHITECTURE



INFLUENCE OF SUBSTRATE TYPE ON THE GROWTH OF *OSMUNDA REGALIS* L. SPOROPHYTES UNDER GREENHOUSE CONDITIONS

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Abstract

*Ferns constitute a group of vascular plants relevant for the study of the evolution of terrestrial plants due to their morphological and physiological adaptations, which confer them high ecological versatility. This adaptive capacity explains their distribution in a variety of habitats. In accordance with these characteristics, the present study examined the influence of the cultivation substrate on gravimetric and morphometric growth parameters and on the accumulation of metabolites by *Osmunda regalis* sporophytes in one-year potted cultures under greenhouse conditions. Six types of substrates (S1-S6), with different characteristics from specific ornamental mixtures for shrubs, cacti, aquatic or acidophilic plants, to natural peat, were tested. The plants developed on natural peat substrate (S6, presented the best morphometric characteristics, with the highest concentrations of metabolites, whereas variant S2 (enriched with microelements) had the best development of the vegetative part, with the lowest content of polyphenols, being the least stressed variant. Overall, the results showed that the optimal substrate should be selected according to the intended objective: vegetative growth or phytochemical potential.*

Key words: ferns, greenhouse, *Osmunda regalis*, secondary metabolites, substrate.

INTRODUCTION

Ferns are an ancient lineage of vascular plants, with an evolutionary history spanning more than 360 million years, as documented by the fossil record (Shen et al., 2018; DiMichele & Phillips, 2002). Over time, they have diversified and adapted to an impressive range of habitats and are now represented by approximately 12,000 species (PPG I, 2016). They occur in terrestrial, aquatic, epiphytic, and lithophytic environments, on diverse substrates, from soils to rock surfaces and tree bark, and under climatic conditions ranging from tropical and temperate to semi-arid regions (Anderson, 2021; Dubuisson et al., 2009).

The adaptive potential of ferns derives from the integration of specialized morpho-physiological traits. Rhizomes function both as storage organs and support vegetative growth (Tani & Kudo, 2003; 2005), fine roots facilitate efficient nutrient uptake (Wang et al., 2019), and adaptable leaves enable photosynthesis under varying light conditions (Weng & Wong, 2015). Over time, ferns have developed complex morphophysiological mechanisms that have enabled them to adapt to a wide range of

environmental conditions (Alejo-Jacuinde et al., 2022). This adaptation has involved not only structural modifications of vegetative organs, but also physiological and biochemical adjustments, including those related to oxidative metabolism. In this context, the synthesis of secondary metabolites, particularly phenolic compounds and flavonoids, contributes significantly to cellular protection and to plant responses to abiotic stress. The present study represents a continuation of previous research on the *in vitro* propagation and acclimatization of *Osmunda regalis* (Aldea et al., 2025), a species that has disappeared from the spontaneous flora of Romania (Boșcaiu et al., 1994; Dihoru & Dihoru, 1994) and is naturally found mainly in humid and shaded habitats, such as peat bogs, marshes, lake shores, and forests, on peat-rich soils (Birks et al., 1991; Matchutadze, 2014). In the current research, we aimed to analyze the influence of substrate type on the growth and development of sporophytes cultivated in pots under greenhouse conditions. The gravimetric and morphometric parameters, as well as the accumulation of proteins and secondary metabolites belonging to the classes of polyphenols and flavonoids, with antioxidant

activity, were assessed in order to identify the most favourable substrate variant for plant development.

MATERIALS AND METHODS

Plant Material and Growth Conditions

The biological material used in this experiment consisted of sporophytes of the species *Osmunda regalis*, obtained *in vitro* by sexual reproduction according to our previous works (Mitoi et al., 2024; Aldea et al., 2025). Acclimatization was carried out following the protocol described in the previous study (Aldea et al., 2025). The acclimated sporophytes were grown in pots for one year in a greenhouse on six types of substrates, differentiated according to pH and composition:

- S1 - pH 5.5, substrate for planting and propagation, consisting of 80% peat and 20% humus, enriched with N, P, and K;
- S2 - pH 5.5, substrate for ornamental shrubs, with bark, enriched with basic nutrients, microelements (B, Cu, Mn, Mo, Zn, Fe), N, P, and K;
- S3 - pH 6.7, substrate for aquatic plants, with peat, quality compost, sand, and clay, enriched with N, P, and K;
- S4 - pH 3.5- 5, substrate for acidophilic plants, based on natural peat;
- S5 - pH 4.6 substrate for cacti, with a high proportion of porous material, consisting of a mixture of peat, siliceous sand, and expanded granules, supplemented with small amounts of N, P, and K;
- S6 - pH 4.2, natural peat substrate.

All substrates, except S6, were obtained from Agro CS Romania SRL (<https://agrocs.ro/>). The S6 substrate was collected from a natural peatland. Each experimental variant was performed in six replicates.

The plants were maintained in plastic pots under greenhouse conditions at a temperature of $22 \pm 2^\circ\text{C}$, with natural photoperiod and relative humidity of 40-45%. Watering was provided manually every two days, keeping the substrate slightly moist.

Gravimetric and morphometric parameters

To assess the response of sporophytes to the different substrates, the following morphometric and gravimetric parameters were assessed for

each individual: plant weight, whole plant length, number of fronds per individual, length of the dominant frond, root length, and volume. Growth rates for the whole plant and root parameters were calculated as follows:

$$\text{growth rate} = \frac{(\text{final value} - \text{initial value})}{\text{initial value}}$$

For dry weight determination, approximately 1 gram of sample represented by fronds, rhizomes, or roots was dried at 70°C , for 10 h in an Ecocell oven (MMM Group, Germany). The dry weight of each sample was expressed as grams of dry weight (DW) reported to 1 g of fresh material (FW) - g DW/g FW. The moisture content (Mc) was calculated as a percentage from fresh weight using the formula: $\text{Mc} (\%) = (\text{FW} - \text{DW})/\text{FW} \times 100$.

All gravimetric measurements were performed using an analytical balance (Radwag AS 220/C/2, Radom, Poland), while whole plant, dominant frond, and root lengths were recorded in cm using a measuring tape. Root volume was determined via the volumetric method, using 10 mL graduated cylinders for initial measurements and 250 mL cylinders for final measurements.

Biochemical Analyses

Protein extraction

Cytosolic soluble proteins were extracted from fronds and roots using 50 mM phosphate buffer, pH 7.0 with 2 mM EDTA, 4% PVP at a ratio of 1:3 (w/v). The homogenates were centrifuged at 15,000 rpm for 10 min, and the resulting supernatants were used for protein quantification.

Protein Content Analysis

Total protein content was determined using the Bradford assay (1976), with Coomassie Brilliant Blue G-250 protein stain. Appropriately diluted samples reacted with the dye solution, and the absorbance was recorded at 590 nm. Protein concentrations were calculated by interpolating the measured absorbances against a bovine serum albumin (BSA) standard curve ($100\text{--}1000 \mu\text{g/mL}$; $R^2 = 0.996$), and results were expressed as $\mu\text{g BSA/g FW}$.

Secondary Metabolites Extraction

Plant material was separated into root, rhizome, and leaf samples, ground and homogenized with methanol at a ratio of 1:10 (w/v). Extractions were prepared by maceration at room temperature, in the dark, with constant agitation

at 169 rpm for 72 h. Subsequently, the samples were centrifuged at $11,000 \times g$ for 15 min at 4°C , and the resulting supernatants were used for biochemical analyses.

Total polyphenolic content assay

The total phenolic content of the extracts was determined using the classic Folin-Ciocalteu method (Stankovic et al., 2012). Appropriately diluted extracts were mixed with the Folin-Ciocalteu reagent (Sigma Aldrich-Merck, Germany), followed by the addition of a freshly prepared 7.5% Na_2CO_3 solution. After incubation for 30 minutes at room temperature, the absorbance of the samples was measured at 765 nm. The results were quantified by extrapolation from a gallic acid calibration curve (concentrations: 10-100 $\mu\text{g/mL}$, $R^2 = 0.9968$) and expressed as milligrams of gallic acid equivalents per gram of fresh weight (mg GAE/g FW).

Flavonoid Content Assay

Flavonoid concentration was assessed using a spectrophotometric method based on the interaction of flavonoid compounds with aluminium ions, according to Cai et al. (2010). The signal intensity was measured at 510 nm and correlated with a calibration curve prepared using a rutin standard (100-1000 $\mu\text{g/mL}$, $R^2 = 0.9996$). Results were expressed as mg rutin equivalents per gram of fresh weight (mg RE/g FW).

Determination of Antioxidant Activity Using the DPPH Method

The antioxidant activity was analysed using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method, following the procedure described by Marxen et al., 2007. The sample was subjected to a reaction with methanol and DPPH solution and incubated in the dark for 30 minutes. The absorbance was measured at 515 nm, and the results were expressed as mM Trolox equivalents per gram of fresh weight (mM TE/g FW), based on a Trolox calibration curve (50-150 $\mu\text{g/mL}$, $R^2 = 0.9873$).

Determination of Antioxidant Activity Using the FRAP Method

The reduction of ferric ions ($\text{Fe}^{3+} \rightarrow \text{Fe}^{2+}$) by the compounds present in the extract was monitored using the FRAP method, according to Simirgiotis et al. (2013), by measuring the absorbance at 593 nm after 30 minutes of incubation in the dark. Results were calculated based on a Trolox calibration curve (0.2-1 mM,

$R^2 = 0.9979$) and expressed as mM Trolox equivalents per gram of fresh weight (mM TE/g FW).

All absorbance measurements were performed using a Spectronic Helios Gamma UV-Vis spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

Each experimental variant consisted of three biological replicates for all biochemical analyses.

Statistical analyses

The experimental data were subjected to normality and homogeneity of variance tests, after which a Two-Way Analysis of Variance (ANOVA) was performed to assess the statistical significance of the combined effect of the two independent variables (type of organ and type of substrate). To determine the differences between means, we used the Tukey HSD test. All analyses were performed using MS Excel (Microsoft) and RStudio (packages emmeans, ggplot2 and multcomp). The results were reported as means $\pm$ standard deviation (SD) for all analysed parameters. The p -values considered statistically significant were $p < 0.05$, $p < 0.01$ and $p < 0.001$.

RESULTS AND DISCUSSIONS

The comparative analysis of vegetative development highlights the influence of substrate type on the growth of *Osmunda regalis* sporophytes cultivated in pots for one year after acclimatization (Figure 1).

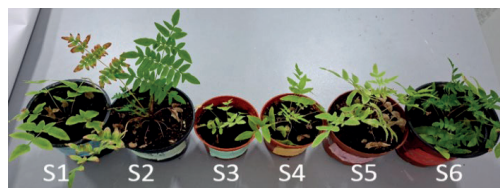


Figure 1. Influence of different substrate types on the growth of *O. regalis* potted cultures under greenhouse conditions

The gravimetric and morphometric analyzed parameters supported the macroscopic observations noted between substrate variants. Thus, the highest gravimetric whole-plant growth rate was recorded in variants S2 (45.24 ± 8.02) and S6 (44.22 ± 7.20), followed by S5 (41.34 ± 3.28) and S1 (39.99 ± 6.70), while the

other two variants had significantly lower values. The variant cultivated on substrate S3 showed the lowest gravimetric growth rate value of 7.07 ± 1.13 (Table 1). The plant growth rate in length displayed maximum values for variants S1 (5.50 ± 1.35) and S5 (5.16 ± 0.59), followed by S2 and S6 (4.77 ± 1.83 and 4.77 ± 1.12), with approximately similar values (Table 1). These results suggest that plant elongation does not

always directly reflect total biomass accumulation; in our case, some substrates supported gravimetric growth more efficiently than linear expansion. The significant differences were noted only for plants cultivated on S1 and S5 substrates in comparison with plants with slower growth cultivated on substrate S4 (Table 1).

Table 1. Gravimetric and morphometric parameters in one-year potted cultures of *O. regalis*

Variant	Gravimetric growth rate	Plant growth rate in length	Length of the dominant frond (cm)	Number of fronds/individuals	Root growth rate in length	Root growth rate in volume
S1	39.99±6.70 ^a	5.50±1.35 ^a	9.90±1.15 ^{ab}	9.5 ± 2.34 ^{ab}	7.16±1.88 ^a	23.17±6.21 ^a
S2	45.24±8.02 ^a	4.77±1.83 ^{ab}	12.30±1.68 ^a	10.33 ± 2.58 ^a	5.74±2.44 ^a	23.86±6.84 ^a
S3	7.07±1.13 ^c	4.16±1.36 ^{ab}	5.25±0.94 ^c	5.83 ± 0.75 ^c	7.52±4.01 ^a	10.94±4.99 ^b
S4	20.71±1.50 ^b	3.89±0.52 ^b	8.22±0.96 ^b	6.33 ± 1.03 ^{bc}	6.70±1.48 ^a	10.94±2.22 ^b
S5	41.34 ± 3.28 ^a	5.16±0.59 ^a	11.72±0.98 ^a	9.83 ± 3.43 ^{ab}	7.38±1.39 ^a	25.25±5.05 ^a
S6	44.22 ± 7.20 ^a	4.77±1.12 ^{ab}	11.12±1.49 ^a	9.16 ± 1.16 ^a	7.46±2.04 ^a	24.14±6.11 ^a

The mean ± standard deviation (SD) followed by different letters in the column were considered statistically significant with $p < 0.05$.

A similar response was observed regarding fronds' development. The dominant frond length reached the highest values in variants S2 (12.30 ± 1.68 cm), S5 (11.72 ± 0.98 cm), and S6 (11.12 ± 1.49 cm), whereas in S3 and S4 significantly lower values were recorded (Table 1). The number of fronds was also highest in S2, S5 and S6, suggesting that these substrates supported not only the elongation of the fronds but also the initiation of new organs. Also, in S3 and S4 significantly lower values were registered (Table 1), indicating a slower development of the vegetative part.

Compared with frond parameters, roots showed a differentiated response. Surprisingly, root length showed the highest value in variant S3 (7.52 ± 4.01) and the lowest in variant S2, but the differences between variants were not significant due to the high SD values. In contrast, root volume differentiated more clearly between efficient and restrictive substrates. The highest values were observed in S5 (25.25 ± 5.05), S6 (24.14 ± 6.11), S2 (23.86 ± 6.84), and S1 (23.17 ± 6.21), whereas S3 and S4 registered significantly lower root volumes (Table 1). This contrast between root length and root volume suggests that, under unfavourable substrates, the root system may invest in exploratory elongation but without sufficient differentiation of a robust absorptive apparatus capable of sustaining an adequate flow of water and minerals. From a functional perspective, root

volume appears to be a better indicator of the capacity to support aerial biomass than root length alone (Figure 2).



Figure 2. The appearance of *O. regalis* potted plants cultivated on different types of substrates

Substrate type significantly influenced water distribution and the degree of dry matter accumulation in organs (fronds, rhizomes, and roots). Thus, following separation (Figure 3) we monitored the differentiated accumulation of dry matter, water content, and metabolites' concentration in these organs.



Figure 3. The appearance of *O. regalis* potted plants: fronds (a), rhizome (b) and roots (c)

In fronds, the highest values of the DW were recorded in S4 (0.407 ± 0.007) and S3 (0.363 ± 0.046), while significantly lower values were obtained in S2, S5 and S6 (Table 2). In contrast, the highest fronds' water content was determined for S6 ($78.64 \pm 0.45\%$), followed by S5 ($77.60 \pm 0.80\%$) and S2 ($76.03 \pm 4.08\%$), while S4 and S5 variants had the lowest values (Table 2). The interpretation of these data should be coupled with plant growth rates. Thus, the variants with the highest dry matter content had the lowest water content and were the least productive in terms of biomass. This association

suggests that the decrease in biomass accumulation in variants S3 and S4 could be due to the reduction of water content, rather than growth inhibition. However, the lowest growth parameters in these variants indicate that the relatively higher dry matter accumulation was not associated with a more active metabolism, but rather with reduced tissue hydration and structural changes induced by substrate-related stress. In these variants, the reduction of water content could be due to nutritional imbalance or inadequate aeration determined by the substrate composition and granulation.

Table 2. Dry weight and water content of *O. regalis* fronds, rhizomes, and roots from one-year potted cultures

Variant	Fronds		Rhizomes		Roots	
	g DW/g FW	% Water Content	g DW/g FW	% Water Content	g DW/g FW	% Water Content
S1	0.266 ± 0.070^{bc}	73.36 ± 7.00^{ab}	0.245 ± 0.037^b	75.54 ± 3.74^a	0.198 ± 0.021^b	80.17 ± 2.06^a
S2	0.240 ± 0.041^c	76.03 ± 4.08^a	0.288 ± 0.026^{ab}	71.16 ± 2.60^{ab}	0.248 ± 0.019^a	75.15 ± 1.92^b
S3	0.363 ± 0.046^{ab}	63.70 ± 4.64^{bc}	0.331 ± 0.022^a	66.92 ± 2.17^b	0.241 ± 0.020^{ab}	75.90 ± 2.01^b
S4	0.407 ± 0.007^a	59.29 ± 0.73^c	0.263 ± 0.089^{ab}	73.73 ± 8.89^{ab}	0.261 ± 0.025^a	73.89 ± 2.47^b
S5	0.224 ± 0.008^c	77.60 ± 0.80^a	0.270 ± 0.023^b	73.01 ± 2.29^a	0.187 ± 0.028^b	81.31 ± 2.83^a
S6	0.214 ± 0.005^c	78.64 ± 0.45^a	0.269 ± 0.016^b	73.13 ± 1.61^a	0.239 ± 0.023^{ab}	76.08 ± 2.27^b

The means $\pm$ standard deviation (SD) followed by different letters in a column were considered statistically significant $p < 0.05$.

This dry matter accumulation, in association with the decrease in water content in variants S3 and S4 can also be observed at the rhizome and root level (Table 2), but, additionally, dehydration in variant S2 was observed at the rhizome and root level. Overall, the obtained data indicate that the less favourable substrates S3 and S4 modified the water content and the proportion of dry matter in rhizomes and roots, in correlation with the inhibition of plant growth. In the case of variant S2, the substrate produced dehydration in the root system, but in association with promoting the development of the vegetative system (Table 1).

The results obtained in the case of variant S2 suggest that a more aerated substrate structure, associated with an adequate supply of macro- and microelements, may favour long-term pot cultivation more efficiently than a substrate based exclusively on peat and humus. In contrast, substrate S3, intended for aquatic plants, recorded the weakest growth response, although it maintained relatively high values for root elongation and water content. This behaviour may be explained by the substrate composition, which includes clay and compost, as well as by its higher pH, factors that may increase compaction and reduce rhizosphere

aeration. For a hygrophilous species such as *O. regalis*, high humidity is not sufficient in the absence of an adequate physical structure of the substrate. Under these conditions, the root system may respond through exploratory elongation, without, however, developing a sufficiently absorptive capacity to support the overall growth of the plant.

A relevant contrast was also observed in the case of S4, the substrate for acidophilous plants. Although its acidity falls within the favourable range for the species, the lower conductivity and the lack of available values for N, P, and K suggest a weaker nutrient supply. Thus, the data indicate that acidity alone does not guarantee optimal growth, as performance depends on the interaction between substrate reaction, physical structure, and nutrient availability. Taken together, these observations support the idea that substrate pH may play an important role in plant growth, mineral nutrition, and physiological response, in agreement with Ferrarezi et al. (2022), who showed that the availability of several nutrients to plants varies depending on substrate pH.

Unlike these results, our experimental variants with the best growth parameters displayed the lowest DW, in association with the highest water content. The substrates that significantly promoted

water accumulation, in association with higher growth parameters, were S6, S5 and S2 in fronds, S1, S6 and S5 in rhizomes, and S5 and S1 in roots (Tables 1 and 2). Interestingly, variant S5 supported a high biomass and a large root volume, suggesting that good drainage and efficient aeration of the root zone may compensate, at least partially, for a less favourable nutritional status, while the root system may respond through exploratory elongation without developing a sufficiently absorptive capacity to support the overall growth of the plant.

The results showed that the substrate is a determining factor in the balance between growth and nutritional requirements in *O. regalis*. The variants that supported the highest biomass accumulation and superior fronds development, namely S2, S1, S5, and S6, probably ensured a more favourable balance between water retention, aeration, and nutrient availability. This interpretation is consistent with our previous studies on the acclimatization of *O. regalis*, which reported a superior performance on substrates rich in peat and organic matter (Aldea et al., 2025), as well as with research on other fern species, where amended organic substrates stimulated growth and the accumulation of secondary metabolites (Pietrak et al. 2022).

The biochemical analyses provided new insights into the growth of the vegetative and root systems, highlighting that the substrate also affects plant metabolism. Thus, protein content varied depending on both the substrate and the analysed organ. In fronds, significantly higher protein concentrations were identified in variants S2, S6, and S5, in comparison to variants S1 and S3 (Figure 4). At root level, the significant values were recorded for S6, S2 and S4 variants compared with S1, S3 and S5 (Figure 4).

Differences between frond and root proteins' accumulation suggest that certain substrates (S5) supported a more efficient vegetative development, with more intense metabolic activity, reflected in a higher protein biosynthesis.

In some variants, roots had a significantly higher protein content than fronds (S6, S4 and S1), which could indicate an accentuated metabolic activity of the root system under pot culture

conditions. These data showed a more intense root activity related to nutrient absorption and adaptation to substrate conditions.

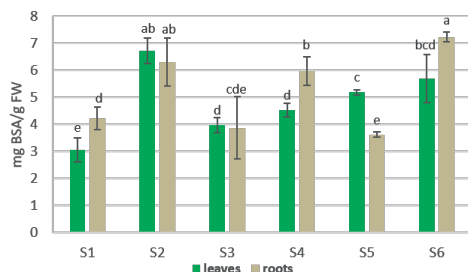


Figure 4. The protein concentrations in frond and root extracts from *O. regalis* potted plants cultivated on different types of substrates. The mean $\pm$ SD followed by different letters were considered statistically significant with $p < 0.05$

The literature indicates that ferns represent important sources of phenolic compounds (Mitoi et al., 2024; Langhansová et al., 2021). Our study showed that the properties of the cultivation substrate influenced the differential accumulation of secondary metabolites and antioxidant activity in *O. regalis* fern plants. In all variants, rhizomes presented the highest values for polyphenols, flavonoids and antioxidant activity, suggesting their important role in storage and regeneration (Klimešová et al., 2023). Fronds displayed the lowest values, while roots presented intermediate levels, which indicates a differentiated distribution of phenolic compounds among plant organs. A higher phenolic content in underground organs, compared with leaves, was also reported by other authors in several fern species (Valizadeh et al., 2015; Shin and Lee, 2010; Pietrak et al., 2025).

In our experiments, the significantly higher polyphenol content was recorded in the rhizomes of plants grown on substrate S6, followed by those on substrates S5 and S1, compared to variant S2, which presented the lowest polyphenols' concentration (Figure 5a). In roots and fronds, the variants with higher polyphenol content were S6, S3, S4 and S5, which showed significant differences compared with the other variants, S2 and S1 (Figure 5a).

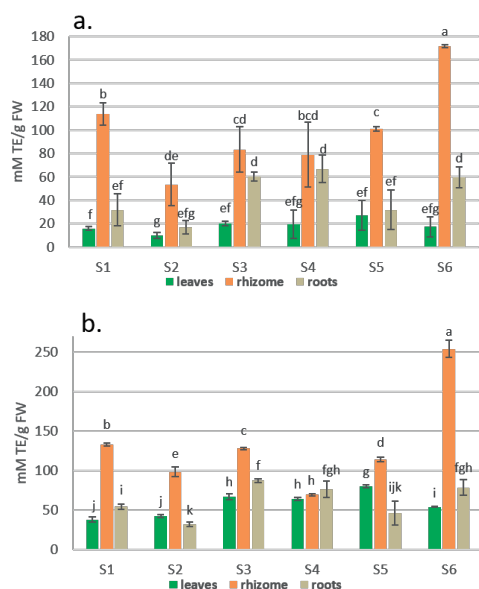


Figure 5. The polyphenols (a) and flavonoids (b) content in frond, rhizome and root extracts from *O. regalis* potted plants cultivated on different types of substrates. The means $\pm$ SD followed by different letters were considered statistically significant $p < 0.05$

A relatively similar accumulation pattern was observed for the flavonoids' content. The same variant S6 recorded the highest values in the rhizome, but, also in this case, at the fronds and roots' level, suggesting that the natural peat substrate favoured flavonoid accumulation and, implicitly, the intensification of secondary metabolism compared with the other variants. Interestingly, variant S1 presented higher values for both metabolite classes' accumulation in the rhizome, while the fronds and roots measured significantly lower concentrations (Figure 5a and b). The antioxidant activity determined by the DPPH method was correlated with the polyphenol content, with the same variation pattern noticed between substrate variants. Thus, the most significant value was seen in variant S6, at the rhizome level, followed by variants S1 and S5. Generally, higher antioxidant activity at the rhizome and roots level could suggest an intensification of antioxidant protection mechanisms in the underground organs. The variation pattern of antioxidant activity determined by the FRAP assay was not poorly correlated with the metabolites' determinations. In the case of rhizomes, all obtained values were

significantly different, with the maximum value for variant S6 (Figure 6b). By comparison with the DPPH determinations, fronds showed a higher FRAP antioxidant activity (Figure 6a and b), depending on the substrate type, with a significantly increased activity in variant S5. A possible explanation might be that fronds are a reservoir of more Fe^{3+} -compounds that must be protected from oxidation reactions.

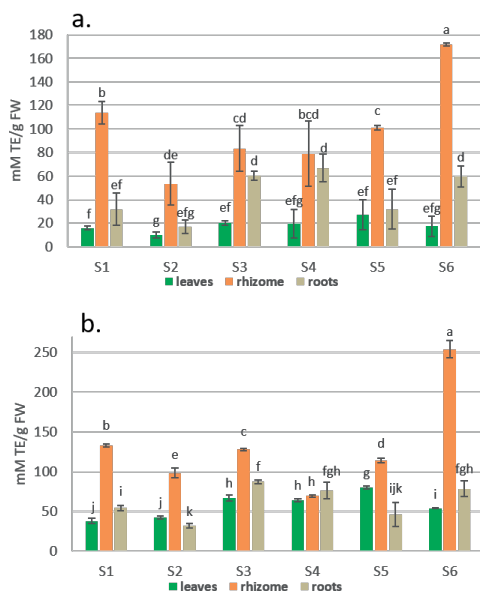


Figure 6. The DPPH (a) and FRAP (b) antioxidant activity in frond, rhizome, and root extracts from *O. regalis* potted plants cultivated on different types of substrates. The means $\pm$ SD followed by different letters were considered statistically significant with $p < 0.05$.

Correlated with the growth parameters, our biochemical data indicated that substrate type influenced not only vegetative and root system development but also the metabolic resources targeting secondary metabolism and antioxidant protection. These observations are consistent with other studies conducted on fern species, which have shown that substrate modifications may differentially affect growth, the accumulation of secondary metabolites, and antioxidant activity, depending on the species and the dose of the applied amendment (Pietrak et al., 2022). Substrate variants that did not necessarily support the highest biomass accumulation (S3 and S4) may have favoured the synthesis of phenolic compounds and the intensification of

antioxidant activity, reflecting a reorientation of metabolism toward adaptation and cellular protection. Also, variants with the best development of the root system (S6 and S5) possessed a rich phenolic content and high antioxidant activity. Our results are in agreement with the growth–defence trade-off concept (Sun et al., 2020). However, the data obtained in the present study also showed that this relationship is not absolute. Certain substrate variants (S6 and S5) simultaneously supported both biomass accumulation and an increase in secondary metabolite content, highlighting a species- or substrate-specific response.

CONCLUSIONS

Substrate type had a direct influence on the plant growth, root development, and biochemical response of *Osmunda regalis* sporophytes in potted cultures.

The variants S1 and especially S2 proved to be the most favourable for vegetative development, while variants S5 and especially S6 had the best root development, in association with higher polyphenol content and antioxidant activity. In contrast, S3 and S4 proved to be less favourable for growth, highlighting the importance of physical structure and nutrient availability in cultivation substrates.

Root volume proved to be more relevant than root length for supporting aerial biomass. The rhizomes showed the highest levels of phenolic compounds and antioxidant activity, as we expected.

The most suitable variants for *ex situ* multiplication, for obtaining plants with optimal development and higher biomass, were substrates S6, natural peat, and S2, enriched with microelements.

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DESIGNING AND LANDSCAPING OF A SUSTAINABLE PRIVATE GARDEN USING NATIVE SPECIES

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Abstract

Present paper analyzes the process of designing and setting up a residential garden in Botoșani County, with the main goal of creating an aesthetic and functional space adapted to the climatic rigors of North-Eastern Romania. The central objective is to identify an optimal mix of native species and technical landscaping solutions that can withstand the extreme thermal variations specific to the area. The execution methodology presented follows a precise technical sequence: land preparation works, the execution of the paving works, the installation of the pergola, the planting of woody vegetation and flowering species, and the sowing of the lawn. The results confirm that compliance with technical standards in the early phases of the construction site minimizes the risks of degradation and maximizes the aesthetic benefits. The paper concludes that a strategically planned execution represents the optimal solution for the development of resilient individual gardens, capable of generating a favourable microclimate and increasing the environmental value of the property.

Key words: native species, sustainability, garden, landscape, design, environment.

INTRODUCTION

Landscaping of private gardens is an important field of landscape architecture, having the role of harmoniously combining aesthetic with functional and ecological elements. The use of sustainability principles in the design of green spaces becomes a necessity, not just an aesthetic option (Sádaba et al., 2025; Pașcu et al., 2019; Zlati et al., 2024).

Given the current situation of global climate change and accelerated urbanization, the realization of private landscaping has evolved from a purely aesthetic approach to one focused on sustainability. The private garden has become a micro-ecosystem that must fulfil multiple functions: conservation of local biodiversity, efficient management of water resources, and regulation of the urban and peri-urban microclimate (Peter, 2010; Pașcu et al., 2022; Manapragada et al., 2025).

Private gardens play a significant role in conserving biodiversity, improving the local microclimate, and improving the quality of life (Pașcu et al., 2021; Peng et al., 2025).

The essential element in achieving these objectives is the use of plant species adapted to

local conditions, able to develop harmoniously without excessive consumption of resources (Sandu, 2013; Bernardis et al., 2022; Zaharia et al., 2003; Iliescu et al., 2002). In this type of compositions, also can be included underused tree species that have high ornamental value, such as *Mespilus*, *Cornus*, etc. (Grădinaru et al., 2007; Negrea et al., 2011; Zlati et al., 2021).

The paper analyses the design and execution of a private garden located in the town of Brăești, Botoșani County, with the aim of creating a sustainable, functional, and aesthetic green space integrated into the existing natural and architectural context. The project is based on the rational use of plant material, the coherent organization of the space, and the observance of modern principles of landscape design (Iliescu, 2001; Petrescu, 1983).

In the north-eastern region of Romania, and specifically in Botoșani County, landscape design faces rigorous challenges caused by the temperate-continental climate with excessive nuances, characterized by large thermal amplitudes and periods of prolonged drought (Cojocariu et al., 2022).

Thus, the use of local species in landscape design represents the foundation of the sustainability of

the arrangement (Bernardis, 2010, 2011, 2012). The species are genetically adapted to the pedological conditions and the local water regime, requiring a low cost for maintenance (Grădinariu et al., 2011; Zlati et al., 2011). Their integration into the layout contributes not only to the biological stability of the space, but also to maintaining the identity of the regional cultural landscape (Tadesse et al., 2019).

The integration of native species in the design of sustainable private gardens can represent a solid strategy from an ecological and economic point of view, with significant potential for adapting to current climate challenges.

In the design of a residential garden, the analysis of the soil is a fundamental step because the physical, chemical, and biological properties determine the success of the ornamental vegetation and the lawn. In the Botoșani area, North-Eastern Romania, the dominant soils are leached chernozems, phaeozems, locally cambic or clayey chernozems. These soils have a general clay-clay texture, good water retention capacity, an alkaline or neutral pH, and a moderate humus content (Galeș et al., 2016).

Site works (excavations, transport of materials) can cause compaction of the upper horizon, which causes a decrease in porosity, reduced water infiltration, and superficial development of the root system; therefore, it is recommended to scarify the soil to 30-40 cm and incorporate organic materials.

MATERIALS AND METHODS

Brăești commune is located in Botoșani County. Botoșani County is located in the North-East of the country, at 48 degrees north latitude and 16 degrees east longitude. It borders Iași County to the south, Suceava County to the west, and Ukraine (Figure 1).

The component villages of the commune are Brăești, Poiana, Popeni și Vîlcele.

Brăești commune is located in the plateau units, respectively the Moldavian Plateau. Geomorphologically, it is located in the plain subunit represented by the hilly plain of Jijia (also known as the Moldavian plain in classical literature).

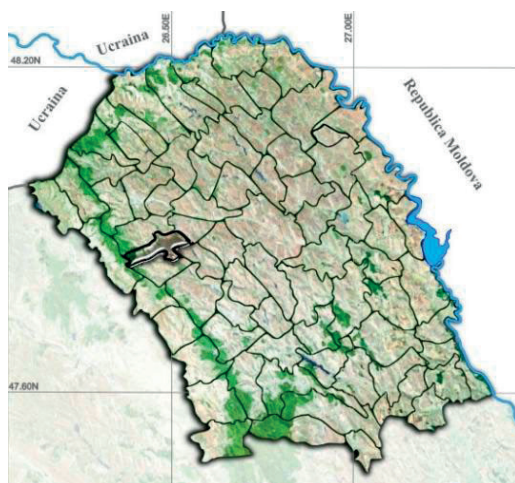


Figure 1. Location of Brăești

The territory of Brăești commune, Botoșani County, falls within the pedological zone of the chernozems of the Moldavian Plateau. The dominant soils are cambic chernozems developed on loessoid deposits, characterized by a well-developed mollic A horizon (0-25/30 cm) with a humus content of approximately 3.8-4.5% and pH values between 6.8 and 7.2. Beneath it is a Bv and Cca horizon. The predominantly loamy-clayey texture gives the soil a field capacity of approximately 30% and a good water retention capacity. However, under the conditions of the excessive continental climate in northeastern Romania, periods of drought during the summer can generate a water deficit in the superficial layer of the soil, which justifies the implementation of efficient irrigation systems within residential landscaping (<https://www.meteoblue.com>).

The increase in soil water content causes a significant improvement in plant morphological parameters and crop yield, highlighting the importance of technologies that contribute to soil water conservation, especially in regions frequently affected by drought periods (Galeș et al., 2016). It was also observed that soil moisture tends to increase with depth due to water infiltration and reduced evaporation. These characteristics highlight the important role of soil textural and structural properties in regulating the hydric regime.

The land is largely stabilized by acacia saplings and terraced plantations. Relief is one of the main factors involved in runoff and erosion processes, so the analysis of the length and slope of slopes is of particular interest.

From a climatic point of view, Botoșani County is located in the northeastern part of the country and is influenced by the continental climate of Eastern Europe, and less by Central Europe or Southwest and South, although most precipitation comes from Western and Northwest Europe, caused by air masses from the interior.

The commune presents, in its forested area, elements specific to the steppe and forest-steppe continental zone, and the hydrographic network has created conditions for the existence of aquatic vegetation. Three vegetation zones are distinguished on the commune's territory: the forest zone, the forest-steppe zone, and the steppe zone.

The land proposed for development is located in Brăești, Botoșani County, with a total area of 1300 sqm, of which: 150 sqm is the building area, 250 sqm is the paved area, and 900 sqm is intended for green space (Figure 2).



Figure 2. Inclusion in the geographical area

A single-family house with a ground floor plus an attic floor was built on the site. It is located 8 meters from the car and pedestrian entrance on the main street. The front of the house to the street measures 10 meters.

To implement the proposed design, a special design program for landscape design was used, namely Real Time Landscaping Architecture Pro, version 2016.

This program uses 2D and 3D techniques to reproduce the reality designed for the studied space. Within the program, thanks to a library with various elements, all the component parts of the designed space can be found, thus an overall image of the space and the proposed layout could be achieved.

As a planning tool for designing and landscaping using native species, SWOT analysis was used, focusing on the main Strengths, Weaknesses, Opportunities, and Threats of the location and the design theme. It was used as a planning tool to ascertain the feasibility of the project.

Strengths are related to:

- High ecological adaptability because native species are adapted to local pedoclimatic conditions (soil, rainfall regime, temperature), which determine an increased rate of capture and optimal development without intensive interventions.
- Reduced external inputs because the chosen species are much more resistant than intensive horticultural ornamental or exotic species. These lead to better tolerance of extreme phenomena (drought, late frost), in the context of climate change.
- The increase in local biodiversity is supported and contributes to ecosystem stability and the restoration of local food chains.
- Identity and cultural value by strengthening the regional landscape and promoting local plant heritage.
- Lower costs in the long term. Although the initial investment may be comparable to conventional solutions, maintenance costs decrease significantly over time.

Main weaknesses are related to limited aesthetic appeal, limited commercial availability, and the need for interdisciplinary expertise. Some native species may be perceived as having less ornamental value compared with intensively improved exotic species, which is not always true.

Opportunities we identify are related to the possibility of creating a replicable model by developing a plant selection algorithm that can be applied in other regions. Thus, private gardens can become demonstration models for the community.

Integrating the idea into emerging concepts and in correlation with Nature-Based Solutions and regenerative design is also a good opportunity.

The main **threats** are linked to the lack of availability of native species in large commercial nurseries and to the fact that invasive species can compete with or eliminate native species.

Taking into account the proposed design directions and the specific architecture of the building, the layout was created, perfectly combined with various dendro-floricultural species, which provide dynamism to the space, elegance, and harmony.

The program offers the possibility of creating 3D details and presentation videos, thus creating a clear image of the layout, with both visual and auditory effects.

Following a detailed investigation of the land and its component elements, the strengths and weaknesses of the existing development could be established, thus identifying the needs of the landscape proposal.

At the moment, the space is not arranged and does not meet any requirements; therefore, we must consider highlighting the land by using different arrangement methods, creating relaxation and play areas for children. We must take into account that the surface is small, thus the arrangement is compact, useful, and economical.

The free land intended for development is divided as follows:

- to the left of the entrance to the property, there is a rectangular area of approximately 100 sqm.
- on the side of the property, near the garage, there is a strip of land of approximately 30 sqm.
- behind the house, there is a raised or raised area of approximately 1070 sqm.

The goal of this work was to create a landscape that would fulfil both aesthetic and functional functions, using native species. The work aims to satisfy two mandatory aspects in the arrangement of recreational spaces: combining a harmonious style with a functional style through the use of trees and decorative plant species; and creating an oasis of peace and relaxation.

The objectives of this work are the following:

- correct execution of the preparations of the land to be landscaped;
- use of dendrological and floricultural species selected for decorative purposes;

- execution, care, and maintenance of the landscaping.

Climatic conditions, terrain, available funds, and, last but not least, the beneficiary's requirements are taken into account when implementing the proposed objectives.

The style adopted in the design is distinguished by a mixed landscape style, characterized by free compositions, irregular shapes, and winding lines. By implementing this project, we aim to reproduce a garden characterized by nature, where each chosen element fits perfectly into the area, and the plants are arranged in simple groups in irregular shapes, with balanced asymmetry.

The design was carried out on the land of an inherited house. The house was preserved 100%, with all the traditional elements (Figure 3).



Figure 3. The architectural style of the house

The main criteria in choosing the species were:

- drought resistance;
- low-temperature resistance;
- soil adaptability;
- decorative elements.

The native plants from the North-East of Moldova are already adapted to the clayey or chernozemous soils here; they resist brilliantly

the harsh winter frosts and the summer drought periods (Savé, 2009).

The landscape proposal is exemplified in Figure 4. The areas with plants were mainly delimited with curved lines on the lawn side. To avoid the growth of unwanted weeds in the areas with plants, the soil was mulched using agrotextile; then, crushed stone and fir cones were spread on top. Crushed stone with a size of 6-12 cm was used because, when maintenance work is carried out with a blower to remove plant debris, leaves, etc., the stone should not be thrown into the lawn.

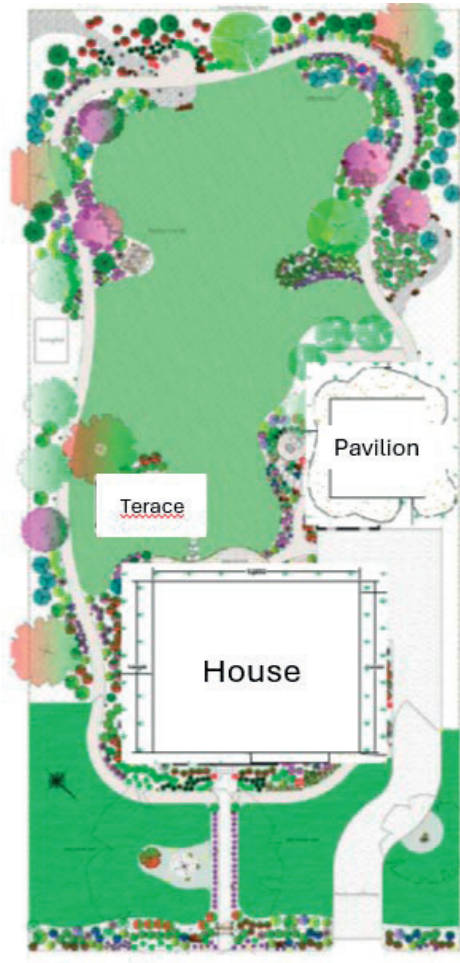


Figure 4. Landscaping proposal

Research into the field of native plants fundamentally shifts planting design from a purely aesthetic practice to an evidence-based ecological strategy. By moving away from decorative plant selection toward a deeper

understanding of indigenous species, functional ecosystems can be created that are more resilient, sustainable, and better integrated into the local environment.

The interest in how these species grow and change over the years allows designers to create long-term planting plans that evolve gracefully, rather than requiring constant care or replanting.

Main decorative features of the vegetation are presented in Table 1.

Table 1. Main decorative features of the vegetation

Specie	Exposure	Decorative features
<i>Cornus</i> sp.	Sun/Penumbra	Red branches during winter
<i>Acer</i> sp.	Sun/Penumbra	Autum colours
<i>Stipa</i> sp.	Sun	Texture and movement
<i>Salvia</i> sp.	Sun	Vibrant purple flowers
<i>Sedum</i> sp.	Sun/Penumbra	Texture/groundcover

For the implementation of the project, a series of dendrological and herbaceous species were used, as mentioned in Table 2.

Table 2. List of plant material proposed for the landscape solution

Nr. crt.	Scientific name
1	<i>Tilia tomentosa</i>
2	<i>Quercus robur</i>
3	<i>Carpinus betulus</i>
4	<i>Acer platanoides</i>
5	<i>Fraxinus excelsior</i>
6	<i>Salix alba</i>
7	<i>Acer tataricum</i>
8	<i>Crataegus monogyna</i>
9	<i>Mespilus germanica</i>
10	<i>Cornus sanguinea</i>
11	<i>Sambucus nigra</i>
12	<i>Ulmus minor</i>
13	<i>Cornus sanguinea</i>
14	<i>Ligustrum vulgare</i>
15	<i>Corylus avellana</i>
16	<i>Hiphophae rhamnoides</i>
17	<i>Rosa canina</i>
18	<i>Cotinus coggygria</i>
19	<i>Lonicera xylosteum</i>
20	<i>Acer campestre</i>
21	<i>Quercus cerris</i>
22	<i>Tilia platyphyllos</i>
23	<i>Rhamnus frangula</i>
24	<i>Viburnum opulus</i>
25	<i>Centaurea cyanus</i>
26	<i>Stipa pennata</i>
27	<i>Salvia pratensis</i>
28	<i>Viola odorata</i>
29	<i>Sedum</i> sp.

RESULTS AND DISCUSSION

Ecological argumentation. The scientific basis from which our research started was habitat restoration, especially in the current context of

climate change and the shortage of resources. Scientific understanding of which native plants support specific pollinators (bees, butterflies) and local wildlife is critical. This transforms isolated garden beds into vital nodes in a larger regional ecological network, aiding biodiversity conservation. Thus, the design incorporates species with persistent fruits (*Rosa canina*, *Cornus* sp., *Mespilus* sp.), turning the garden into a year-round bird sanctuary.

One the most significant impacts is the cultural shift it enables. Research on the ecological value of plants enables designers to better communicate the meaning of the landscape to beneficiaries. A planting design is no longer just a collection of plants; it becomes an educational tool that showcases the beauty of the region's natural heritage and the necessity of ecological stewardship.

Economical argumentation: reduced inputs. Because native plants are adapted to local soils and pests, designers can justify a lower-maintenance regime that avoids the use of synthetic pesticides and fertilizers, which is better for the local watershed.

After the final design and execution of the landscaping, a schedule was drawn up with areas regarding the garden arrangement (Table 3) and the final expenses resulting from the arrangement (Table 4).

Table 3. Surfaces for garden landscaping

Surface	Sqm	% of total area
Total surfaces	1300	100
Built surface, from which:	400	30.77
- Buildings	150	11.53
- Alleys	250	19.23
Green space	400	30.77
Lawn surface	500	38.46

Table 4. Final expenses

Nr. crt.	Name of the work	U.M.	Lei	Total costs - lei
1	Lawns	Sqm	500	11880
2	Planting the woody material vegetal lemnos	Sqm	95	3000
3	Flower species	Sqm	25	250
Total costs for landscaping				15130.00

Sustainability means low costs. Total cost for the arrangement and execution of the private garden in the town of Brăești, Botoșani County, is 13882.00 lei.

Ornamental argumentation. The use of native species preserves the spirit of the place and

creates visual harmony between the garden and the surrounding natural landscape.

A very important aspect is related to the high aesthetic value and to emphasizing local identity. Sustainability does not mean a "neglected" look, but a new visual perspective. The use of native species preserves the spirit of the place and creates visual harmony between the garden and the surrounding natural landscape. Proper design provides year-round visual interest by using winter textures or persistent fruits, not just summer flowers.

Last but not least, the value of the theme can also reside in its psychological and educational impact, because the garden becomes a space for authentic connection with nature. Studies show that biodiverse ecosystems have a stronger therapeutic effect than rigid, artificial landscapes. The owner becomes a custodian of nature, observing natural cycles without trying to dominate them.

Present proposal. For the example presented, to carry out the landscaping works, the following stages were completed:

- Land preparation works. Land development includes the following activities: clearing the land; clearing the land of shrubs and bushes; digging and removing the vegetation layer; levelling the land; carrying out excavations.
- Execution of paving works. Before installing the pavers, the quality of the base layer will be carefully checked, including the regularity of the last layer in longitudinal and transverse sections and the way in which compaction is carried out. The implementation of the sidewalks was preceded by the implementation of the curb frames.

- Woody vegetation planting works. For planting woody species, the land is marked out according to the development plan for the planting period after the completion of all other components of the green space. On the site of the stakes, 50x50x50 holes are made for trees, 40x40x40 for isolated shrubs, and trenches for hedges. Watering was done after planting using approximately 20 litres of water/plan (Bernardis et al., 2022).

- Lawn installation. For the lawn, it is considered the use of *Sedum* sp. (Figure 6) and *Viola* sp. (Figure 7). The advantage of using these species is that they ensure good ground cover and, most importantly, no irrigation is needed, especially

taking into consideration that the lawn surface is almost 40% of the garden. Thus, it is created a *green mulch* effect that mimics a forest floor, cooling the soil and naturally preventing weed growth without chemicals. Another argument for the use of native plants, especially those with deep, robust root systems, is that they act as a "sponge" for the landscape, facilitating better rainwater infiltration, reducing surface runoff, and preventing erosion.



Figure 6. The aspect of the lawn using *Sedum* sp.



Figure 7. The aspect of the lawn using *Viola* sp.

The choice of native species was made because they are biologically adapted to the climatic conditions of the region (soil, rainfall regime, and extreme temperatures), once established, local species require minimal to no irrigation compared to exotic species. Also, these plants have developed natural resistance to local pests, eliminating the need for pesticides or synthetic fertilizers. A comparison between the traditional residential design and the native species-based design is summarized in Table 5.

Table 5. Comparison between traditional vs. native design

Feature	Traditional residential design	Native species design
Watering	Frequent; relies on irrigation systems.	Minimal once established; it relies on rainfall.
Fertilizer	Regular applications of synthetic NPK.	Rarely needed; plants adapt to local soil.
Wildlife fauna (birds, butterflies)	Often repelled or ignored.	Actively invited as part of the "garden show."
Winter interest	Often "cut back" to bare soil.	Stalks and seed heads left for winter beauty and insect habitat.

CONCLUSIONS

The current study shows us that setting up a sustainable private garden using native species is not only a project of landscape aesthetics, but also an act of conservation of local resources. The most obvious conclusion is related to increased resource efficiency and reduced maintenance. An important conclusion is related to the potential of these species to restore micro-ecosystems. A sustainable garden functions as an *ecological corridor* that can support pollinators. Native species provide specific food and shelter for bees, butterflies, and birds in the area. Also, the roots of native plants, often much deeper than those of the classic lawn, improve soil structure and prevent erosion. So, the shift to sustainable landscaping represents a paradigm: the transition from the garden viewed as a *decorative object* that consumes resources to the garden viewed as a living system that produces ecological benefits.

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FLOWER AND FLOWERING PECULIARITIES OF SOME *SALVIA* SPECIES CULTIVATED IN ROMANIA

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Abstract

This study addresses the morphological peculiarities of the flower and flowering in several Salvia species, a genus of significant biological, ornamental, and utilitarian importance. The genus Salvia belongs to the family Lamiaceae, which represents a plant group with remarkable morphological diversity, characterized by its bilabiate flowers and the unique staminal lever mechanism, adapted to various specific modes of pollination. The morphological and functional analysis of the flower in the Salvia species under investigation (Salvia officinalis, Salvia splendens, Salvia farinacea, and Salvia coccinea, cultivated in Romania) aims to identify specific traits by relating them to the general floral plan of the genus Salvia and, respectively, the family to which it belongs, Lamiaceae. This paper integrates data from both Romanian and international specialized literature, which will subsequently be complemented by original experimental results. These results focus on the elements of the floral structure (bracts, calyx, corolla, androecium, gynoecium, fruit) and the phenology of flowering.

Key words: Lamiaceae, Salvia, flower, androecium, ornamental.

INTRODUCTION

The importance of flowers in contemporary society is undeniable, as they represent a constant presence in everyday human life, both in public and private spaces, and occupy a special place in the arts - painting, poetry, and music. In some countries, the appreciation of flowers is reflected in their elevation to the status of national symbols, such as the chrysanthemum in Japan, the tulip in the Netherlands, and the rose in the United States of America (Toma Fl., 2009).

The diversity of floral morphology and function can be explained as an adaptation of plants to pollinators and to different reproductive strategies (Han-Wen Xiao et al., 2023).

The family *Lamiaceae*, belonging to the angiosperms (*Magnoliophyta*), is distinguished by the morphological and functional coherence of its flowers, which represent a classical model for studying evolutionary adaptations associated with entomophilous pollination.

Among the representative genera of the family *Lamiaceae*, *Salvia* holds a central position due to its high species diversity and the morphological innovations that have contributed to the adaptive radiation of the

group (Claßen-Bockhoff, 2007; Walker & Sytsma, 2007).

The present study aims to provide a comparative characterization of floral structure and the flowering process in four representative species of the genus *Salvia* cultivated under field conditions, namely *Salvia officinalis*, *Salvia splendens*, *Salvia farinacea*, and *Salvia coccinea*.

Salvia species are widely used in the design of flower beds, roundabouts, and floral patches, and are also cultivated as potted plants and in planters for balconies and terraces (Toma Fl., 2003).

Owing to their drought tolerance, *Salvia* plants may represent ideal ornamental species for use in arid and semi-arid areas, particularly within the context of xeriscaping - a landscaping or gardening approach that reduces or eliminates the need for irrigation. Native plant species are especially suitable for xeriscaping, as they exhibit low water requirements, excellent adaptability to local soil and climatic conditions, high ornamental value, and contribute to the preservation of landscape character. Furthermore, *Salvia* species may also prove valuable for use on urban green roofs (Papafotiou et al., 2021).

Through the analysis and synthesis of data from the specialized literature, combined with the integration of original results obtained from experimental research, this study seeks to highlight the morpho-functional traits that support the adaptability of *Salvia* flowers to environmental factors specific to south-eastern Romania. This perspective contributes to a better understanding of the relationships between floral structure, pollination ecology, and ornamental value, while also providing arguments for the use of the genus *Salvia* in the improvement and diversification of ornamental and medicinal crops.

Floral Structure in *Lamiaceae*

The flower, as a functional unit of sexual reproduction, balances the reproductive requirements of the species by achieving a compromise between pollen presentation as a floral attractant and pollen economy for successful reproduction (Claßen-Bockhoff, 2007).

A complete flower is composed of the following elements: pedicel, receptacle, floral envelope (perianth), androecium, and gynoecium (Săvulescu, 2009).

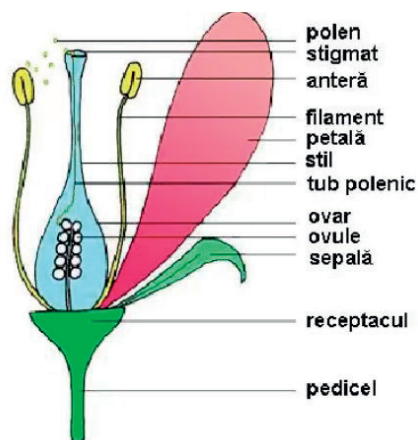


Figure 1. Morphology of a Complete Flower, according to Săvulescu, 2009

Source: <https://www.scribd.com/doc/236016038/Elena-Savulescu-Botanica-Morfologia-I#page=90>

The family *Lamiaceae*, also known as *Labiatae*, represents one of the largest and most extensively studied families of dicotyledons, many of whose members are recognized for their medicinal, aromatic, and ornamental

importance. The floral architecture of *Lamiaceae* has been regarded by several authors (Claßen-Bockhoff, 2007; Rather et al., 2018) as a representative example of the evolutionary adaptation of floral morphology to specialized entomophilous pollination.

This family, comprising over 7,000 species and approximately 230 genera (Harley et al., 2004), includes predominantly aromatic, herbaceous plants, and more rarely woody forms represented by shrubs and subshrubs, characterized by quadrangular stems and opposite leaves lacking stipules. The inflorescences are typically verticillate, a diagnostic feature of the family; the flowers are zygomorphic, hermaphroditic, and arranged in cymose inflorescences. The calyx is gamosepalous, frequently bilabiate, while the corolla is gamopetalous, bilabiate, and associated with a double perianth. The androecium is didynamous, consisting of four stamens - two with longer filaments and two with shorter filaments - inserted on the corolla tube. The gynoecium is bicarpellate and syncarpous, with a superior ovary, and the fruit is a schizocarpic mericarp, typically a tetrachene. Members of the family are characterized by the presence of glandular hairs that secrete essential oils (Toma, 2003; Pădure, 2006; Ciocărlan, 2009; Georgescu, 2014).

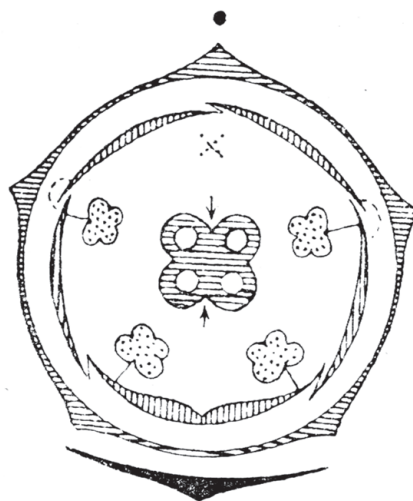


Figure 2. Floral Morphology in *Lamiaceae* (ex.: *Lamium*). Public domain, after Strasburger et al., 1900
Source: https://commons.wikimedia.org/wiki/File%3ALamium_flowerdiagram.png?utm

The corolla is gamopetalous and bilabiate, organized into a concave upper lip (formed by two fused petals) and a trilobed lower lip (formed by three connate petals), a fundamental morphological adaptation for guiding pollinators (Claßen-Bockhoff, 2007).

The androecium typically consists of four didynamous stamens (two longer and two shorter), inserted on the corolla; however, in numerous genera, a reduction in stamen number to two functional stamens is observed, a phenomenon interpreted as an evolutionary innovation (Walker & Sytsma, 2007).

The gynoecium is bicarpellate and syncarpous, but appears four-lobed due to the formation of four false ovary locules. The gynobasic style and bifid stigma are diagnostic features of the family (Pădure, 2006).

At the base of the ovary there is a well-developed annular nectary, described by Konarska et al. (2022) as a secretory tissue rich in epithelial cells containing sugar-filled vacuoles.

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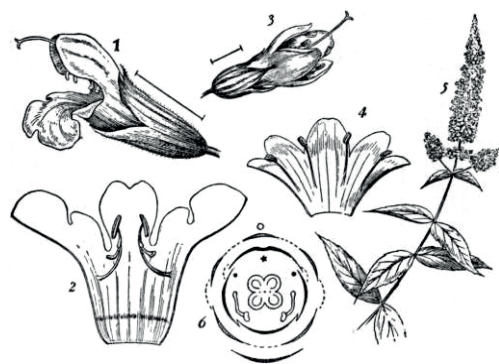


Figure 3. Floral diagram and floral details in *Salvia officinalis* (Encyclopaedia Britannica, 1911, public domain)

Source: https://commons.wikimedia.org/wiki/File%3AEB1911_-_Labiatae_-_Fig._2.%E2%80%94%2C_Flower_of_Sage_%28Salvia_officinalis%29.jpg?utm

Particular Features of the Flower in the Genus *Salvia*

Belonging to the family *Lamiaceae*, the genus *Salvia* comprises over 700 annual and perennial species, herbaceous or suffrutescent, characterized by hollow, quadrangular stems,

and originating from warm and temperate regions of the world (Toma Fl., 2009).

In the following figure, a schematic longitudinal section of a “typical” *Salvia* flower is presented, illustrating the functioning of the staminal lever mechanism (Reith et al., 2006).

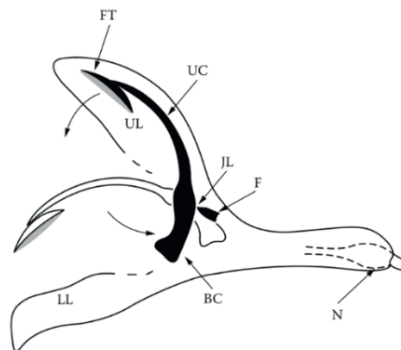


Figure 4. Schematic longitudinal section of a “typical” *Salvia* flower showing the functioning of the staminal lever mechanism. For clarity, the style and second stamen are omitted. Black: staminal lever arm in unreleased position, white: in released position. UL: upper lip of the flower, LL: lower lip of the flower, F: filament, JL: jointlike ligament, UC: upper part of the elongated connective with the fertile theca (FT), BC: basal, sterile part of the connective, N: nectar glands. (Modified from Speck, T. et al., in *Deep Morphology: Toward a Renaissance of Morphology in Plant Systematics*, T. Stuessy, T., F. Hörandl, F., and Mayer, V., Eds., Koeltz, Königstein, 2003, p. 241)

Source: <http://www.anthonhyherrel.fr/publications/Herrel%20et%20al%202006%20Ecology%20&%20Biomechanics.pdf>

The androecium, composed of two fertile stamens, employs a mobile connective that functions as a lever. Upon contact with an insect, the connective arm rotates the anther, depositing pollen onto the dorsal surface of the pollinator (Claßen-Bockhoff et al., 2004). In subsequent flowers, the stigma collects pollen from the same body region, ensuring efficient nototribic pollination (Walker & Sytsma, 2007).

The gynoecium is four-lobed, with a gynobasic style and a bifid stigma. The annular nectary located at the base of the ovary secretes nectar rich in sugars and volatile oils (Konarska et al., 2022), which attract pollinators.

Corolla shape and size vary among species: *Salvia officinalis* and *Salvia nemorosa* exhibit violet corollas with short tubes, adapted to bee pollination, whereas *Salvia splendens* has a red corolla and *Salvia coccinea* presents a pinkish-

milky tubular corolla, both adapted to bird pollination (Kriebel et al., 2020).

The genus *Salvia* includes species that possess only two stamens, each with a single fertile theca (Georgescu, 2014).

With regard to the *Salvia* species that constitute the subject of the present research, the specialized literature has identified several approaches addressing the aspects of interest, as follows:

Salvia splendens

The plant is native to Brazil, where it is perennial and locally used as a medicinal and culinary plant. It does not tolerate freezing temperatures but can be grown as an annual in colder climates (Clebsch et al., 2003). Under the climatic conditions of Romania, it is cultivated as an annual species, flowering from June until late autumn, when the first frosts occur (Toma Fl., 2009).

Salvia splendens is an attractive and valuable ornamental plant, widely used in flower beds and frequently employed for decorating parks and urban green spaces. It is suitable for borders, rock gardens, and container cultivation. The species flowers profusely and is well adapted for large-scale plantings in public areas. It effectively fills empty spaces and is often used in combination with other summer flowers. It displays a particularly striking appearance when associated with blue lobelia, purple-flowered ornamental sunflower cultivars, or yellow marigolds. *Salvia splendens* grows best on sandy-loam, fertile soils that are well cultivated and moderately moist (Błażewicz-Woźniak et al., 2012).

The plant is branched from the base and exhibits a pyramidal growth habit, reaching heights of 25-50 cm. The stem and shoots are intensely green, with violet to dark purplish hues at the base. Leaves are petiolate, oval to elliptic, with crenate margins and an acuminate apex, arranged oppositely along the stem. The flowers are bilabiate, predominantly red in colour, and arranged in terminal spiciform racemes. The flowers persist for a relatively short period; however, after their abscission, the decorative effect is maintained by the persistent, cup-shaped calyx, divided apically into three teeth and coloured in vivid shades of red, pink, white, or violet. Flowering occurs from June until late

autumn, coinciding with the onset of frost (Toma Fl., 2009).



Figure 5. *Salvia splendens* (SCARLET SAGE) botanical illustration (Favourite flowers of garden and greenhouse /by Edward Step, public domain)
Source: [https://commons.wikimedia.org/wiki/File:Favourite_flowers_of_garden_and_greenhouse_\(10593932836\).jpg](https://commons.wikimedia.org/wiki/File:Favourite_flowers_of_garden_and_greenhouse_(10593932836).jpg)

Salvia splendens, characterized by a bright red calyx and corolla, is cultivated as an ornamental plant (Munteanu et al., 2007; Munteanu et al., 2016).

Salvia officinalis

Also known locally as *jaleș* or *garden sage*, is a perennial subshrub or shrub native to the eastern Mediterranean region, characterized by a lignified rhizome that is branched within the soil and bears numerous roots. The stem reaches 30-80 cm in height, is strongly branched, lignified at the base, and quadrangular in its upper portion. The plant has a characteristic aroma, while the leaves exhibit a bitter, aromatic taste. *Salvia officinalis* is cultivated as an ornamental, aromatic, and medicinal plant (Muntean, 1990; Munteanu et al., 2007; Georgescu, 2014).

It is a perennial subshrub with a well-developed, highly branched root system, extending both vertically and horizontally, a feature that confers

increased resistance to water deficit. The stem is covered with fine, greyish-white, appressed hairs; the leaves are elongate-ovate to lanceolate, with prominent reticulate venation on the abaxial surface. The flowers are blue-violet in colour and are arranged in verticillasters with a spiciform appearance. Flowering occurs in June-July. The fruits are dark brown to blackish tetrachenes, ovoid in shape, grouped in fours at the base of the calyx (Luchian, 2017).

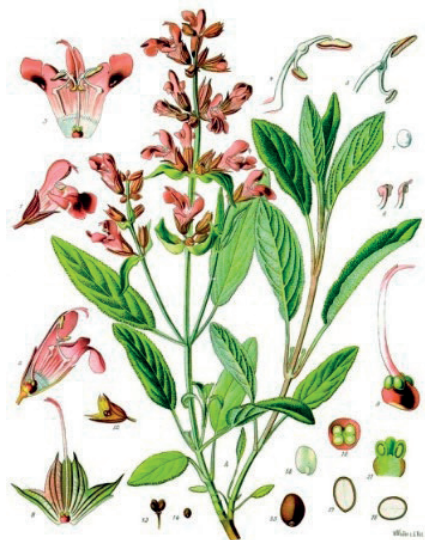


Figure 6. *Salvia officinalis* (JALES) - botanical illustration (List of Koehler Images /by Walther Otto Müller, public domain)

Source:https://commons.wikimedia.org/wiki/File:Salvia_officinalis_-_K%C3%B6hler%E2%80%93Medizinal-Pflanzen-126.jpg

Salvia coccinea

The plant grows as a compact shrub, reaching a height of 60-70 cm, and is entirely covered with small, grey hairs. The leaves are ovate-cordate, dark green on the adaxial surface and greyish-green on the abaxial surface. The flowers are small, bright red in colour, and arranged in spaced verticillasters forming terminal spikes. The calyx is green, cup-shaped, and divided apically into three short teeth. Flowering occurs from July until late autumn (Toma Fl., 2009). This species is an excellent ornamental plant for public green areas and private gardens and exhibits moderate tolerance to salinity (Grzeszczuk et al., 2018).



Figure 7. *Salvia coccinea* Buchoz – herbarium (T. F. Cheeseman/Place of creation-Cook Islands. Collection Auckland War Memorial Museum Tamaki Paenga Hira) Source:[https://commons.wikimedia.org/wiki/File:Salvia_coccinea_Buchoz_\(AM_AK94432\).jpg](https://commons.wikimedia.org/wiki/File:Salvia_coccinea_Buchoz_(AM_AK94432).jpg)

Salvia farinacea



Figure 8. *Salvia farinacea* – botanical illustration (PLATE 119 ADDISONIA,

The New York Botanical Garden - 1918)

Source:[https://commons.wikimedia.org/wiki/File:Addisonia_colored_illustrations_and_popular_descriptions_of_plants_\(1916-1964\)\)_\(16771647711\).jpg](https://commons.wikimedia.org/wiki/File:Addisonia_colored_illustrations_and_popular_descriptions_of_plants_(1916-1964))_(16771647711).jpg)

Salvia farinacea reaches a height of 50-60 cm and is strongly branched from the base. The

leaves are ovate-lanceolate and borne on long petioles. Both the flowers and the calyx are small, arranged in verticillasters within terminal spiciform inflorescences, and are covered with a thin, farinose, waxy layer. The flowers, like the calyx, are coloured in various shades of white, blue, or violet. Flowering occurs from July until late autumn, extending into October–November (Toma Fl., 2009).

According to the description provided by George V. Nash in *Addisonia – Colored Illustrations and Popular Descriptions of Plants*, vol. 3 (1918), *Salvia farinacea* is a perennial species whose self-sown seeds germinate readily in spring, producing an abundance of seedlings that require vigorous thinning.

MATERIALS AND METHODS

The research was conducted on four species of the genus *Salvia*. Considering, on the one hand, the high species diversity within the genus *Salvia* and, on the other hand, the specific characteristics of its members, the study was designed so that the selected species would cover as much as possible of the existing range with respect to the duration of the biological cycle, namely annual, biennial, and perennial plants (Toma Fl., 2009).

Accordingly, perennial and annual species were selected for the present study, as follows:

– *Salvia officinalis* - a perennial plant with underground organs resistant to low temperatures, capable of flowering and fruiting every year (Draghia L., 2011). Also referred to as perennial (*vivacious*) plants, these species have a biological cycle extending over several years and are characterized by the presence of a dormancy period induced by adverse environmental conditions in their area of origin (Toma Fl., 2009).

– *Salvia splendens*, *Salvia coccinea*, and *Salvia farinacea* – annual plants in which the entire biological cycle is completed within a single growing season (Draghia L., 2011). These species are characterized by rapid growth and highly decorative inflorescences. In particular, *Salvia splendens* is well recognized for its prominent role in ornamental plantings, flowering continuously throughout the summer and until late autumn (Toma Fl., 2009).

The experimental field was established in Brănești, Romania, located in the south-eastern region of the country. Soil preparation included ploughing to a depth of 25-30 cm, followed by the preparation of planting plots.

The species were cultivated in plots with an area of 1.2 m² (1.2 m in length × 1.0 m in width), with a spacing of 0.5 m between rows and between plants within the row. The plots were arranged linearly, forming a Latin rectangle design, with each variant replicated three times and arranged in a randomized layout.

To ensure planting material, seeds were procured from certified suppliers of planting material, namely *Salvia splendens* cv. Blaze of Fire, *Salvia farinacea* cv. Blue Victoria, *Salvia coccinea* cv. Summer Jewel Pink, and *Salvia officinalis*.

Seed germination was carried out in the greenhouses of the Faculty of Horticulture within the University of Agronomic Sciences and Veterinary Medicine of Bucharest. Seedlings were transplanted approximately three weeks after emergence; at the same time, the apical growth tip was pinched in order to promote improved plant branching. Transplanting into the open field was performed in May, after the risk of late frosts had passed.

Within the field maintenance technology applied to the experimental plot, particular attention was given to irrigation, given its major importance for plant growth and development. Immediately after transplanting, individual watering of the seedlings was carried out. Subsequently, irrigation during the growing season was performed by furrows, as required, taking into account plant water requirements in relation to their phenological stage, environmental factors, and the period of the year (Toma Fl., 2009).

In addition, maintenance of the experimental field involved weed control and soil loosening; for this purpose, specific practices such as hoeing and manual weeding were applied whenever necessary.

Plant material was collected at the full flowering stage and consisted of a sample of 10 flowers from each studied species, harvested from the experimental field under similar conditions of light exposure, moisture, and soil characteristics.

Morphological analyses focused on the identification of the distinctive characteristics of the flower (calyx, corolla, androecium, gynoecium, fruit), the inflorescence, and the flowering period. Standard macro and microscopic description methods were employed, with morphological observations and measurements performed in millimetres (mm), as well as microphotographs taken using a light microscope. Flower dissections were carried out manually using a blade and forceps.

RESULTS

Salvia splendens

The inflorescence of *Salvia splendens* is a pyramidal spike composed of 15-22 intensely red flowers.

The bracts are modified leaves, intensely red in colour, positioned at the base of the floral peduncle. They have an average length of 7.5 mm, with entire margins and an acuminate apex. The epidermis contains anthocyanin pigments responsible for the intense coloration, conferring a decorative appearance that persists even after corolla wilting.

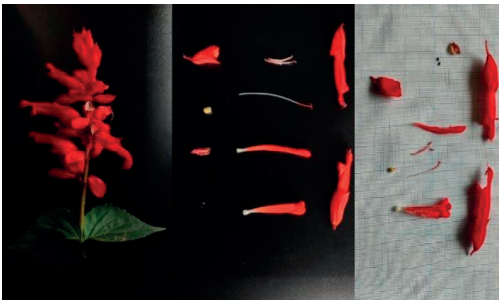


Figure 9. Inflorescence and flower in *Salvia splendens*
Source: original

The *calyx* is tubular, intensely red in colour, with an average length of 19 mm and a basal diameter of 4-5 mm. It is composed of five fused sepals ending in acute lobes. Its role is to protect the corolla and internal organs during the bud stage, and after fertilization it remains attached to the peduncle, enclosing the fruit.

The *corolla* has an average length of 37.3 mm and is formed of connate petals arranged in a tubular, bilabiate structure. The upper lip, measuring approximately 37.2 mm in length, is curved and covers the androecium and

gynoecium, while the lower lip is about 30.2 mm long, trilobed, and serves as a landing platform for pollinators. The intense red coloration is due to anthocyanin accumulation, and the elongated tubular shape represents an adaptation to entomophilous pollination.

Table 1. Morphometric data (biometric-morphological)
Salvia splendens

Floral elements	M.S. (mm)	R.V. (mm)	Morphological observations
Bracts	7,5	7-8	Intensely red in color, oval in shape, and prominent at the base of the peduncle
Sepal calyx	19	18-20	Tubular, persistent, dark red, composed of five fused sepals
Corolla – petals upper lip (labium superius) lower lip (labium inferius)	37,2	36-39	Tubular, bilabiate, intensely red, with a curved upper lip
Androecium – stamens – filament	17	16-18	Two fertile stamens, with red filaments and transversely oriented anthers
Androecium – stamens – anthers	3	3	The anthers are basifixed, with distinct thecae
Gynoecium – style and stigma	31,2	29-32	Style filiform; stigma bifid
Gynoecium – ovary	4	4	Superior, tetralocular ovary
Fruit (nutlets)	2	2	Four small, oval, black, glossy nutlets

M.S. - Medium size;
R.V. - Range of variation.

The *androecium* consists of two fertile stamens positioned beneath the upper lip of the corolla. The filaments are slender, red in colour, with an average length of 17 mm, and bear transversely oriented anthers. This structure forms a lever-like mechanism characteristic of the genus *Salvia*.

The *gynoecium* is bicarpellate, bearing a filiform style with an average length of 31.2 mm and an ovary approximately 4 mm in diameter, divided into four locules, each containing a single ovule.

The stigma is bifid and slightly exceeds the corolla. The position and length of the style facilitate effective contact between the stigma and pollinators.

The *fruit* is a tetranutlet, composed of four small, oval, black nutlets with an average size of 2 mm. At maturity, the nutlets are released through the opening of the calyx.

Flowering period - following transplanting and pinching performed to stimulate branching, the first flowers appeared approximately four weeks later, with flowering progressing dynamically from the base towards the apex of the inflorescence. Peak flowering occurs during July-September, when average temperatures of 27-30°C are favourable for blooming. Under open-field cultivation conditions in the lowland area of south-eastern Romania, *Salvia splendens* exhibits an extended flowering period.

Salvia officinalis

Salvia officinalis exhibits a terminal inflorescence (at the apex of the stem), composed of flowers arranged in verticillasters (2–6 flowers per whorl), protected by bracts. The flowers have a bilabiate corolla of blue-violet colour and are arranged so as to form a spike or raceme at the end of the stem.



Figure 10. Inflorescence and flower in *Salvia officinalis*
Source: original

The *bracts* are modified leaves, greenish in colour, positioned at the base of the floral peduncle. They have an average length of 11 mm, an ovate to lanceolate shape, with an acute apex, green with slightly violaceous tinges at the base, and are prominent at the peduncle base.

The *calyx* is tubular, green in colour, with an average length of 9 mm, slightly pubescent, and a basal diameter of 4-5 mm. It is formed of fused sepals terminating in acute lobes. Its function is

to protect the corolla and internal organs at the bud stage, and after fertilization it remains attached to the peduncle, enclosing the fruit.

The *corolla* has an average length of 24 mm and is composed of connate petals arranged in a tubular, bilabiate structure of bluish-lilac colour, with a velvety texture. The upper lip is narrow, slightly arched, approximately 24 mm long, covering the androecium and gynoecium, while the lower lip is about 25 mm long, trilobed, with a larger median lobe serving as a landing platform for pollinators.

Tabel 2. Morphometric data (biometric-morphological)
Salvia officinalis

Floral elements	M.S. (mm)	R.V. (mm)	Morphological observations
Bracts	11	10-12	Ovate to lanceolate in shape, with an acute apex, green in colour with slightly violaceous tinges at the base, and prominent at the base of the peduncle.
Sepal calyx	9	8-10	Tubular, persistent, greenish in colour.
Corolla – petals upper lip (labium superius) lower lip (labium inferius)	24	23-25	Tubular at the base, bilabiate, bluish-lilac in colour, with a concave upper lip and a trilobed lower lip bearing a larger median lobe.
Androecium – stamens – filament	20	19-21	Two fertile stamens, with white to violet filaments, finely pubescent, and transversely oriented anthers.
Androecium – stamens – anthers	2	2	Golden-yellow in colour.
Gynoecium – style and stigma	25	24-26	Style filiform; stigma bilobed
Gynoecium – ovary	4	4	Ovary tetralobed in shape, greenish in colour.
Fruit (nutlets)	2	2	Four small, oval, black, glossy nutlets

M.S. - Medium size;
R.V. - Range of variation.

The *androecium* consists of two fertile stamens located beneath the upper lip of the corolla, modified into a staminal lever mechanism

characteristic of the genus *Salvia officinalis*. When an insect enters the corolla to collect nectar, it touches the lower arm of the stamen, causing the fertile theca to be lowered onto the insect. Pollen is deposited on the back of the pollinator and subsequently transferred to the next flower. The filaments are slender, lilac in colour, with an average length of 20 mm, and bear transversely oriented anthers.

The *gynoecium (pistil)* is bicarpellate, bearing a filiform style with an average length of 25 mm, slightly exceeding the corolla, and an ovary approximately 4 mm in diameter, divided into four locules, each containing a single ovule. The stigma is bifid and protrudes beyond the corolla. The position and length of the style facilitate effective contact between the stigma and pollinators.

The *fruit* is a tetranutlet, composed of four small, oval, black nutlets with an average size of 2 mm. At maturity, the nutlets dry and are released through the opening of the calyx.

The *flowering period* occurs in the second year after planting; following pinching performed to stimulate branching, the first flowers appear from May to July, opening progressively from the base towards the apex of the inflorescence. The intense violet coloration of the corolla combined with the silvery-green foliage confers a pleasant ornamental effect, making the species suitable for borders, planters, and mixed green spaces.

Salvia coccinea



Figure 11. Inflorescence and flower in *Salvia coccinea*
Source: original

Salvia coccinea cv. Summer Jewel Pink is a compact plant characterized by an elongated, lax spiciform inflorescence, with tubular flowers grouped in terminal racemes measuring 15-25

cm in length. The flowers are approximately 2-3 cm long and exhibit a white to pink coloration, with a bilabiate corolla in which the upper lip is relatively straight and the lower lip is more open.

The flower is zygomorphic and hermaphroditic, borne on a pedicel attached to the inflorescence axis at the level of a small green bract. Flowering extends from early summer until autumn. The flowers are attractive to butterflies, bees, hummingbirds, and other pollinators.

The *bracts* are modified leaves, green in colour, positioned at the base of the floral peduncle. They have an average length of 2.5 mm and an acuminate apex.

Tabel 3. Morphometric data (biometric-morphological)
Salvia coccinea cv. Summer Jewel Pink

Floral elements	M.S. (mm)	R.V. (mm)	Morphological observations
Bracts	2.4	2-3	Intensely green in colour, oval in shape, and prominent at the base of the peduncle
Sepal calyx	9	8 – 10	Tubular, persistent, green in colour, tridentate
Corolla – petals upper lip (labium superius) lower lip (labium inferius)	24	23-25	Tubular, bilabiate, milky-pink in colour, with a narrow upper lip
Androecium – stamens – filament	30.8	30-32	Two fertile stamens, with white to pink filaments and transversely oriented anthers
Androecium – stamens – anthers	2	2	Whitish or slightly yellowish in colour.
Gynoecium – style and stigma	31.2	30 -33	Style filiform; stigma bifid
Gynoecium – ovary	4	4	Superior, tetralocular ovary
Fruit (nutlets)	2	2	Four small, oval, black, glossy nutlets

M.S. - Medium size;
R.V. - Range of variation.

The *calyx* is tubular, green in colour, with an average length of 9 mm, slightly pubescent, and a basal diameter of 4-5 mm. It is composed of

fused sepals terminating in acute lobes. Its function is to protect the corolla and internal organs at the bud stage, and after fertilization it remains attached to the peduncle, enclosing the fruit.

The *corolla* has an average length of 24 mm and is formed of connate petals arranged in a tubular, bilabiate structure of milky-pink colour, with a velvety texture. The upper lip is narrow, slightly arched, measuring approximately 24.2 mm in length and covering the androecium and gynoecium, while the lower lip is about 23 mm long, trilobed, and serves as a landing platform for pollinators.

The *androecium* consists of two fertile stamens positioned beneath the upper lip of the corolla, modified into a staminal lever mechanism characteristic of the genus *Salvia*. When an insect enters the corolla to collect nectar, it contacts the lower arm of the stamen, triggering the downward movement of the fertile theca onto the insect. Pollen is deposited on the dorsal surface of the pollinator and subsequently transferred to the next flower. The *filaments* are slender, pink in colour, with an average length of 31 mm, slightly protruding from the corolla tube, and bear transversely oriented anthers.

The *gynoecium (pistil)* is bicarpellate, bearing a filiform style with an average length of 31 mm, slightly exceeding the corolla, and an ovary approximately 4 mm in diameter, divided into four locules, each containing a single ovule. The stigma is bifid and protrudes beyond the corolla. The position and length of the style facilitate effective contact between the stigma and pollinators.

The *fruit* is a tetranutlet, composed of four small, oval, black nutlets with an average size of 2 mm. At maturity, the nutlets are released through the opening of the calyx.

The *flowering* period extends from May to October following transplanting and pinching performed to stimulate branching. Flowering is abundant and prolonged, progressing from the base towards the apex of the inflorescence. The pastel pink coloration of the corolla combined with the intense green foliage confers a pleasant ornamental effect, making the species suitable for borders, planters, and mixed green spaces. Pruning is required to stimulate continuous flowering, which may extend from spring until the first frost.

Salvia farinacea

The plant bears flowers arranged in elongated, spiciform inflorescences with an average length of 22 cm, organized in verticillasters along the floral axis. The flowers are hermaphroditic, zygomorphic, and bilabiate.

The *bracts* measure approximately 3 mm in length, are greenish-grey with bluish tinges at the base, elliptic, thin, caducous, and slightly farinose (with a whitish, powdery appearance). They have a protective role and fall after flowering.

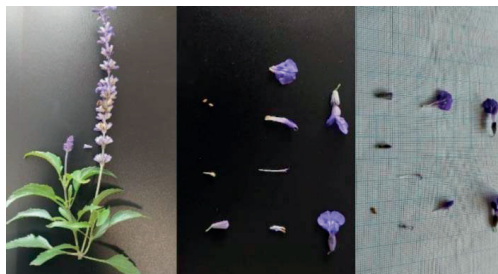


Figure 12. Inflorescence and flower in *Salvia farinacea* cv. Blue Victoria
Source: original

The *calyx* is tubular, with an average length of 8 mm, persistent, blue-violet in colour, and covered with greyish-white hairs, giving it a farinose (powdery) appearance. It bears short, unequal teeth and remains decorative even after corolla abscission, displaying a greyish-bluish colour.

The *corolla* has an average length of 16 mm; it is tubular, narrow at the base and widening towards the upper part, bilabiate, and of an intense blue colour. The upper lip is concave, forming a hood that covers the androecium, while the lower lip is trilobed, with a large, flat median lobe. The corolla has a velvety texture, and the corolla tube displays nectar guide markings.

The *androecium* consists of two fertile stamens positioned beneath the upper lip of the corolla, modified into a staminal lever mechanism characteristic of the genus *Salvia*. When an insect enters the corolla to collect nectar, it contacts the lower arm of the stamen, triggering the downward movement of the fertile theca onto the insect. Pollen is deposited on the dorsal surface of the pollinator and subsequently transferred to the next flower. The stamens have short filaments, with an average length of 3 mm,

and an elongated, articulated connective measuring 6-8 mm, which functions as a staminal lever.

Tabel 4. Morphometric data (biometric-morphological)
Salvia farinacea cv. Blue Victoria

Floral elements	M.S. (mm)	R.V. (mm)	Morphological observations
Bracts	3	2-4	Greenish-grey in colour with bluish tinges at the base, elliptic, thin, caducous, and slightly farinose (with a bluish powdery appearance); protective in function and falling after flowering.
Sepal calyx	8	7-9	Tubular, persistent, blue-violet in colour, covered with greyish-white hairs (farinose), with short, unequal teeth; remaining decorative after corolla abscission, displaying a greyish-blue colour.
Corolla – petals upper lip (labium superius) lower lip (labium inferius)	16	15-17	Tubular, narrow at the base and widening towards the upper part, bilabiate, of intense blue colour; the upper lip is concave, forming a hood that covers the androecium, while the lower lip is trilobed, with a large, flat median lobe. The corolla has a velvety texture, and the corolla tube exhibits nectar guide markings.
Androecium – stamens – filament	3	3-5	Two fertile stamens, with short filaments and an elongated connective measuring 6-8 mm in length.
Androecium – stamens – anthers	2	2	Stamens glabrous, bluish to pale violet in colour.
Gynoecium – style and stigma	13	12-14	Style filiform; stigma bifid, with slightly curved lobes.
Gynoecium – ovary	4	4	Superior, tetralocular ovary
Fruit (nutlets)	1	1	Four small, oval, black, glossy nutlets

M.S. - Medium size;
R.V. - Range of variation.

The *gynoecium (pistil)* is bicarpellate with fused carpels, bearing a filiform style with an average length of 13 mm, slightly exceeding the corolla, and an ovary approximately 4 mm in diameter, divided into four locules, each containing a single ovule. The stigma is bifid.

The *fruit* is a tetranutlet, composed of four small, oval, black nutlets with an average size of 2 mm. At maturity, the nutlets are released through the opening of the calyx.

The *flowering period* extends from June to October, with flowering lasting approximately five months. Flowers open progressively from the base towards the apex of the inflorescence, ensuring continuous blooming. Removal of senescent inflorescences is required to promote re-flowering. The high spike density, large inflorescence size, extended flowering duration, intense blue coloration with violet tinges, and good field performance confer a pleasant ornamental effect, making the species suitable for borders and mixed green spaces.

DISCUSSIONS

The analysis of the morphological results of the inflorescences of the six *Salvia* species included in this study highlights, on the one hand, their structural and chromatic diversity and, on the other hand, their adaptive strategies, while also emphasizing their ornamental value.

With regard to shared characteristics, it was found that all species included in the study exhibit spiciform inflorescences composed of flowers arranged in verticillasters along the entire length of the floral axis.

The distinguishing elements that highlight the differences among species are related to the length of the floral axis, the number of floral verticillasters, and flower density, as follows:

- *Salvia splendens* cv. Blaze of Fire develops a pyramidal spiciform inflorescence composed of 15-22 intensely red flowers, with an extended flowering period from July until the first frost, which confers exceptional ornamental value.

- *Salvia officinalis* exhibits a terminal inflorescence (at the stem apex), consisting of flowers grouped in verticillasters (2-6 flowers per tier) and protected by bracts. The flowers have a bilabiate, blue-violet corolla and are arranged to form a spike or raceme at the end of the stem. Flowering occurs in the second year

after planting, from May to July, progressing from the base towards the apex of the inflorescence. The intense violet coloration of the corolla combined with the silvery-green foliage confers a pleasant ornamental effect, making the species suitable for borders, planters, and mixed green spaces.

– *Salvia coccinea* cv. Summer Jewel Pink is a compact plant with an elongated, lax spiciform inflorescence, bearing tubular flowers grouped in terminal racemes measuring 20–22 cm in length. The flowers are approximately 2–3 cm long, white to pink in colour, and bilabiate, with a straighter upper lip and a more open lower lip. The pastel pink corolla and the intensely green foliage provide a pleasant ornamental appearance, making the species suitable for borders, planters, and mixed green spaces. It exhibits a prolonged flowering period from May until the first frost.

– *Salvia farinacea* cv. Blue Victoria bears flowers arranged in elongated spiciform inflorescences with an average length of 22 cm, organized in verticillasters along the floral axis. Flowers open progressively from the base towards the apex of the inflorescence, ensuring continuous flowering. Blooming extends from June until the first frost. The high spike density, inflorescence size, long flowering duration, intense blue coloration with violet tinges, and good field performance confer high ornamental value, making the species suitable for borders and mixed green spaces.

CONCLUSIONS

The species investigated in this study demonstrate, on the one hand, morphological unity at the genus level, and on the other hand, pronounced diversity in inflorescence structure and floral coloration.

At the same time, the size and shape of the bracts, calyx, and corolla represent key elements in species identification and in the assessment of their ornamental value.

The staminal lever mechanism of the androecium is evolutionarily conserved, confirming the taxonomic affiliation with the genus *Salvia* and the efficiency of entomophilous pollination.

The gynoecium, through its tetralobed ovary and the formation of four nutlets, consistently

preserves the fruit type characteristic of the family *Lamiaceae*.

Based on the observed features, it can be concluded that each species exhibits valuable ornamental traits adaptable to various types of landscape design. The combinations of colors, textures, and forms provide diverse possibilities for use in public green spaces, as well as in private gardens and thematic landscape arrangements.

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INDOOR THERAPEUTIC GARDENS AS SUPPORTIVE ELEMENTS IN HEALTHCARE ENVIRONMENTS

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Abstract

People are spending less time in contact with natural elements in contemporary built environments, a situation that raises particular concerns in healthcare settings. Previous studies indicate that exposure to vegetation and nature-inspired design can support both physical recovery and psychological well-being. This paper examines the role of indoor therapeutic gardens as supportive components of hospital environments, with an emphasis on landscape and horticultural design. The research is based on a review of the literature on biophilic design and healing landscapes, combined with an analysis of international case studies and a site-specific evaluation of a medical recovery hospital in north-eastern Romania. Based on these approaches, the paper proposes a conceptual indoor garden solution that integrates vegetation, natural materials, daylight, and spatial organization, while complying with health and safety requirements. The results suggest that properly managed indoor greenery can reduce stress, improve environmental quality, and support patient recovery, while also contributing to better working conditions for medical staff. The study highlights the importance of landscape architecture and horticulture in developing healthier, more sustainable hospital environments.

Key words: therapeutic gardens, biophilic design, healthcare environments, landscape architecture, horticultural design.

INTRODUCTION

Modern healthcare environments are increasingly designed with attention not only to medical treatment but also to the quality of the surrounding environment. Hospitals are often perceived as stressful places, where patients may experience anxiety, discomfort, and psychological pressure during treatment and recovery. In this context, the integration of natural elements into healthcare facilities has become an important topic of research in healing environments and environmental design.

Several studies suggest that contact with vegetation and natural elements can contribute to improved physical and psychological well-being. Exposure to plants or views of nature has been associated with reduced stress, lower blood pressure, decreased anxiety, and improved patient satisfaction in hospital environments (Jiang et al., 2018; Zlati et al., 2022). For example, the study by Park & Mattson (2009) found that patients recovering in rooms with indoor plants reported lower levels of pain, anxiety, and fatigue than those in standard hospital rooms.

Beyond passive exposure to plants, therapeutic gardens represent a broader approach to integrating nature into healthcare settings. Therapeutic gardens are designed spaces that combine vegetation, sensory stimuli, and accessible spatial layouts to support healing processes and improve well-being (Stoica & Istrate, 2023). According to Yeo et al. (2020), indoor nature-based interventions such as horticultural activities, indoor plants, and garden environments can improve mood, social interaction, and overall quality of life, especially for older adults in care environments.

The concept of therapeutic landscapes is closely related to the principles of biophilic design, which emphasize the human need for connection with nature within built environments. Research indicates that natural elements integrated into interior spaces can create calmer and more restorative environments, which may positively influence both patients and healthcare staff. In this context, indoor therapeutic gardens represent a practical design strategy that can introduce vegetation, natural materials, daylight, and multisensory experiences into hospital

interiors (Grecu et al., 2023; Uwajeh et al., 2019).

Despite the growing interest in healing environments, indoor therapeutic gardens remain relatively limited in many healthcare facilities, particularly in Eastern European contexts. Their implementation often requires careful adaptation to medical regulations, spatial constraints, and maintenance requirements. Landscape architecture and horticulture, therefore, play an important role in designing feasible solutions that integrate vegetation within clinical environments while respecting sanitary and functional standards.

The present study examines the potential of indoor therapeutic gardens as supportive elements in healthcare environments. The research focuses on the Medical Recovery Hospital Arcadia located in Bârnova commune, north-eastern Romania. Based on a literature review, case study observations, and landscape design principles, the paper proposes a conceptual indoor garden intervention tailored to the specific conditions of this medical facility. The proposed design explores how vegetation, spatial organization, and natural elements can contribute to a more supportive and restorative hospital environment for patients, visitors, and medical staff.

MATERIALS AND METHODS

The present research combines literature analysis with a case study approach in order to explore the role of indoor therapeutic gardens in healthcare environments and to develop a landscape design proposal adapted to a real medical facility.

The study's theoretical framework is based on a review of the scientific literature on healing environments, biophilic design, and therapeutic gardens in healthcare settings. Previous studies examining the effects of indoor plants and nature-based interventions in medical environments were analyzed to identify the main design principles and potential benefits of integrating vegetation in healthcare facilities. Particular attention was given to studies that investigated the physiological and psychological effects of indoor plants and therapeutic garden environments on patients and

healthcare staff (Park & Mattson, 2009; Uwajeh et al., 2019; Yeo et al., 2020).

The empirical component of the research is represented by a case study conducted at the Medical Recovery Hospital Arcadia, located in Bârnova commune, near the city of Iași, north-eastern Romania. The building and its interior spaces were analyzed to identify opportunities to introduce vegetation and nature-inspired elements within the hospital environment. The analysis considered spatial configuration, circulation areas, lighting conditions, and the functional requirements specific to a medical recovery facility.

Based on the results of the literature review and the spatial analysis of the case study site, a conceptual design proposal for an indoor therapeutic garden was developed. The design process followed principles commonly associated with therapeutic landscapes and healing environments, including the integration of vegetation, natural materials, daylight access, and spatial organization that supports relaxation and visual contact with greenery. The proposed intervention was adapted to the hospital building's existing architectural structure, while considering hygiene requirements and the operational conditions specific to healthcare environments.

The methodological approach, therefore, combines theoretical research with applied landscape design, allowing the study to explore both the scientific background of therapeutic gardens and their practical implementation in real healthcare contexts.

RESULTS AND DISCUSSIONS

Reference Case Studies of Therapeutic Healthcare Environments

To identify relevant design strategies for integrating nature into healthcare environments, several international hospital examples incorporating biophilic design principles and therapeutic garden elements were analysed. These reference projects provided insights regarding spatial organisation, plant integration, the use of natural materials, and the role of visual contact with nature in medical facilities. One relevant example is Crown Sky Garden, located on the 11th and 12th floors of the Ann & Robert H. Lurie Children's Hospital in Chicago.

The project was designed by the landscape architecture studio Mikyoung Kim Design and represents an indoor therapeutic garden created specifically for paediatric patients and their families. The space integrates several principles of biophilic design, including natural light, vegetation, natural materials, and areas for relaxation and contemplation (Nemes & Mikyoung Kim Design, n.d.). Large floor-to-ceiling windows allow natural daylight to penetrate the interior space. At the same time, elements such as bamboo vegetation, wooden seating structures, and sculptural installations create an atmosphere similar to a small indoor forest (Figure 1). Curved resin panels serve as both decorative and functional elements, acting as planters and spatial dividers that offer a sense of privacy and comfort (Nemes & Mikyoung Kim Design, n.d.).



Figure 1. The waiting area at Ann & Robert H. Lurie Children's Hospital (Nemes & Mikyoung Kim Design, n.d.)

Another relevant reference project is The Royal Children's Hospital in Melbourne, Australia, a healthcare facility that integrates natural elements throughout both interior and exterior spaces. The design emphasizes visual contact with nature, with more than 80% of patient rooms offering views of surrounding green areas or landscaped courtyards (Figure 2). The hospital also incorporates terraces, gardens, and transitional spaces that connect interior environments with outdoor natural areas. In addition, interior spaces are designed around thematic concepts inspired by Australian landscapes, integrating graphics, art, and natural imagery that reflect local ecosystems. These design strategies help create a supportive,

calming atmosphere for patients, visitors, and healthcare staff (HKS Architects, n.d.).



Figure 2. View of natural surroundings from a patient room at The Royal Children's Hospital (HKS Architects, n.d.)

A third example is the Shengavit Medical Centre in Armenia, where biophilic design principles were applied to transform waiting areas and public spaces into environments that resemble comfortable living spaces rather than traditional hospital interiors (Figure 3) (Archangel Architectural Bureau, 2018).

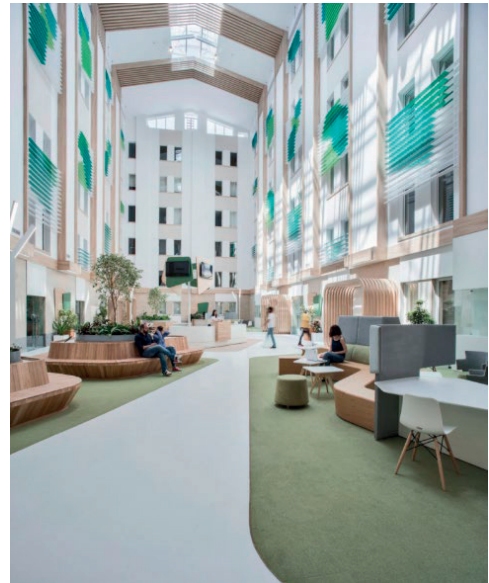


Figure 3. The waiting area at Shengavit Medical Centre (Archangel Architectural Bureau, 2018)

The design uses neutral colour palettes combined with warm accents such as green and blue tones, which are associated with calmness and emotional balance. Natural materials, such as wood, are used extensively for furniture and

decorative panels, while organic forms and soft lighting create a welcoming and relaxing atmosphere. The project demonstrates how relatively simple design interventions based on environmental psychology and colour theory can significantly improve the perception of healthcare spaces (Archangel Architectural Bureau, 2018).

Another important example is the Lucile Packard Children's Hospital Stanford, located in California, where therapeutic gardens and outdoor terraces are integrated throughout the hospital complex (Figure 4). The project

includes rooftop gardens, individual terraces for patients and staff, and landscaped areas covering approximately 1.4 hectares. These spaces incorporate a wide range of plant species, including trees, shrubs, and flowering plants, which help create a natural and restorative environment. Large glazed surfaces allow daylight to penetrate interior spaces, while the extensive use of wood and natural colours enhances the connection between the built environment and the surrounding landscape (Allan & Perkins & Will, n.d.).

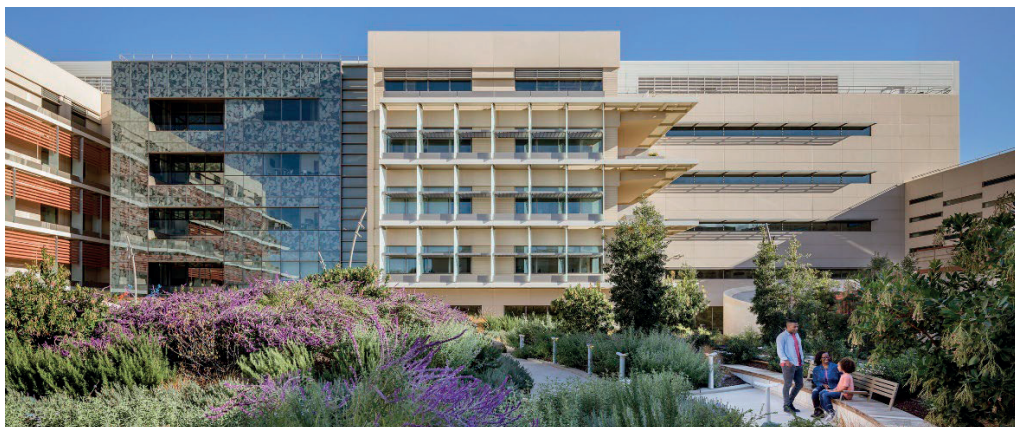


Figure 4. The therapeutic garden on the roof of Lucile Packard Children's Hospital Stanford (Allan & Perkins & Will, n.d.)

The analysis of these projects highlights several common design principles that support the creation of therapeutic healthcare environments. These include integrating vegetation into interior spaces, providing access to natural light, using natural materials such as wood, creating visual connections to nature, and creating spaces dedicated to relaxation and contemplation. These principles were considered during the development of the landscape proposal for the Arcadia Medical Recovery Hospital.

Case Study: Arcadia Medical Recovery Hospital

The case study selected for this research is the Medical Recovery Hospital Arcadia, located in Bârnova commune, near the city of Iași, in north-eastern Romania. The hospital is part of a private medical network and provides specialised services in medical recovery and rehabilitation. Facilities of this type typically host patients for extended periods during

treatment and recovery, which increases the importance of the surrounding environment and the overall quality of interior spaces.

In healthcare environments dedicated to rehabilitation, patients often spend considerable time not only in treatment rooms but also in circulation areas, waiting spaces, and transitional interior zones. These spaces, therefore, play an important role in shaping the perception of the hospital environment and may influence patient comfort, stress levels, and overall well-being. Previous research indicates that environmental factors such as access to daylight, visual contact with vegetation, and the presence of natural materials can contribute to improved psychological comfort and patient satisfaction in healthcare facilities (Mohajer & Movahed, 2026).

The Arcadia Medical Recovery Hospital building offers several interior areas that could potentially accommodate elements of indoor greenery and nature-inspired design. Among

these, circulation zones and waiting areas represent spaces with high user interaction, frequently used by patients, visitors, and medical staff. Such areas provide opportunities for introducing vegetation without interfering with medical activities or clinical procedures. The building's spatial characteristics, including natural light sources, interior openness, and available transitional spaces, make it feasible to consider integrating a therapeutic indoor garden (Figure 5). Introducing vegetation and natural elements in these areas may help create a calmer, more welcoming atmosphere within the hospital, while maintaining the functional requirements specific to healthcare facilities.

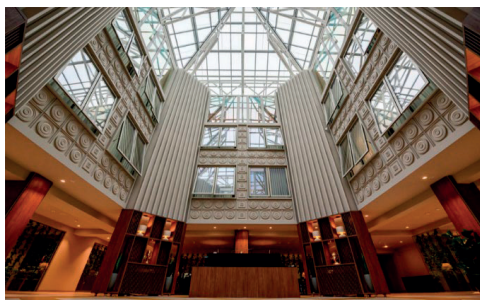


Figure 5. The central area inside Arcadia Medical Recovery Hospital
(<https://secp2024.feg.ro/event/vizita-la-spitalul-de-recuperare-arcadia-barnova/>)

Based on observations conducted within the hospital building and design principles identified through analysis of reference case studies, a conceptual proposal for an indoor therapeutic garden was developed. The design aims to introduce vegetation, natural materials, and spatial elements that encourage relaxation and visual contact with nature, while respecting hygiene requirements and the operational conditions specific to medical recovery centres.

Spatial Analysis of the Interior Environment

The spatial analysis of the Arcadia Medical Recovery Hospital focused on identifying interior areas that could accommodate vegetation and nature-inspired design elements without compromising the facility's functional requirements. Healthcare buildings must respect strict circulation rules, hygiene standards, and accessibility requirements; therefore, any landscape intervention must be carefully adapted to these conditions.

Particular attention was given to circulation spaces, waiting areas, and interior transitional zones. These areas are frequently used by patients, visitors, and medical staff, and they are spaces where environmental design can visibly impact the hospital's overall atmosphere. In many healthcare buildings, such spaces are primarily designed for movement or waiting and often lack visual stimuli or natural elements. Introducing vegetation in these areas may improve spatial quality while maintaining their functional role.

Natural light availability was another important factor considered during the spatial analysis. Adequate daylight is essential for maintaining healthy indoor vegetation and creating comfortable environments for hospital users. Areas near windows or glazed surfaces were therefore considered more suitable for integrating plant compositions and landscape elements.

In addition to lighting conditions, the spatial analysis also considered accessibility and safety requirements. Circulation routes must remain clear to allow easy movement of patients with reduced mobility, medical equipment, and hospital staff. For this reason, the proposed design solutions focus on using controlled planting areas, integrated planters, and clearly defined spatial zones that do not obstruct circulation (Figure 6).



Figure 6. Close-up of proposed planters (original)

The analysis also highlighted the importance of visual contact with vegetation. Even indirect exposure to plants or green elements can help create a more calming environment and can improve the perception of healthcare spaces (Ulrich, 1984). Therefore, the proposed intervention focuses not only on the physical

presence of vegetation but also on ensuring that greenery is visible from multiple points within the interior space.

Based on the spatial analysis and design principles identified in the reference case studies, several interior areas within the hospital were considered suitable for the integration of an indoor therapeutic garden. These findings formed the basis for the conceptual landscape proposal developed for the Arcadia Medical Recovery Hospital.

Conceptual Design of the Indoor Therapeutic Garden

Based on the spatial analysis of the Arcadia Medical Recovery Hospital and the design principles identified through the reference case studies, a conceptual proposal for an indoor therapeutic garden was developed. The intervention aims to introduce natural elements into the hospital interior, creating a more calming and supportive environment for patients, visitors, and medical staff (Figure 7).

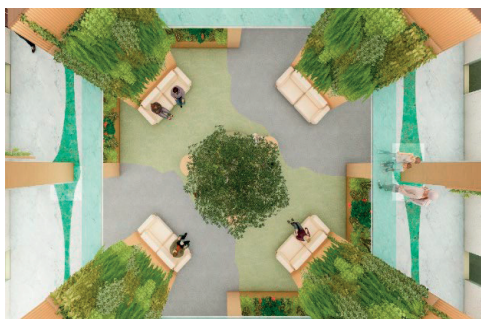


Figure 7. Aerial view of the proposed concept (original)

The proposed design focuses on integrating vegetation, natural materials, and spatial elements to encourage visual contact with nature. Indoor plants are the central component of the intervention, as vegetation can help improve environmental quality and psychological comfort for hospital users. Previous studies indicate that the presence of plants in healthcare environments can reduce stress and anxiety and may contribute to improved recovery conditions for patients (Park & Mattson, 2009; Yeo et al., 2020).

The spatial organisation of the indoor garden is designed to maintain clear circulation routes while introducing areas dedicated to relaxation

and visual interaction with greenery (Figure 8). Vegetation is arranged in controlled planting areas and integrated planters that define different zones within the interior space without obstructing movement. These planted areas serve as both aesthetic elements and spatial organizers, contributing to a more welcoming atmosphere.



Figure 8. Relaxation and waiting area (original)

The selection of plant species for the indoor therapeutic garden was based on several criteria, including adaptability to interior conditions, low maintenance requirements, and the ability to contribute to a calming visual environment. Species that tolerate indirect light and stable indoor temperatures were prioritised, as these conditions are typical for hospital interiors. Among the proposed plants are *Sansevieria trifasciata* (snake plant), *Zamioculcas zamiifolia* (ZZ plant), and *Epipremnum aureum* (golden pothos). These species are widely used in interior landscaping because of their high tolerance to varying light conditions and relatively low water requirements. Their vertical or cascading growth forms also help create visual structure within planted areas without obstructing circulation.

Additional species were selected to increase visual diversity and create a more natural and relaxing atmosphere. Plants such as *Chlorophytum comosum* (spider plant), *Nephrolepis exaltata* (Boston fern), and *Asplenium nidus* (bird's nest fern) introduce softer textures and more dynamic foliage forms, which enhance the perception of greenery within the interior space. Decorative foliage plants such as *Calathea orbifolia* (calathea) and *Philodendron hederaceum* (heartleaf

philodendron) were also considered for their ornamental leaves and adaptability to indoor environments. In addition, *Hedera helix* (English ivy) can be used in vertical or cascading arrangements, contributing to the visual continuity of the planted areas. The combination of these species supports the creation of a balanced interior planting composition that integrates aesthetic, functional, and therapeutic qualities within the hospital environment.

Natural materials were also considered an important component of the design proposal. Elements such as wood and natural textures were selected to reinforce the connection between the built environment and natural landscapes (Figure 9). Research on therapeutic environments suggests that natural materials can contribute to creating more comfortable and restorative interior spaces (Scott et al., 2016; Uwajeh et al., 2019; Vladimir & Cojocariu, 2024).



Figure 9. The vertical wall of ivy and the glass pillar (original)

Another important aspect of the design concept is the relationship between vegetation and light. Adequate natural light supports plant growth and enhances the visual perception of greenery within interior spaces. Large windows and well-lit areas, therefore, play an important role in integrating indoor vegetation and creating a more open and pleasant hospital environment (Figure 10).

The conceptual proposal developed for the Arcadia Medical Recovery Hospital aims to transform interior spaces typically associated with waiting or circulation into environments that offer moments of relaxation and a visual connection to nature. By introducing vegetation

and nature-inspired design elements, the indoor therapeutic garden enhances the spatial quality of the hospital while maintaining the functional requirements specific to healthcare facilities.



Figure 10. Close-up of the glass roof (original)

CONCLUSIONS

The integration of natural elements within healthcare environments has become an important topic in recent research on healing environments and environmental design. The present study explored the potential of indoor therapeutic gardens as supportive components of hospital interiors, with particular attention to landscape architecture and horticultural design principles.

The analysis of international reference projects demonstrated that the presence of vegetation, natural materials, and visual connections with nature can contribute to creating more comfortable and restorative healthcare environments. These examples highlight the role of biophilic design strategies in improving spatial quality and supporting the well-being of patients, visitors, and medical staff.

The case study conducted at the Arcadia Medical Recovery Hospital in Bârnova, Romania, showed that interior circulation areas and transitional spaces can provide suitable opportunities to integrate vegetation without interfering with medical functions. Based on the spatial analysis and the identified design principles, a conceptual proposal for an indoor therapeutic garden was developed.

The proposed design aims to introduce vegetation and nature-inspired elements into the hospital environment in a controlled, functional manner. Such interventions may help reduce stress, improve environmental quality, and

create a more welcoming atmosphere for hospital users.

Overall, the study highlights the potential contributions of landscape architecture and horticulture to the design of healthier, more supportive healthcare environments. Further research and practical implementations may help expand the integration of therapeutic gardens into hospital buildings, particularly in regions where such approaches remain relatively limited.

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RESEARCH ON THE EVOLUTION OF URBAN GREEN SPACES IN ROMANIA (2019-2025)

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Abstract

Urban green spaces are an essential component of the population's quality of life and the sustainability of cities, playing a major role in mitigating the effects of climate change. This research analyzes the evolution of urban green spaces in Romania between 2019 and 2025, using a methodology based on temporal and spatial comparative analysis, with reference to mapped data, complemented by official statistics provided by the National Institute of Statistics. The analysis is conducted at the level of development regions, examining both the evolution of the total area of urban green spaces and the green space per capita indicator. The study highlights disparities between more developed urban areas and those with limited resources and identifies general trends of growth or reduction in green infrastructure. The outcomes provide an overview of Romania's urban green infrastructure and offer valuable information for urban planning and public policy development, supporting strategies aimed at maintaining and expanding green spaces.

Key words: green infrastructure, urban sustainable development.

INTRODUCTION

Urban green spaces represent an essential component of sustainable urban development, contributing to environmental protection, climate regulation, biodiversity conservation, and the improvement of population well-being. In recent decades, accelerated urbanization and the continuous expansion of built-up areas have considerably transformed the spatial structure of cities, increasing pressure on urban ecosystems and reducing the availability of natural and semi-natural areas within urban environments. In this context, green infrastructure has become an increasingly important element of urban planning policies aimed at improving environmental quality and enhancing urban resilience to climate change.

Specialized literature emphasizes that urban green spaces generate multiple ecological, social, and economic benefits. These areas contribute to reducing urban heat island effects, improving air quality, regulating stormwater runoff, and supporting urban biodiversity. At the same time, access to green spaces is associated with improved physical and mental health, recreational opportunities, and a higher quality of life for urban residents (Humă, 2022; Oprea et al., 2023). Consequently, the preservation and development of urban green infrastructure are

increasingly integrated into European strategies concerning sustainable urban development and climate adaptation policies.

At the European level, the EU Biodiversity Strategy for 2030 highlights the importance of expanding urban green infrastructure and promoting nature-based solutions within cities to improve urban resilience and environmental sustainability (European Commission, 2020).

In addition, the European Environment Agency underlines that equitable access to urban green spaces represents an important objective for reducing social and territorial disparities in urban areas (European Environment Agency, 2022).

These strategic orientations encourage national and local authorities to integrate green infrastructure into urban development policies and territorial planning frameworks.

In Romania, the dynamics of urban green spaces have become increasingly relevant in the context of rapid urban expansion, demographic concentration in major urban centers, and the intensification of real estate development pressures. National legislation establishes a minimum target regarding green space availability per inhabitant, emphasizing the importance of maintaining and expanding urban green areas within the built environment (Monitorul Oficial no. 764, 2009).

Nevertheless, important territorial disparities continue to exist between Romanian cities in terms of green space distribution, accessibility, and management practices.

Previous studies conducted in Romania indicate that the evolution of urban green spaces is influenced by demographic factors, local urban planning policies, land-use transformations, and the administrative management of urban territories (Badiu et al., 2016; Șorcaru, 2015). Furthermore, the dynamics of these spaces are closely connected with local urban planning instruments and territorial development strategies, including General Urban Plans (PUG) and Zonal Urban Plans (PUZ). These planning mechanisms strongly influence the balance between real estate densification and the preservation of green infrastructure, frequently favoring commercial development and built-up expansion at the expense of ecological continuity and urban green networks (Badiu et al., 2016).

Recent research also suggests that official statistical data regarding urban green areas may present methodological limitations, as cadastral records do not always fully reflect the ecological reality observed at the local level. In some cases, reported increases in green space areas may result from administrative reclassification processes, updates of urban green registers, or modifications of territorial boundaries rather than from the actual physical expansion of vegetation-covered surfaces.

The present study analyses the evolution of urban green spaces in selected Romanian municipalities during the period 2019-2024 using official statistical data provided by the National Institute of Statistics through the TEMPO Online database.

The research focuses on the municipalities of Bucharest, Galați, Iași, Arad, and Brașov, selected due to their different territorial dimensions, demographic characteristics, and urban development dynamics. A comparative temporal and spatial approach was applied to identify the main trends regarding the evolution of urban green infrastructure and green space availability per inhabitant.

This objective is consistent with recent national assessments conducted by Stanciu et al. (2025), which emphasize that monitoring macro-regional disparities is essential for

understanding the uneven evolution of sustainable development across Romania's development regions.

The novelty of the study derives not only from the comparative assessment of urban green space dynamics in selected Romanian cities, but also from the critical interpretation of official statistical data in relation to the limitations of cadastral reporting systems and the broader urban development context. The results provide relevant information for understanding territorial disparities in urban green infrastructure and may support the development of more effective urban planning policies oriented toward sustainable urban development and environmental protection.

MATERIALS AND METHODS

For the preparation of the bibliographic documentation, open-access scientific articles indexed in recognized academic databases, including Clarivate, Google Scholar, and ResearchGate, were consulted, together with official reports published by national authorities and relevant European institutions in the fields of environment, urban planning, and sustainable development. The analysis of the legislative framework was based on the examination of national and European regulations concerning the management and development of urban green spaces, to establish the normative context and the reference standards regarding green space availability in relation to the number of inhabitants.

The data regarding the evolution of urban green space areas in Romania during the period 2019-2024 were obtained from the official statistics provided by the National Institute of Statistics (NIS) through the TEMPO Online platform, which provides updated statistical series at national and local administrative levels.

The analysis focused on selected Romanian municipalities, namely Bucharest, Galați, Iași, Arad, and Brașov, chosen due to their different demographic, territorial, and urban development characteristics.

The collected data were organized into a database and processed using descriptive statistical methods. Subsequently, the information was graphically represented to highlight the temporal evolution and territorial

variations of urban green spaces within the analysed administrative units.

However, an important methodological limitation of the NIS dataset derives from its predominantly cadastral character, as the statistical records reflect legally registered urban green surfaces rather than the actual ecological conditions observed on-site (Badiu et al., 2016). In addition, the rapid development of metropolitan areas and the continuous modification of functional urban boundaries may influence the statistical baseline, meaning that some increases in reported green space areas may reflect administrative reclassification processes rather than the effective physical expansion of urban vegetation.

The interpretation of the results was carried out through comparative analysis between different years and between the selected municipalities, to identify the main territorial disparities and temporal dynamics regarding urban green infrastructure. To improve the reliability of the analysis and validate the obtained results, the statistical information was compared and correlated with data and conclusions from specialized scientific literature, including studies published in the fields of urban planning, environmental management, urban ecology, and sustainable development.

This methodological approach facilitated the integration of official statistical data with previous scientific findings, contributing to the formulation of more robust interpretations regarding the evolution and distribution of urban green spaces in Romania.

RESULTS

The analysis was based on statistical data regarding the annual evolution of urban green space areas in the municipalities of Braşov, Iaşi, Galaţi, Bucharest, and Arad during the period 2019-2024 (Table 1).

According to the methodology of the National Institute of Statistics (NIS), urban green spaces include parks, public gardens, public squares, areas planted with trees and shrubs, urban forests, cemeteries, and other recreational areas located within the built-up perimeter of localities, excluding agricultural land, greenhouses, and plant nurseries (NIS, 2022). The reported values are expressed in hectares (ha).

Table 1. Evolution of urban green space areas in selected Romanian municipalities (2019-2024)

Urban green spaces	2019	2020	2021	2022	2023	2024
Total (ha)	26,958	29,136	31,319	31,508	33,350	33,419
Braşov (ha)	351	352	357	372	1,365	1365
Iaşi (ha)	827	913	1,005	990	1,017	993
Galaţi (ha)	1,050	1,087	1,051	1,059	1,059	1,059
Bucharest (ha)	4,506	4,506	4,506	4,506	4,506	4,506
Arad (ha)	524	528	540	614	1,020	1,020

Source: Authors' processing based on NIS data (2026).

At the national level, official statistical data indicate an increase in the total area of urban green spaces from 26,958 hectares in 2019 to 33,419 hectares in 2024, corresponding to an increase of approximately 24% during the analysed period. This evolution suggests a gradual expansion of urban green infrastructure in Romania, consistent with European objectives regarding climate adaptation and sustainable urban development promoted through the EU Biodiversity Strategy for 2030 (European Commission, 2020). At the same time, these macro-regional dynamics and the general upward trend of reported green surfaces are consistent with recent findings from Romanian specialized literature, which emphasize that, despite the overall national increase, the territorial distribution of urban green spaces remains uneven across Romania's development regions (Stanciu et al., 2025). The year 2019 was considered the reference point for analysing the subsequent evolution of urban green spaces in the selected municipalities (Table 2).

Table 2. Green space area in selected areas, 2019 (ha)

City	Green spaces in selected areas, 2019 (ha)
Bucharest	4,506
Galaţi	1,059
Arad	614
Braşov	372
Iaşi	990

Source: Authors' processing based on NIS TEMPO Online data (2026).

In this year, the municipality of Bucharest ranks first in terms of total green space area, with 4,506 hectares. The difference compared to the other cities is considerable, as the following positions are occupied by Galaţi with 1,050 hectares, Iaşi with 827 hectares, Arad with 524 hectares, and Braşov with 351 hectares (NIS, 2026).

This distribution reflects both the territorial size of the cities and the way in which local administrations manage urban green

infrastructure. In 2020 a moderate increase in green space areas can be observed in most of the analysed cities. Iași records the largest relative increase, with the green space area increasing from 827 to 913 hectares. Galați increases from 1,050 to 1,087 hectares, and Arad from 524 to 528 hectares. Brașov shows a very small variation, from 351 to 352 hectares. The municipality of Bucharest remains constant at 4,506 hectares. In 2021 the increasing trend continues in some cities, especially in Iași, where the green space area reaches 1,005 hectares, representing an increase of over 21.5% compared to 2019. Arad increases to 540 hectares, and Brașov to 357 hectares. At the same time, Galați records a temporary decrease to 1,051 hectares, indicating a possible update of the green space register or changes in the reporting method (National Institute of Statistics NIS, 2026). In 2022 the evolution of green spaces becomes more pronounced in some cities (Table 3).

Table 3. Urban green space per inhabitant in selected Romanian cities, 2022 (m²/inhabitant)

City	Green spaces in selected Romanian cities, 2022 (m ² /inhabitant)
Bucharest	26.15
Galați	21.39
Arad	14.97
Brașov	6.79
Iași	12.93

Source: Authors' processing based on NIS TEMPO Online data (2026).

In 2022, Arad reached 614 hectares of urban green spaces, representing a significant increase compared to previous years. Brașov recorded 372 hectares, while Galați returned to approximately 1,059 hectares. At the same time, Iași registered a slight decrease to 990 hectares, whereas the municipality of Bucharest maintained a constant value of 4,506 hectares. At national level, the green space per inhabitant indicator varied between approximately 2.69 and 52.38 m²/inhabitant, with a national average of approximately 15.16 m²/inhabitant according to NIS data. This indicator suggests that access to urban green spaces depends not only on their total surface area, but also on population density and urban demographic structure. The year 2023 marked a more visible change in the evolution of urban green spaces in several of the analysed municipalities (Table 4).

Table 4. Green space area in analysed areas, 2023 (ha)

Source: Authors' processing based on NIS data (2026).

City	Green spaces in analysed areas in 2023 (ha)
Bucharest	4,506
Galați	1,059
Arad	1,020
Brașov	1,365
Iași	1,017

In 2023, Arad recorded a substantial increase in urban green spaces, from 614 to 1,020 hectares, while Brașov reached 1,365 hectares following a considerable increase compared to previous years (NIS, 2026). Iași registered a slight increase to 1,017 hectares, whereas Galați and Bucharest remained relatively stable.

At national level, the total urban green space area reached approximately 33,350 hectares, with an average value of approximately 794 hectares per analysed administrative unit.

In the same year, the national average of green space per inhabitant in Romania was approximately 16.10 m²/inhabitant, while the recorded values varied between approximately 2.71 m²/inhabitant and over 52 m²/inhabitant according to NIS data.

Table 5. Green space per inhabitant in selected Romanian cities, 2023 (m²/inhabitant)

City	Green spaces per inhabitant in selected cities (sqm), 2023
Bucharest	26.12
Galați	21.43
Arad	24.85
Brașov	24.69
Iași	13.14

Source: Authors' processing based on NIS data (2026).

Comparing the data presented in Tables 3 and 5 regarding green space area per inhabitant in 2022 and 2023 for the five analysed cities, several different development patterns can be identified. In Bucharest, the indicator remained relatively constant, decreasing slightly from 26.15 m²/inhabitant in 2022 to 26.12 m²/inhabitant in 2023. Galați recorded a marginal increase, from 21.39 to 21.43 m²/inhabitant. More substantial increases were observed in Arad, where the indicator rose from 14.97 to 24.85 m²/inhabitant, and in Brașov, where the value increased from 6.79 to 24.69 m²/inhabitant. In Iași, the evolution remained moderate, with a slight increase from 12.93 to 13.14 m²/inhabitant.

Overall, the data indicate that most of the analysed municipalities recorded increases in green space availability relative to population in

2023, the most important changes being observed in Arad and Braşov.

It should be emphasized that the substantial statistical increases recorded in Braşov and Arad during 2023 should not be interpreted exclusively as immediate physical expansions of urban vegetation surfaces. Instead, these developments may also reflect administrative updates of local Green Space Registers and the inclusion of previously unclassified urban areas in official statistical records (Badiu et al., 2016). These localized dynamics are consistent with the findings of Stanciu et al. (2025), which indicate that although the national aggregate shows an overall increase in urban green spaces, their territorial distribution remains uneven across Romania's development regions.

In 2024, Bucharest maintained a constant green space area of 4,506 hectares, while Galaţi remained at approximately 1,059 hectares. Iaşi recorded a slight decrease to 993 hectares, whereas Arad and Braşov maintained the values registered in the previous year, namely 1,020 hectares and 1,365 hectares respectively (Table 6).

Table 6. Green space per inhabitant in selected Romanian cities, 2024 (m²/inhabitant)

City	Green spaces per inhabitant in selected cities (sqm), 2024
Bucharest	26.17
Galati	21.43
Arad	24.85
Brasov	24.69
Iasi	13.14

Source: Authors' processing based on NIS data (2026).

In 2024, the national average of green space per inhabitant reached approximately 16.12 m²/inhabitant, while the recorded values at national level varied between approximately 2.71 and 52.34 m²/inhabitant according to NIS data.

The data regarding green space availability per inhabitant in 2024, summarized in Table 6, highlight important differences between the analysed municipalities. Bucharest recorded the highest value, at 26.17 m²/inhabitant, being the only analysed city exceeding the threshold of approximately 26 m²/inhabitant considered the recommended minimum level under Romanian legislation. Arad and Braşov registered similar values, namely 24.85 m²/inhabitant and 24.69 m²/inhabitant respectively, indicating a relatively high level of green space availability in relation to population size. Galaţi recorded an intermediate value of 21.43 m²/inhabitant,

suggesting a moderate level of green infrastructure availability. In contrast, Iaşi registered the lowest value, namely 13.14 m²/inhabitant, indicating a lower level of green space availability compared with the other analysed municipalities. Overall, the data indicate an uneven territorial distribution of urban green spaces, reflected in significant differences regarding the accessibility of these areas for urban residents. Furthermore, the green space per capita indicator may also be influenced by demographic and methodological limitations. The NIS census records the population according to official residence addresses rather than actual dwelling locations. In dynamic metropolitan regions such as Bucharest–Ilfov, daily commuting flows, unregistered residential mobility, and suburban migration may increase the effective urban population using the existing infrastructure, potentially reducing the actual green space availability per effective user (Dumitrache et al., 2016). Overall, the chronological analysis highlights differentiated patterns regarding the evolution of urban green spaces in Romania. The municipalities of Arad and Braşov registered the most substantial increases in green space areas during the analysed period, whereas cities such as Galaţi and Bucharest maintained relatively stable values. Iaşi occupies an intermediate position, characterized by moderate fluctuations in urban green space areas. In the context of European policies promoting sustainable urban development, the expansion and balanced distribution of urban green infrastructure remain important objectives for improving urban quality of life and increasing the resilience of cities to climate change.

The comparative analysis of the evolution of urban green space areas between 2019 and 2024 highlights significant differences between the analysed municipalities (Table 7).

Table 7. Evolution of urban green space areas in the analysed municipalities (2019-2024)

City	2019 (ha)	2024 (ha)	Difference (ha)	Evolution (%)
Bucharest	4,506	4,506	0	0%
Galati	1,050	1,059	+9	+0.9%
Iasi	827	993	+166	+20.1%
Arad	524	1,020	+496	+94.7%
Brasov	351	1,365	+1014	+288.9%

Source: Authors' processing based on NIS data (2026).

The comparative analysis of the selected municipalities highlights different patterns

regarding the evolution of urban green infrastructure during the period 2019-2024. In 2019, the recorded values were 4,506 hectares in Bucharest, 1,050 hectares in Galați, 827 hectares in Iași, 524 hectares in Arad, and 351 hectares in Brașov. The subsequent evolution of these indicators suggests different rhythms of development and management of urban green spaces across the analysed municipalities.

In the case of Bucharest, the total green space area remained constant throughout the analysed period, maintaining a value of 4,506 hectares between 2019 and 2024. This stability suggests the absence of significant expansions of urban green infrastructure within the Romanian capital. From the perspective of percentage evolution, the variation remained practically null (0%), reflecting the influence of high construction density and intense urban development pressures that may limit the possibilities for expanding green spaces.

However, this apparent statistical stability may conceal more complex ecological dynamics. While official cadastral records maintain relatively constant green space surfaces through the inclusion of peripheral green patches or fragmented urban parks, previous remote sensing analyses based on near-infrared spectral combinations have identified significant reductions in mature vegetation cover in certain inner-city areas affected by urban densification and real estate development processes (Badiu et al., 2016; Lillesand et al., 2015). These observations suggest that administrative statistical records may not fully capture the ecological transformations occurring within major Romanian urban areas (Necula et al., 2023).

Galați registered a relatively stable evolution, characterized by moderate fluctuations in green space area. Between 2019 and 2024, the green space surface increased slightly from 1,050 hectares to approximately 1,059 hectares, corresponding to a total increase of approximately 0.9%. Although limited, this increase suggests the maintenance of existing urban green infrastructure in the context of ongoing urban development pressures. This relatively stable evolution is also associated with the preservation and gradual integration of peri-urban forest areas within the municipality's green infrastructure system (Geacu et al., 2022).

Iași recorded a more dynamic evolution during the analysed period. The green space area increased from 827 hectares in 2019 to 993 hectares in 2024, representing an overall increase of approximately 20%. The most visible growth occurred between 2019 and 2021, when the reported green space area exceeded 1,000 hectares. These developments may reflect local urban policies oriented toward the extension and consolidation of urban green infrastructure.

Arad registered one of the most significant increases among the analysed municipalities. The green space area increased from 524 hectares in 2019 to 1,020 hectares in 2024, corresponding to an increase of approximately 95%. Similarly, Brașov recorded the highest relative increase, from 351 hectares in 2019 to 1,365 hectares in 2024, representing an increase of approximately 289%. In both municipalities, the most substantial changes were recorded in 2023, suggesting either the expansion of urban green infrastructure or the administrative updating of local green space inventories and registers.

The comparative analysis suggests the existence of three general patterns regarding the evolution of urban green spaces. The first pattern includes municipalities characterized by relatively stable dynamics, such as Bucharest and Galați, where green space areas remained almost constant throughout the analyzed period. The second pattern is represented by municipalities with moderate growth, such as Iași, where the expansion of green infrastructure occurred gradually. The third pattern includes municipalities with accelerated growth, such as Arad and Brașov, where the reported green space areas increased substantially during the final years of the analysed period.

The interpretation of these developments should also consider the green space per inhabitant indicator, which reflects the accessibility of urban green infrastructure for the population. According to the data presented in Tables 3, 5, and 6, the national average increased from approximately 15.16 m²/inhabitant in 2022 to 16.10 m²/inhabitant in 2023 and 16.12 m²/inhabitant in 2024. Although this evolution suggests a moderate improvement in population access to green spaces, the recorded values

remain relatively low compared with certain international standards.

Overall, the analysed data indicate a general tendency toward the expansion of urban green infrastructure in Romania, although the pace and territorial distribution of this process differ significantly between municipalities (Figure 1). While municipalities such as Arad and Braşov registered substantial increases in reported green space areas, other cities displayed more stable dynamics. These territorial differences reflect both local urban development strategies and the demographic and economic characteristics of each municipality.

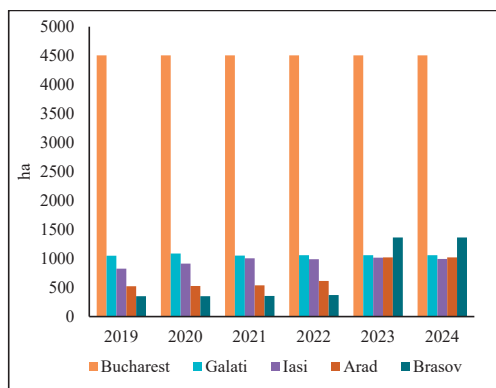


Figure 1. Evolution of Urban Green Spaces in Selected Romanian Cities (2019-2024)
Source: Authors, using NIS (2026)

From the perspective of European sustainable urban development policies, green infrastructure represents an important component for improving environmental quality and increasing urban resilience to climate change. The EU Biodiversity Strategy for 2030 emphasizes the importance of integrating green infrastructure into urban planning policies and expanding urban ecological networks (European Commission, 2020). In this context, the evolution of urban green spaces in Romania suggests a gradual alignment with these European objectives, although their implementation remains uneven at the territorial level.

The evolution of urban green space areas should be analysed not only from the perspective of total surface area, but also in relation to urban population size and the accessibility of these spaces for residents. In the context of accelerated urbanisation and increasing

population density in major cities, the green space per inhabitant indicator represents an important instrument for evaluating urban environmental quality and living conditions.

According to the statistical data and thematic maps developed using information provided by the National Institute of Statistics, the average value of green space per inhabitant in Romania was approximately 15.16 m² in 2022, increasing slightly to approximately 16.10 m² in 2023 and 16.12 m² in 2024. These values indicate a moderate improvement in population access to urban green spaces, although significant territorial disparities continue to exist.

The analysed NIS data also reveal important variations between Romanian administrative units, with green space per inhabitant values ranging from approximately 2.7 m² to over 52 m² per inhabitant. This uneven distribution reflects the influence of demographic structure, urban morphology, and local urban planning policies on the availability and accessibility of green infrastructure. In densely populated cities, even relatively large green space areas may correspond to low per capita values, limiting residents' effective access to these spaces.

From the perspective of international standards, the World Health Organization recommends a minimum of at least 9 m² of green space per inhabitant to ensure basic health and environmental benefits for the population, while optimal values may reach approximately 50 m² per inhabitant. In addition, European studies regarding urban green infrastructure recommend that residents should have access to a green area located within a maximum distance of 300 meters from their homes in order to facilitate frequent recreational and physical activities (European Environment Agency, 2022).

In Romania, the legislation regarding the administration of urban green spaces establishes a target of approximately 26 m² of green space per inhabitant, according to the provisions of Law no. 24/2007 concerning the regulation and administration of green spaces within the built-up area of localities (Monitorul Oficial no. 764, 2009). Comparing these standards with the values recorded at the national level indicates that many Romanian cities still do not reach this threshold, highlighting the need for more efficient urban planning policies oriented

toward the expansion, protection, and balanced distribution of urban green infrastructure.

CONCLUSIONS

The comparative analysis of the analysed municipalities indicates that the evolution of urban green spaces is influenced by local urban planning conditions and territorial development strategies. While municipalities such as Arad and Braşov recorded substantial increases in green space areas during the final years of the analysed period, cities such as Bucharest and Galaţi displayed relatively stable dynamics, which may reflect territorial constraints, urban densification processes, or stronger development pressures. Iaşi occupies an intermediate position, characterized by moderate increases and temporary fluctuations in urban green space areas.

In the context of European policies promoting sustainable urban development, green infrastructure is considered an essential component for improving environmental quality, reducing urban heat island effects, and increasing the resilience of cities to climate change. The European Union Biodiversity Strategy for 2030 emphasizes the importance of integrating green infrastructure into urban planning policies and expanding urban ecological networks. In this context, the evolution of urban green spaces in Romania suggests a gradual alignment with these objectives, although the implementation process remains uneven across municipalities.

Overall, the results of the analysis indicate a general upward trend in the development of urban green spaces in Romania. Nevertheless, their territorial distribution and accessibility remain uneven. At the same time, the interpretation of official statistical data requires caution, particularly in relation to the methodological limitations of cadastral reporting systems. Future urban planning policies and environmental monitoring frameworks should better integrate geospatial analyses, satellite-based vegetation indicators, and demographic dynamics associated with metropolitan expansion to provide a more accurate representation of urban ecological conditions (Badiu et al., 2016; Dumitrache et al., 2016).

The interpretation of NIS data should therefore consider the fact that official statistics primarily reflect cadastral records of legally registered green surfaces rather than the complete ecological reality observed on-site. For example, the statistical stability of green spaces in Bucharest contrasts with evidence from remote sensing analyses indicating important reductions in mature vegetation cover in some urban areas, despite the simultaneous creation of new parks. In addition, changes in the territorial configuration of cities through metropolitan expansion, together with demographic processes such as commuting, suburban migration, and the discrepancy between official residence and actual dwelling location, may also influence the interpretation of the green space per inhabitant indicator.

To improve urban environmental quality and comply with European standards regarding urban green infrastructure, it is necessary to strengthen urban planning policies oriented toward the expansion, protection, and balanced distribution of green spaces within Romanian cities.

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INTEGRATED ANALYSIS OF MORPHOLOGICAL, PHYTOCHEMICAL, AND TECHNOLOGICAL CHARACTERISTICS OF TAXONS OF THE GENUS *LAVANDULA* L., WITH REFERENCE TO THE CULTIVAR 'LAVINIE DE GRĂDINĂ'

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Abstract

The article presents the results of a biomorphological, phytochemical, and technological study on the cultivation of *Lavandula angustifolia* Mill., the cultivar 'Lavinie de Grădină', registered at the Botanical Garden. The research focused on describing the biomorphological characteristics of the cultivar and on assessing the content and composition of its essential oil – a key resource for therapeutic and industrial applications. The plants are characterized by strongly branched stems, opposite linear leaves, and aromatic violet flowers arranged in spike inflorescences. Both generative and vegetative propagation methods were examined, along with primary cultivation practices. Generative propagation can be achieved using stratified seeds or seeds treated according to a specialized protocol, whereas vegetative propagation is performed through cuttings, lignified annual shoots, and layering. The inflorescences constitute the main raw material due to their high essential-oil yield (4.25%), consisting mainly of linalool (50.0%) alongside other aromatic compounds with relevant biological activities. This phytochemical profile provides the cultivar with a broad spectrum of therapeutic properties. The cultivar is early-maturing and exhibits tolerance to drought and overwintering, making it a valuable local source of raw material.

Key words: lavender, aromatic plants, biomorphology, essential oil, cosmetics.

INTRODUCTION

In the context of prevailing trends in cosmetology and modern naturopathy, there is an increasing emphasis on the use of plant-based preparations alongside synthetic alternatives. This shift is driven by the growing recognition of the beneficial properties of medicinal plants, which have been rediscovered and reaffirmed through scientific research, as well as by an acknowledgment of their integral role in contemporary human life. The widespread use of phytotherapeutic and natural cosmetic products reflects a high interest in efficient and promising alternatives for promoting health and beauty. Following a period of stagnation, there has been a notable resurgence in the application of natural essential oils and products across various sectors, including phytotherapy, aromatherapy, the fragrance industry, and the food industry. This trend is part of a global movement towards gradually replacing synthetic compounds with natural counterparts. A similar evolution is also evident at the national

level, where major companies in the fragrance industry now incorporate natural essential oils in concentrations ranging from 60 to 90% in the production of finished goods. Regarding aromatic and medicinal plants, the quality of raw materials is evaluated based on the content of biologically active compounds, the accumulation of which is influenced by ecological factors, species distribution, cultivation techniques, and the intrinsic biological value of the plant. In this context, the introduction, conservation, enhancement, and valorization of aromatic, medicinal, and spice species housed within the Botanical Garden represents a key area of research for the long-term future (Teleuță A., 2008).

Among the species of particular significance, lavender (*Lavandula angustifolia* Mill.) has been, and continues to be, of considerable interest. It is extensively studied within the Botanical Garden as both an aromatic and medicinal plant, distinguished by its high concentration of biologically active compounds, particularly essential oil, which has diverse

applications in the perfume, cosmetics, and pharmaceutical industries. The collection of "Alexandru Ciubotaru" National Botanical Garden (Institute) also includes other species such as *Lavandula dentata* L., *Lavandula angustifolia* Mill. subsp. *pyrenaica* (DC.) Guinea, and *Lavandula pinnata* Lundmark, in addition to cultivars like *Lavandula angustifolia* Mill. 'K-90' and 'Lavinie de grădină', the latter has officially been registered at the NBGI.

Lavandula angustifolia (lavender) is a species native to North Africa and the mountainous regions of the Mediterranean basin. The earliest documented use of lavender dates back to Ancient Egypt, where it was utilized in both religious rituals and personal care practices. Subsequently, in Persia, Greece, and Ancient Rome, lavender became a significant component in cosmetic formulations and hygiene practices, revered for its claimed ability to purify both the body and the mind. The flowers were used to fragrance and disinfect public baths, as well as to scent indoor spaces (Rasch A., 2010). Currently, lavender is cultivated extensively, primarily for use in the cosmetic industry. Over time, the plant has been utilized as a natural remedy for a range of conditions, including anxiety, insomnia, depression, acne, nausea, skin irritations, and headaches, as well as in the prevention of hair loss. Lavender essential oil is widely used in aromatherapy due to its beneficial effects on overall well-being (Pârvu C., 2006; Gladstar R., 2015). It possesses therapeutic properties and is recommended for the treatment of wounds, burns, varicose ulcers, and eczema. Recent studies have demonstrated the antibacterial activity of lavender oil against microorganisms such as *Escherichia coli*, *Bacillus subtilis*, and *Staphylococcus aureus*, as well as its antifungal efficacy against *Candida albicans* (Ștefănescu R., 2018; Hajhaschemi V., 2003).

The pedoclimatic conditions of the Republic of Moldova are particularly beneficial to lavender cultivation, facilitating high yields of both flowers and essential oil. In the context of growing concerns about the sustainable use of natural resources, there is an increasing need for the development and implementation of effective strategies for utilizing plant resources, both in the economic sector and in medical practice, to positively impact public health (Gonceariuc M., 2008; Musteață Gh., 2007).

Numerous studies confirm the influence of environmental factors and cultivation conditions on the production and quality of the essential oil derived from *Lavandula angustifolia* Mill. during the flowering period. Factors such as high temperatures and light intensity are crucial in determining both the yield and the chemical composition of the essential oil. The literature suggests that the optimal harvest time for obtaining high-quality lavender oil occurs when approximately 60% of the flowers are in bloom, with air temperatures exceeding 26°C and no precipitation in the ten days before harvest (Păun E., 1995; Hassiotis C., 2014).

Quantitatively, the European Pharmacopoeia identifies the primary components responsible for the characteristic scent of lavender essential oil as linalool (20-45%) and linalyl acetate (25-47%). Other compounds present in smaller quantities include eucalyptol ($\leq 2.5\%$) and camphor ($\leq 1.2\%$), with the latter being considered a factor that may degrade the quality of the oil. Lavandulol ($\geq 0.1\%$) and lavandulyl acetate ($\geq 0.2\%$) are recognized as specific markers of lavender oil, contributing to its distinct aromatic profile (Ceașescu et al., 1988; Lis-Balchin, 2002; Herraiz-Peñalver et al., 2013).

Differences in the composition of essential oil derived from lavender cultivated in Syria compared with that cultivated in Romania underscore the significant influence of pedoclimatic conditions, genetic factors, and extraction technologies on the phytochemical profile. Syrian lavender is characterized by elevated concentrations of linalool (34.70%), camphor (12.77%) and eucalyptol (11.50%), indicating the plant's adaptation to a more arid climate and higher temperatures, which favor the biosynthesis of oxidized monoterpenes (Gassan al Wassouf, 2018).

In contrast, the essential oils obtained from lavender cultivated in Romania exhibit a more balanced profile, with linalool (20.60-35.99%) and linalyl acetate (12.58-19.65%) predominating, along with notable concentrations of lavandulyl acetate (3.74-10.48%) and other aromatic compounds, including *t*-p-3-ocimene, α -terpineol and nerol. These compounds contribute to a more refined fragrance, which is highly valued in the perfume industry (Robu S., 2011). These regional

variations further substantiate the statement that the chemical profile of lavender essential oil serves as a sensitive indicator of ecological conditions and cultivation practices. The selection of cultivation areas and the optimization of agricultural techniques are therefore crucial in guiding production toward specific cosmetic or therapeutic applications.

MATERIALS AND METHODS

The investigations were conducted between 2014 and 2025, with the experiments carried out in the aromatic plant collection at the NBGI. The study encompassed the following species: *Lavandula dentata* L., *Lavandula angustifolia* Mill. ssp. *pyrenaica* (DC.) Guinea, *Lavandula pinnata* Lundmark, and *Lavandula angustifolia* Mill., cultivar 'Lavinie de grădină'. The primary focus of the research was on the 'Lavinie de grădină' lavender cultivar, with emphasis on evaluating the plants' resistance of the plants to climatic conditions, determining optimal propagation methods, analyzing essential oil content and its chemical composition, and developing specific cultivation guidelines for the registered cultivar 'Lavinie de grădină'. Various propagation methods, seasonal growth rates, and biological potential were tested. Key factors such as vegetation phases, relevant organs for raw material accumulation, plant age, and the seasonal dynamics of essential oil accumulation were assessed, leading to the identification of the optimal harvest period. The plants were cultivated in open fields under ecologically balanced conditions and on a uniform agrotechnical background. Phenological observations and biomorphological studies were performed every three days throughout the entire growing season (Sparks, 2009). Essential oil was extracted via steam distillation using a Clevenger-type hydrodistillation apparatus (Clevenger, 1928), with direct heating and rigorous monitoring. The chemical composition of the essential oil was determined by gas chromatography coupled with mass spectrometry (GC-MS), using an Agilent Technologies 6890N gas chromatograph connected to the 5975 Inert XL Mass Selective Detector (Adams, R., 2017). Phytochemical analyses were conducted at the Biological Research Center "Stejarul", Piatra Neamț, Romania.

RESULTS AND DISCUSSIONS

Among the lavender species monitored and evaluated within the collection of the "Alexandru Ciubotaru" National Botanical Garden (Institute) (NBGI) is *Lavandula dentata* L., a species introduced via international seed exchange from France. Native to the Mediterranean basin, Eritrea, Ethiopia, and the Arabian Peninsula, *L. dentata* thrives under local pedoclimatic conditions, achieving an average height of approximately 60 cm. The plant is characterized by gray-green leaves that form a delicate, velvety background with a characteristic silver sheen. The ornamental value of *L. dentata* is notable, with large flowers of intense violet color that harmonize with the leaves, which feature slightly serrated edges and are arranged in false whorls, forming spike inflorescences. The shoots extend radially from the base, displaying a curved structure and lateral divergence toward the apex. The plant emits a strong, typical lavender fragrance, attributed to its high content of linalool (45.06%), followed by camphor (15.62%) and borneol (8.28%). Under the pedoclimatic conditions of the Republic of Moldova, *L. dentata* has demonstrated resistance to low temperatures of up to -10°C , in particularly cold winters, it was necessary to transfer the plants to protected environments. Its drought tolerance was assessed as moderate. In traditional medicine, *L. dentata* is used to treat gastrointestinal disorders and is recognized as an effective antidepressant (Figure 1.1). Another species evaluated in this study is *Lavandula angustifolia* Mill. ssp. *pyrenaica* (DC.) Guinea, named after the Pyrenees Mountains, where it predominantly occurs on the Spanish side, with a limited distribution in France. This subspecies closely resembles *L. angustifolia* ssp. *angustifolia*, which is found further east in France and Italy. In the collection of NBGI, it was introduced through international seed exchange from Germany and has demonstrated good adaptation to local pedoclimatic conditions. The plants require a southern exposure and full sunlight, reaching the peak flowering phase approximately two weeks earlier than *L. angustifolia* Mill. Introduction studies highlight its high resistance to drought, frost, diseases, and pests. Morphologically, it is

an evergreen shrub with gray-pubescent stems, growing to heights ranging from 30 to 60 cm, and occasionally reaching up to 100 cm, characterized by an intense fragrance. The root system is taproot-like, woody, and branched at the apex. The lower, woody branches are intensely branched and ascending, bearing numerous young shoots. The flowering shoots are tetrahedral, with elongated upper internodes and typically numbering between 100 and 120. The leaves are opposite, sessile, oblong-linear, with curved edges, measuring 2-6 cm in length, pubescent and green or gray-green in color. The flowers are grouped in false whorls, forming spike inflorescences; the corolla, approximately 1 cm long, is typically blue-violet and pubescent. The fruit consists of four nutlets enclosed in a persistent calyx (Figure 1.2)

Lavandula pinnata Lundmark, commonly known as fern-leaf lavender, is another species present and monitored within the collection. It is characterized by its pinnate, fleshy leaves,

which confer a distinct morphological appearance compared with other lavender species. The flowers, which are of an intense violet-blue color, are arranged in short, spike-like inflorescences on slender stems, spirally opening around the floral axis. The flowering period is extended, spanning from early spring to early summer. The plants exhibited rapid growth under fertile soil conditions but also demonstrated the ability to tolerate poor, stony soils. Being native to India and surrounding regions, *L. pinnata* has shown greater tolerance to high temperatures and increased humidity compared with other lavender species, making it particularly suited for warmer climates. However, *L. pinnata* is sensitive to low temperatures and cannot withstand freezing conditions. Thus, under local pedoclimatic conditions, it is cultivated as an annual, with specimens transferred to protected spaces during the cold season to preserve the biological material (Figure 1.3).



Figure 1. General appearance of lavender species: 1. *Lavandula dentata* L.; 2. *Lavandula angustifolia* subsp. *pyrenaica* (DC.) Guineá; 3. *Lavandula pinnata* Lundmark

Lavandula angustifolia Mill. cv. 'Lavinie de grădină' was developed and certified at the "Alexandru Ciubotaru" National Botanical Garden and is officially registered in the Plant Cultivar Catalogue. It was created through individual selection and is characterized by an average harvest period and a plant height of approximately 65 cm. The cultivar can be propagated both generatively and vegetatively. Plants derived from generative seedlings exhibit heterogeneity, displaying variation in size,

flower corolla color, and a shorter lifespan. In contrast, plants propagated vegetatively typically produce between 450 and 500 flowering stems per plant on average.

An experimental plantation of 0.40 ha was established for systematic phenological observations. The research aimed to assess plant resistance to local climatic conditions, determine optimal propagation methods, analyze essential oil content and composition, and develop cultivation guidelines for the

certified cultivar 'Lavinie de grădină'. Various propagation methods were tested, and the plants' seasonal growth patterns and biological potential were evaluated. Key factors, including vegetative phases, organs crucial for raw material accumulation, optimal plant age, and the dynamics of essential oil biosynthesis, were examined to identify the optimal harvest period. The research results indicate that the cultivar 'Lavinie de grădină' is a perennial semi-shrub with a globular shape, developing a thick, deep lignified root system that ensures strong root anchorage and tolerance to variable

pedoclimatic conditions. The stem is highly branched, brown, and features exfoliating bark, reaching heights of 50–70 cm. The young branches are square, pubescent, and gray. From the second year onward, the plants typically produce 450–500 flowering stems per plant. The leaves are opposite, linear, entire, sessile, and acute; the lower leaves are gray and hairy, while the upper leaves are gray-green and less pubescent. The flowers are labiated, intensely aromatic, blue-violet in color, pubescent, and glandular, grouped in spike-like inflorescences typical of the species (Figures 2.1, 2.2).

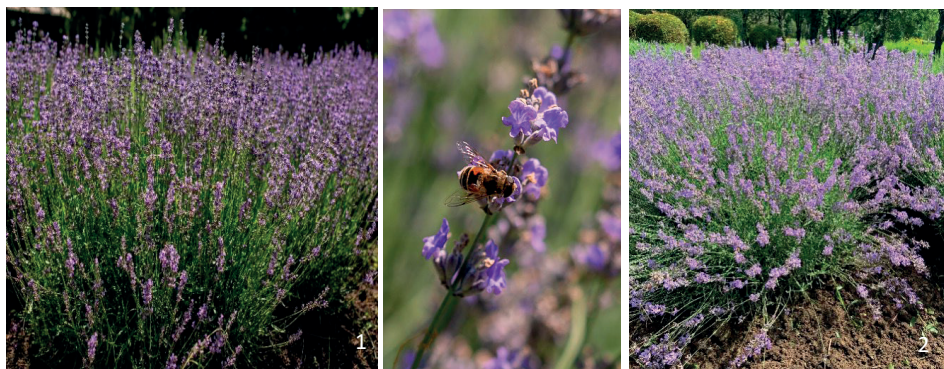


Figure 2. The cultivar *Lavandula angustifolia* Mill. 'Lavinie de grădină':
1. peak the flowering stage; 2. the end of the flowering stage

'Lavinie de grădină' flowers during June and July and is classified as a relatively late-blooming cultivar. The flowering phase typically lasts between 25 and 30 days. The fruits are ovoid nutlets, shiny and brown, arranged at the base of the persistent calyx. This cultivar is valued for its high productivity of raw plant material, good resistance to winter conditions, and the longevity of the plantation. For small areas, where harvesting can be done quickly, the optimal harvesting period is the 75% flowering phase. However, in cases where the harvesting period is extended (due to large areas or limited processing capacity), it is recommended to begin harvesting at the 50% flowering phase and complete it by the 100% flowering phase. The harvested inflorescences are immediately transported to the distillation units. Over several years of testing, the inflorescence yield ranged from 9.2 to 10.8 t/ha, with essential oil content varying between 1.390% and 1.484% (f. m.) and 4.251% (d. m.). Under these conditions, essential oil production

ranged from 105.4 to 145.3 kg/ha. Under favorable climatic conditions, the cultivar can exhibit a secondary flowering period in October. However, during this period, the essential oil content is significantly lower, and the flower stems are much shorter. Flowers produced at this time are primarily suitable for decorative purposes in floral arrangements. According to phenological observations over a 10-year period, the plants showed no signs of damage from low temperatures, drought, diseases, or pests, underscoring the biological stability and high adaptability of the cultivar to local pedoclimatic conditions. In the fifth year of growth, when the bushes reached full vigor, methods for propagating planting material through cuttings and rooted layers were evaluated. These vegetative propagation methods proved effective in producing uniform plantations with higher yields of raw material and essential oil.

Vegetative propagation through rooting lignified cuttings begins with the collection of

planting material, typically performed during autumn (September-October) or spring (March-April) when the plants are in their vegetative rest phase. The cuttings are sourced from healthy, well-developed mother plants aged between 3 and 5 years, exhibiting phenotypic traits that align with cultivar standards. Using a sharp knife or blade, the cuttings are sectioned to minimize tissue damage, ensuring a clean cut just below a vegetative bud. Leaves are carefully removed to prevent injury to the buds. At the base of each cutting, a single slanted wedge-shaped incision is made just beneath the bud to promote callus formation and initiate the rooting process.

The cuttings, typically 5-6 cm in length, should be planted within 1-2 hours of collection. If planting is delayed (but not exceeding 24 hours), the cuttings are placed in containers with clean water to prevent dehydration. The soil

designated for the rooting beds must be free of weeds and preferably located near a reliable water source. It is leveled, broken up, and lightly compacted to ensure a stable substrate. Frames are then placed with a 40-50 cm distance between them to facilitate proper access and ventilation. Within the frames, a layer of well-sieved sand (8-10 cm thick) is spread and thoroughly watered after leveling.

Following watering, rows are marked using specialized markers to guide planting. The cuttings are manually inserted into the substrate, spaced 5-10 cm apart, depending on the vigor of the planting material. They are gently pressed into the substrate to prevent damage to the apical leaves. Moreover, the cuttings are watered abundantly, and the frames are covered with whitewashed glass panes and mats to maintain optimal humidity and protect the cuttings until the first roots emerge (Figure 3).

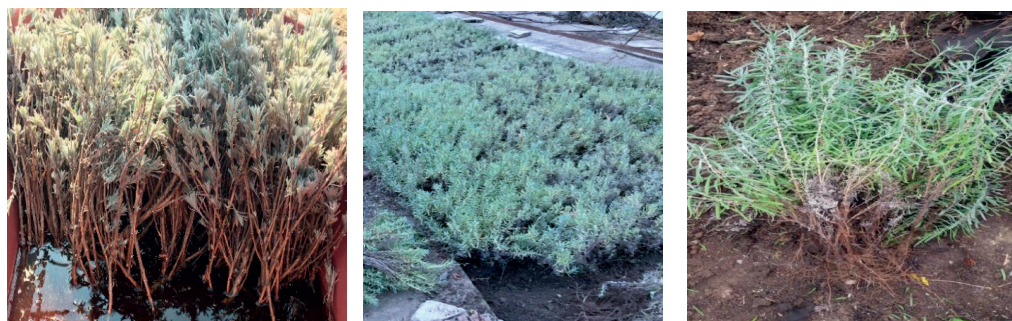


Figure 3. Obtaining rooted cuttings of *Lavandula angustifolia* Mill. cultivar 'Lavinie de grădină'

Once the rooting process is initiated, the cuttings are gradually hardened off by opening the frames for a few hours each day, preferably during the cooler parts of the day, such as morning or evening. When flower stems emerge, they are pruned to approximately half their length to promote branching and ensure balanced growth of the young plants. The rooting success rate typically ranges from 80% to 85%. Following rooting, the cuttings are transplanted into open ground for the establishment of industrial plantations, where field success rates are generally very high, ranging from 90% to 95%.

Horizontal layering (also known as rooted layers) is a simple and cost-effective propagation method that offers significant economic advantages. This technique ensures the production of

genetically pure material, resulting in plants that exhibit the desired characteristics of the cultivar, particularly in terms of both productivity and essential oil quality. Horizontal layering involves rooting the basal portions of lignified stems and branches without severing them.

The process begins by slightly heeling the selected lavender bushes, adding soil from between the rows to form a 4-5 cm mound beneath the lateral shoots. The bush is then carefully opened from the center outward, and the stems are bent radially toward the soil. Additional soil is placed in the center and compacted to form a 10-12 cm layer, ensuring good contact between the bent stems and the substrate. The leaf tips of the bent stems, which extend approximately 8-10 cm, remain above the soil. Soil covering for rooting is typically

carried out in early spring or late autumn, during the vegetative rest periods of plants. Mulching between rows significantly accelerates the rooting process and increases the number of layers produced from each plant. A single

bush can yield 100-120 rooted layers. This method also ensures the production of uniform, vigorous layers with well-developed root systems, resulting in high-quality planting material (Figure 4).



Figure 4. Obtaining rooted layers of *Lavandula angustifolia* Mill. cultivar 'Lavinie de grădină'

The layers are detached with relative ease, resulting in minimal loss or damage to the mother plant. The rootstocks are planted in small holes, positioned at the intersection of the marker. Prior to planting, the rootstocks, having been properly selected and stored, have their roots pruned back to 15-20 cm and are subsequently soaked in a clay solution. The rootstocks are then placed in the soil 5-6 cm below the root collar ring, ensuring that the lower branches are adequately covered. During planting, the roots are carefully arranged, covered with soil, watered, and topped with an additional layer of soil, which is not compacted. Before winter, the seedlings are mounded with a 3-5 cm layer of soil for protection.

To assess the quality of the essential oil obtained from *Lavandula angustifolia* Mill., cv. 'Lavinie de grădină', its chemical composition was analyzed, and a total of 24 chemical components were identified. The analysis was performed on two essential oil samples: one collected during the peak flowering phase and the other during the end of the flowering phase, in order to examine the phytochemical dynamics across different phenological stages. The chemical composition was determined using gas chromatography-mass spectrometry (GC-MS). Compound identification was based on retention times, retention indices, and the comparison of mass spectra with reference data from the literature (Figure 5).

According to the chromatographic analysis, the main constituents of the essential oil include

linalool, which accounts for 48.8% to 50.0% of the composition, with the highest concentration recorded during the mass flowering phase, and linalyl acetate, which ranged from 6.7% to 12.1%. Additionally, α -terpineol was present in amounts ranging from 0.6% to 6.9%, showing a notable decrease toward the end of the blooming period. Lavandulyl acetate was identified in concentrations between 4.0% and 11.3%, with the highest levels recorded at the end of the flowering phase. Eucalyptol (1,8-cineole) was present at 3.6% to 3.9%, exhibiting an increasing trend toward the end of flowering. Geranyl acetate varied from 1.3% to 2.3%, with the highest abundance during the peak flowering phase. Other identified compounds include trans-linalool oxide, cis- β -ocimene, and additional monoterpenes, all of which contribute to the complex aromatic profile of the essential oil.

These findings highlight the significant influence of the phenological phase on the chemical composition of the essential oil produced by *Lavandula angustifolia* Mill. cv. 'Lavinie de grădină'. This is particularly important for determining the optimal harvest time based on the intended therapeutic or cosmetic use of the oil. The chemical composition of the essential oil underscores that adherence to the appropriate harvest timing for this cultivar is essential, as it not only affects the total essential oil yield but also the chemical profile.

RT (min)	Compounds	Area %		Farmacopeea Eur vol. 8.0
		Peak flowering phase	End of flowering phase	
5.85	α -Pinene	0.1	-	
7.19	3-Octanone	0.2	0.3	0.1-2.5%
7.39	β -Myrcene	1.1	1.0	
8.05	Hexyl acetate	0.2	0.2	
8.49	p-Cymene	-	0.3	
8.66	Limonene	0.4	0.3	<1.0%
8.74	Eucalyptol/1,8-Cineole	3.6	3.9	<2.5%
8.91	trans- β -Ocimene	2.9	2.2	
9.32	cis- β -Ocimene	1.5	1.2	
10.19	cis-Linalool oxide	0.8	1.7	
10.78	trans-Linalool oxide	1.1	1.8	
11.25	Linalool	50.0	48.8	20-45%
11.68	1-Octenyl acetate	0.3	1.1	
12.98	Camphor	0.3	0.5	<1.2%
13.90	Lavandulol	1.1	0.2	>0.1%
14.36	4-Terpineol	0.7	0.8	0.1-6.0%
14.90	α -Terpineol	6.9	0.6	<2.0%
17.69	Linalyl acetate	12.1	6.7	25-46%
19.16	Lavandulyl acetate	4.0	11.3	>0.2%
22.24	Neryl acetate	1.4	4.0	
23.03	Geranyl acetate	2.5	1.3	
24.57	β -Caryophyllene	1.5	2.4	
26.06	cis- β -Farnesene	0.6	1.1	
30.95	Caryophyllene oxide	1.4	0.4	
Other compounds		5.6	5.6	

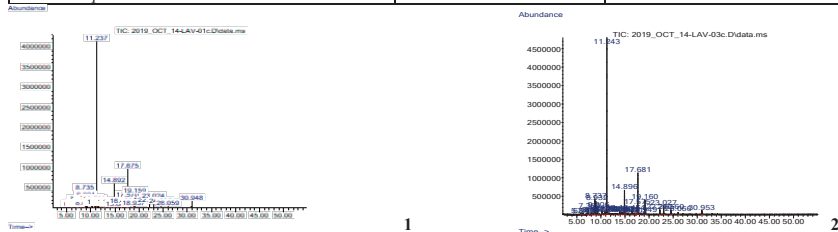


Figure 5: The chemical composition of the essential oil of *Lavandula angustifolia* Mill. cultivar 'Lavinie de grădină' by GC-MS: 1. Chromatogram - peak flowering phase; 2. Chromatogram - end of flowering phase

For plantations established with vegetatively propagated material, where plants exhibit uniform flowering, determining the harvest time becomes relatively simple. The flowering period of this cultivar lasts between 35 and 45 days and can be divided into four distinct phases: bud formation (when the floral buds first appear); early flowering (when approximately 25% of the flowers are open); peak flowering (when 50% of the flowers are open); and end of flowering (when 90% of the flowers have withered). For smaller areas, harvesting can be performed when 70–80% of the flowers are open. For large plantations, harvesting should span 11 to 13 days, beginning when about 55% of the flowers are open and concluding at 95% open flowers. It is important to avoid harvesting during rainy weather, high humidity, or strong winds, as these conditions can negatively affect the quality of the essential oil. Harvesting is best done in the morning hours, between 9:00 and 11:00 AM,

when the concentration of essential oil is at its peak. After 2:00 PM, the essential oil content decreases significantly, making later harvesting less efficient.

CONCLUSIONS

Lavandula angustifolia Mill. cv. 'Lavinie de grădină' is characterized by early technical maturity, high flower yield, and a rich content of essential oils.

This cultivar exhibits a range of specific quantitative and qualitative traits, including plant size, bush shape, inflorescence length, corolla color, inflorescence (spike) length, flower stalk length, and the number of flower stalks.

It is capable of being propagated both generatively and vegetatively, through the use of cuttings and rooted rootstocks, methods which

facilitate the establishment of uniform and productive plantations.

During the peak flowering phase, the essential oil content reaches its highest values, with a maximum of 4.251% (d. m.). The primary compounds identified in the essential oil include linalool (48.8-50.0%), linalyl acetate (6.7-12.1%), α -terpineol (0.6-6.9%), lavandulyl acetate (4.0-11.3%), eucalyptol (1.8-cineole) (3.6-3.9%), geranyl acetate (1.3-2.3%), and other secondary compounds. The productivity of this cultivar is influenced by prevailing pedoclimatic conditions, with yields ranging from 9.2 to 10.8 t/ha for raw material and 105 to 145.3 kg/ha for essential oil.

The primary application of the cultivar 'Lavinie de grădină' is for the extraction of essential oil, which is utilized in a variety of industries, including perfumery, aromatherapy, food production, pharmaceuticals, beekeeping, and ornamental purposes. Furthermore, the cultivar exhibits remarkable resistance to frost, drought, disease, and pests, rendering it a highly resilient crop. Given these traits, it represents a viable and promising alternative crop with substantial potential for exploitation, particularly in the context of ongoing climate change.

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***NEPETA TRANSCAUCASICA* GROSSCH. – A PROMISING AROMATIC PLANT**

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Abstract

*This article presents the results of botanical, ecological, and biochemical research on the aromatic species *Nepeta transcaucasica* Grossch., introduced and studied under the pedoclimatic conditions of the Republic of Moldova. The study aims to highlight the particularities of propagation and to develop the primary elements of its cultivation, thereby contributing to the expansion of knowledge about regional aromatic plants and to the establishment of sustainable crops integrated into modern agricultural practices in the country. *Nepeta transcaucasica* Grossch. is a perennial herbaceous plant studied at the National Botanical Garden as an aromatic, medicinal, ornamental, and melliferous species. The main biologically active component is the essential oil, which contains citronellol, geraniol, citral, eucalyptol, nepetalactone isomer A, and carvacrol - compounds that confer diuretic, analgesic, antitussive, and antimicrobial properties. This essential oil is used in the perfume and cosmetics industries, as well as in soap manufacturing. In gastronomy, the leaves and young shoot tops are used as a spice during the budding and full flowering stages. The plant is also notable for its high ornamental value, drought resistance, and long flowering period, making it an important nectar source for pollinators.*

Key words: aromatic plant, cosmetics, essential oil, introduced plants, medicine.

INTRODUCTION

Aromatic plants have always played a vital role in human life, valued not only for their pleasant fragrance and taste but also for their therapeutic properties. Rich in essential oils and other bioactive compounds, these plants are used across numerous fields - from gastronomy and medicine to cosmetics, agriculture, and industry. Throughout history, they have been regarded as symbols of health and purity, forming an integral part of cultural traditions and customs. Their significance extends beyond practical applications, contributing to the promotion of a natural and balanced lifestyle and reflecting the deep connection between humans and nature. In this context, expanding the raw material base derived from aromatic plants with a high and qualitative essential oil content, studying the biochemical characteristics of new promising essential oil-bearing species, and investigating the seasonal dynamics of biologically active substances depending on the plant organ, phenological phase, and age remain current and relevant research objectives. The study of native aromatic and medicinal plants, as well as the introduction of species from other geographical

regions under the pedoclimatic conditions of the Republic of Moldova, represents a priority area of scientific research within the National Botanical Garden. When introducing new species to an area, interactions among vegetation factors are carefully considered to balance pedoclimatic conditions with the plants' biological requirements. This prevents situations where natural factors enhance biomass at the expense of active compound content. Cultivating new species and expanding the range of available essential oils creates opportunities to discover effective natural agents for health promotion and disease prevention. The genus *Nepeta* L. is one of the largest within the family Lamiaceae, comprising approximately 300 species distributed across temperate regions of the world - particularly in Central and Southern Europe, the Middle East, Central and Southern Asia, and certain areas of Africa and North America. Most *Nepeta* species are endemic, especially in Southwest Asia (Turkey and Iran). Plants of this genus are well known for their medicinal properties and are widely used in traditional medicine to treat various health issues (Formisano, 2011).

Among the numerous genera of aromatic plants represented and studied in the collection, a particularly important role is played by the genus *Nepeta* L., which is represented in the spontaneous flora of the Republic of Moldova by three species: *N. parviflora* Bieb., *N. pannonica* L., and *N. cataria* L. Within the collection of the National Botanical Garden, the following species have been introduced and studied: *Nepeta cataria* L., *Nepeta transcaucasica* Grossch., *Nepeta melissifolia* Lam., *Nepeta grandiflora* M. Bieb., and *Nepeta kokanica* Regel. The subject of this research is the allochthonous species *Nepeta transcaucasica* Grossch., which belongs to the kingdom Plantae, phylum Tracheophyta, class Magnoliopsida, order Lamiales, family Lamiaceae, and genus *Nepeta* (Lawalrée, 1980). This species is native to the Caucasus region - an area characterized by diverse climatic conditions that have favoured the evolution of forms adapted to both high altitudes and specific soil types.

According to specialized literature, this species represents an important natural source of compounds with antimicrobial activity (Öztürk, 2009). The plant exhibits diuretic, analgesic, anti-inflammatory, anxiolytic, sedative, hypnotic, antispasmodic, astringent, antitussive, and antimicrobial effects (Raboteagov, 2014). Characterized by a high content of nepetalactone and other aromatic compounds, this species offers significant potential for utilization across multiple economic sectors. According to recent analyses, 51 constituents have been identified in the essential oil of *N. transcaucasica*, among which the major components are β -citronellol (52.05%), eucalyptol (7.34%), β -citronellal (6.06%), germacrene D (5.45%), β -ocimene (5.14%), β -caryophyllene (3.06%), neral (2.42%), geranial (1.51%), geraniol (1.44%), limonene (0.35%), and β -linalool (0.07%) (Mollova, 2023).

In recent years, interest in aromatic and medicinal plants has increased considerably due to the growing demand for natural, organic, and sustainable products. In this context, the study of the species *Nepeta transcaucasica* Grossch., as a promising aromatic plant under the pedoclimatic conditions of the Republic of Moldova, is particularly relevant for assessing its therapeutic, aromatic, and agronomic

potential. The production of medicines derived from non-toxic plant material provides complex healing effects and supports the body's natural immunity. The cultivation of medicinal and aromatic plants is favoured by the pedoclimatic conditions of the country. The diversity of natural conditions determines a wide range of species that serve as valuable sources of plant material for the pharmaceutical, cosmetic, and food industries (Teleuță, 2008).

The collection of aromatic plants at the "Alexandru Ciubotaru" National Botanical Garden (Institute) comprises over 170 native and allochthonous aromatic taxa, distinguished by their intense fragrance and rich, well-developed flavour.

MATERIALS AND METHODS

The research was conducted over the period 2018-2024, with experiments carried out in the "Aromatic Plants" collection of the "Plant Resources" Laboratory. The species *Nepeta transcaucasica* was introduced and preserved as a promising species under local pedoclimatic conditions. Seed material was obtained through the International Seed Exchange from Crimea. Subsequent stages involved the study of the bioecological and morphological characteristics of the plants under the new growing conditions. Phenological observations were conducted according to established methodologies and recorded once every three days throughout the entire growing season (Sparks, 2009). Harvesting was performed during the period of peak flowering, following standard experimental field procedures (Dospehov, 2011). The study examined propagation methods, seasonal dynamics, and the biological potential of plant growth and development. The influence of phenological phase, plant organ, age, and seasonal dynamics on the accumulation of raw material and essential oil was investigated, and the optimal harvesting period was determined. Plants were cultivated under open-field conditions using ecologically balanced management and standard agrotechnical practices.

The essential oil content of the plants was determined using the steam distillation method, specifically the Ginzberg method, with a Clevenger-type apparatus, and calculated

relative to the absolute dry mass of the plant material (Gosud. Farm., 1968). The chemical composition of the essential oil was analyzed by gas chromatography-mass spectrometry (GC-MS) using an Agilent Technologies 6890N gas chromatograph coupled with a 5975 inert XL Mass Selective Detector. Phytochemical analyses were conducted in collaboration with the “Stejarul” Biological Research Center, Piatra Neamț, Romania.

RESULTS AND DISCUSSIONS

For several years, the genus *Nepeta* has been studied in the experimental field of the Plant Resources Laboratory at the Botanical Garden. Systematic, ecological, biomorphological, and technological studies have assessed the effects of climate and soil on plant development, stem height, flower size, and flowering period to select the best specimens for essential oil extraction. Of particular interest is the allochthonous aromatic species *Nepeta transcaucasica* Grossch., commonly known as catmint, from the Lamiaceae family, introduced to the Botanical Garden in 2018, and studied as both an aromatic and medicinal plant. *Nepeta N. transcaucasica* originates from mountain regions of the Caucasus, where it naturally grows at altitudes between 1,600 and 3,500 m. It is adapted to cold winters and moderate summers and prefers well-drained, neutral to slightly alkaline soils. In the Republic of Moldova, the temperate climate and sandy-clay soils allow excellent adaptation, provided that excessive moisture is avoided, as it can damage the roots. The plant demonstrates good drought resistance due to its pubescence, tolerates low temperatures, and responds favourably to repeated cuttings, which stimulate re-flowering. Under the conditions of the Republic of Moldova, *Nepeta transcaucasica* develops as a perennial herbaceous plant with semi-woody stems; in the first year of development reach a height of 30-40 cm and a diameter of 40-50 cm. Fifteen to eighteen lateral branches arise from the central stem, measuring 51-54 cm long (the

lower branches) and up to 30 cm long (the upper ones). The stems are erect, branched, and densely covered with glandular and simple hairs that secrete an aromatic essential oil. Their surface is slightly pubescent, giving them a fine, velvety texture.

The leaves are opposite, with slightly serrated margins, and range from ovate-lanceolate to elliptical in shape. Leaf size varies along the stem, measuring 3.5 cm long and 2.5 cm wide on the lower branches, and 2.3 cm long and 1.4 cm wide on the upper branches. The leaf surface is covered with short hairs, and coloration ranges from light green on the upper side to pale green or grey on the lower side, due to pronounced pubescence. The veins are well defined, giving the leaves a slightly rough texture to the touch. Leaf petioles are 0.5-1.2 cm long, and the central stem has a diameter of 0.3-0.4 cm. Inflorescences are terminal spikes, composed of hermaphroditic, pentamerous, bilabiate flowers arranged in compound whorls. Flower colour is predominantly lilac, with purple or violet spots on the lower lip. The corolla is tubular and bilabiate, with a slightly concave upper lip and a lower lip formed by three lobes, the central lobe being more prominent. The calyx is pubescent, 0.7 cm long, tubular-campanulate, with acute teeth, and is covered with glandular hairs. The flowers are arranged in multifloral false whorls, each consisting of 18-20 flowers, which emit a strong, pleasant fragrance characteristic of species in the genus *Nepeta*. The fruit is a small, brown, smooth nutlet, approximately 1-2 mm in size, containing fine seeds with good germination capacity. Laboratory research indicates that the weight of 1,000 seeds is 0.70 g. The root system is well developed, comprising a taproot and numerous lateral roots that grow deeply into the soil, providing the plant with stability and increased resistance to drought and low temperatures. Overall, *Nepeta transcaucasica* exhibits a compact habit, characterized by a dense crown resulting from abundant branching and foliage (Figure 1).

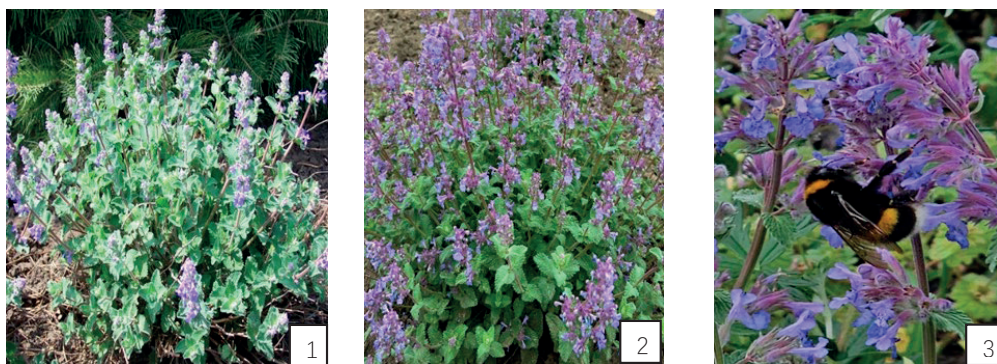


Figure 1. The overall appearance of the species *Nepeta transcaucasica* Grossch.
1. budding phase; 2. peak flowering phase; 3. pollination process

This characteristic gives the plant value not only for its aromatic properties but also for its ornamental appeal, making it highly appreciated in landscaping and garden design.

In the first year of growth, when flower buds begin to develop, the plants are cut to a height of 8-10 cm above the ground. This pruning promotes the development of new shoots and encourages lateral expansion of the bushes. In the second year and in subsequent years, active vegetation typically begins at the end of April, when temperatures reach 7-8 °C. Each plant produces 100-150 perennial shoots, forming low, bushy structures with a diameter of 70-80 cm, with the most vigorous growth occurring in May. The complete shoot development cycle, from emergence to peak flowering, lasts 50-60 days under conditions of sufficient rainfall or on irrigated land, and 70-80 days under non-irrigated conditions or in periods of water deficit. The yield of floral biomass in the first year is 80-150 g of fresh mass per stem,

corresponding to 27-34 g of dry matter. In the second year, the yield per stem increases to 180-210 g of fresh mass and 70-83 g of dry matter.

The study showed that in *Nepeta transcaucasica*, the essential oil content during the peak flowering phase ranged from 0.19% to 0.23%, with an average of 0.20% relative to the green mass of the plant material, or 0.6-0.8% of the absolute dry matter. Among the plant organs, the highest concentration of essential oil was found in the inflorescences, where it reached 1.10-1.22% of the absolute dry matter. The greatest accumulation of essential oil was observed in plants aged 2-5 years. A total of 25 components were identified in the essential oil, the major constituents being citronellol (34.04%), geraniol (11.67%), citral (10.79%), eucalyptol (9.12%), nepetalactone isomer A (8.32%), nepetalactone isomer B (6.33%), carvacrol (4.21%), linalool (2.22%), β -caryophyllene (1.31%) and thymol (0.92%) (Figure 2).

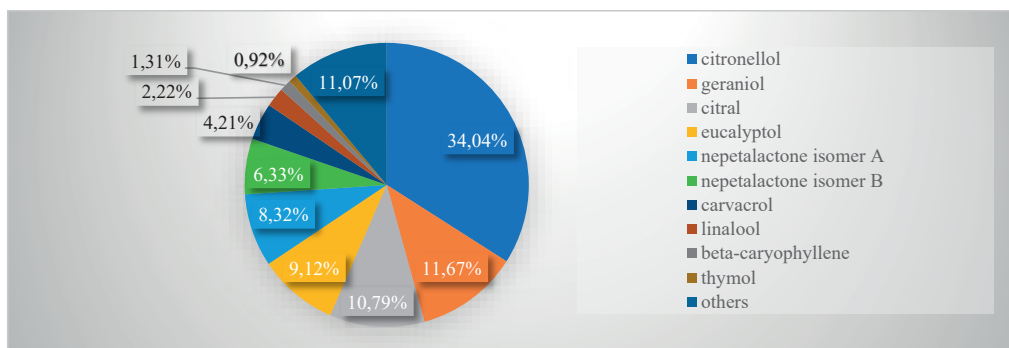


Figure 2. The chemical composition of the essential oil of *Nepeta transcaucasica* Grossch. Determined by GC-MS

Nepeta transcaucasica grows on a wide range of soils, from chernozems to calcareous and stony types. Under cultivation, it performs best on calcareous, humus-rich, well-structured soils with a light to medium texture. During winter, the species tolerates temperatures as low as -25 to -30°C. Although generally undemanding, *N. transcaucasica* responds positively to irrigation under drought and high-temperature conditions. In Moldova, up to three harvests can be obtained annually under irrigated conditions, whereas on non-irrigated land only one, and occasionally two, harvests are possible, with lower productivity.

For the establishment of pilot and industrial plantations, propagation methods have been investigated. The species propagates effectively both vegetatively (by division of mature plants or by cuttings) and generatively (by direct sowing of seeds into the soil in late winter or early spring, or by growing seedlings in greenhouses).

The planting material obtained from cuttings of green annual shoots is rooted in unheated plant nurseries. The cuttings are collected in spring, from the sprouting stage to the budding phase. The optimal length of the cuttings is 8-10 cm, each containing 3-4 internodes. To achieve a high rooting percentage, a temperature of 26-28°C should be maintained. For this reason, rooting is recommended to be carried out in film-covered greenhouses, where temperature, as well as air and substrate humidity, can be controlled. Under these conditions, the rooting success rate ranges from 73% to 80%. Once the root system is formed, the seedlings are transplanted into open fields, following a planting scheme of 25-30 cm between plants and 35-40 cm between rows. The obtained cuttings are planted deeply enough for the root collar to be covered with 3-4 cm of soil, after which the soil around the plants is gently compacted. In subsequent years, crop maintenance consists of mechanical loosening of the soil between and along the rows. Under irrigated conditions, the yield forms within 50-60 days. By the end of May, the plants reach the peak flowering stage, which also corresponds to the optimal harvesting period. The freshly harvested green biomass is immediately transported to processing facilities for distillation or other processing.

Generative propagation is achieved by direct sowing in the field, either in early spring (March-April) or in autumn (September-October). For seedling production, seeds are sown under greenhouse conditions on a suitable substrate composed of sand, perlite, and soil. To ensure uniform and vigorous germination, seeds are recommended to be stored at 20-25°C for 4-6 days. Germination occurs within 2-3 weeks at temperatures of 18-22°C. Seedlings are transplanted into the field after the risk of frost has passed.

In conclusion, the allochthonous species *Nepeta transcaucasica* Grossch., introduced and studied at the National Botanical Garden, can be recommended for cultivation as an aromatic and medicinal plant with valuable biochemical and therapeutic potential. Although current studies have demonstrated multiple possible uses, the species remains insufficiently explored, offering promising prospects for further research and the development of new organic products.

CONCLUSIONS

The pedoclimatic conditions of the Republic of Moldova have proven favourable for the growth and development of the allochthonous aromatic species *Nepeta transcaucasica* Grossch., which completes its full ontogenetic cycle and is characterized by high frost resistance, tolerating temperatures down to -25 to -30°C.

The species exhibits a strong capacity for reproduction, both vegetative – by division and by cuttings – and generative – through direct sowing of seeds into the soil in late autumn or early spring, or by growing seedlings under protected cultivation.

Studies on essential oil accumulation have demonstrated that this process depends on plant age, phenological stage, and plant organ. The maximum essential oil content is recorded during the peak flowering phase, ranging from 0.6% to 0.8% of the absolute dry matter, and reaching 1.10-1.22% in inflorescences. Plants aged 2-5 years contain the highest levels of essential oil.

Under irrigated conditions, the crop can produce up to three harvests per year, whereas under non-irrigated conditions only one or, occasionally, two harvests are obtained, with lower productivity.

The chemical analysis of the essential oil of *Nepeta transcaucasica* Grossch. identified 25 components, the major ones being citronellol, geraniol, citral, eucalyptol, and nepetalactone isomer A.

The high biomass yield and valuable essential oil content confirm the potential of this species as a raw material for the cosmetic industry, where it can be used as an active ingredient in perfumes, moisturizers, and fragrances. These findings justify the need to develop primary cultivation practices for this aromatic plant.

Due to its high melliferous value, attractive ornamental appearance, compact habit, and dense foliage, *N. transcaucasica* can also be recommended as an ornamental species for gardens, borders, and landscape design.

Its adaptability to local conditions, rich content of aromatic compounds, and multiple potential applications further support its inclusion in the economic circuit, contributing to the diversification of aromatic and medicinal plant crops cultivated in the Republic of Moldova.

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SPATIAL ANALYSIS OF GREEN INFRASTRUCTURE IN CLUJ-NAPOCA AND ITS IMPLICATIONS FOR URBAN AIR QUALITY

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Abstract

This research analyzes the spatial distribution and total surface area of green spaces in Cluj-Napoca with the objective of evaluating their contribution to urban air quality. Geospatial datasets were retrieved from the Copernicus platform, including layers on vegetation cover, built-up areas, hydrographic elements, and additional urban features. These data were integrated into AutoCAD and subsequently processed in PyCharm, where spatial analyses enabled the precise delineation of vegetated areas and the assessment of their citywide distribution. On this basis, the green space per capita indicator was computed, offering a quantitative perspective on urban sustainability and the potential role of vegetation in mitigating air pollution. In addition to the quantitative evaluation, the study advances recommendations for the expansion and optimization of green areas in sectors exhibiting insufficient vegetation. These proposals are intended to enhance ecological connectivity, improve air quality, and reinforce the resilience of the urban system. The results emphasize the necessity of continuous monitoring and strategic planning of green infrastructure, underlining the strong linkage between urban development dynamics, ecosystem functioning, and human well-being.

Key words: green space, urban sustainability, AutoCad.

INTRODUCTION

Urban green spaces play a fundamental role in supporting environmental quality, public health, and urban resilience, particularly in rapidly developing metropolitan areas. In this context, the municipality of Cluj-Napoca, located in north-western Romania in the central region of Transylvania, provides a relevant case study for assessing the spatial distribution and structural configuration of urban vegetation within a complex and heterogeneous urban landscape. The city has undergone significant expansion and functional transformation in recent decades, leading to increased pressure on land resources, changes in land-use dynamics, and growing concerns regarding the spatial equity and accessibility of green infrastructure. The relationship between green infrastructure and air quality is particularly relevant in contemporary urban planning, as vegetation can contribute to the reduction of airborne pollutants by capturing particulate matter on leaf surfaces, absorbing gaseous pollutants, and moderating urban heat-island effects that may intensify atmospheric pollution.

This study integrates geospatial datasets derived from official administrative sources, OpenStreetMap building footprints, and high-resolution orthophotography in order to extract, classify, and evaluate the spatial extent of urban green spaces at the neighborhood level. The methodological framework combines ArcGIS Pro-based raster classification using the Iso Cluster Unsupervised algorithm with automated spatial processing performed in the PyCharm environment and subsequent visualization in AutoCAD. (Cooper & Chiaradia, 2020) This workflow enables both the delineation of vegetation patterns and the quantification of district-level indicators related to built-up areas, green space distribution, and urban morphology. Beyond the cartographic identification of vegetated areas, the analysis places particular emphasis on the interpretation of results in relation to World Health Organization (WHO) (WHO, 2017) guidance concerning green-space availability, accessibility, and potential public-health benefits. The main objective of this study is to analyze the spatial distribution and structural configuration of urban green infrastructure in Cluj-Napoca using GIS-based

classification, automated spatial processing, and CAD-based visualization. Specifically, the study aims to identify and classify vegetated areas from high-resolution orthophotographic data, quantify green-space surfaces and built-up footprints at the neighbourhood level, compare the spatial distribution of vegetation across urban districts, and interpret the results in relation to environmental quality and potential air-quality mitigation. The study also seeks to highlight neighbourhoods with reduced green-space availability and to provide a spatially explicit basis for supporting sustainable urban planning strategies focused on ecological connectivity, environmental resilience, and improved urban living conditions.

MATERIALS AND METHODS

The implications for urban air quality were assessed using the spatial distribution and extent of green infrastructure as environmental proxy indicators. Vegetation cover was considered relevant due to its potential role in pollutant retention, particulate matter interception, temperature regulation, and mitigation of urban environmental stress.

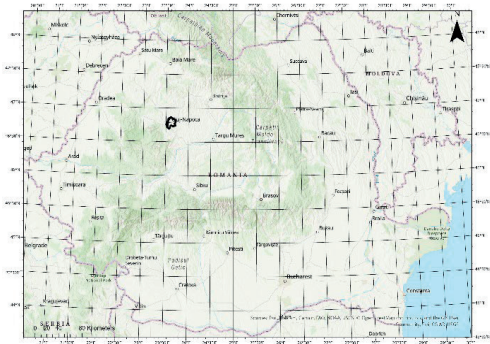


Figure 1. Romania

Therefore, district-level indicators such as green-space area, vegetation percentage, built-up footprint area, and the relationship between vegetated and impervious surfaces were used to evaluate the potential contribution of green infrastructure to urban air-quality improvement. Cluj-Napoca is a municipality located in north-western Romania (Figure 1), more specifically in the central region of Transylvania, covering an area of approximately 180 km².

The administrative boundary of the municipality of Cluj-Napoca (Figure 2), together with the delimitation of its internal neighbourhood units (Figure 3), was acquired through the Geo-Spatial application and managed in Arcgis Pro. (Geo-Spatial, 2025)

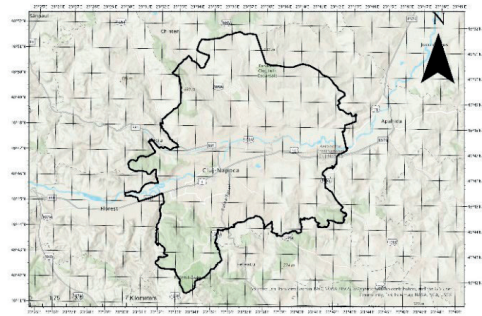


Figure 2. The Administrative Boundary of the Municipality of Cluj-Napoca

This platform provides access to georeferenced vector datasets derived from official territorial and cadastral sources, ensuring the accuracy and reliability of the spatial limits used in the analysis. The downloaded layers served as the foundational geographic framework for subsequent spatial processing and thematic evaluations conducted in this study. The building footprints used in this study were obtained from the GeoFabrik platform, which provides regularly updated extracts of OpenStreetMap (OSM) (OpenStreetMap, 2025) data in vector format. GeoFabrik's (OpenStreetMap Data Extracts, 2025) datasets are derived from the global OSM database and include georeferenced building polygons that are extracted, processed, and organized by geographic region.

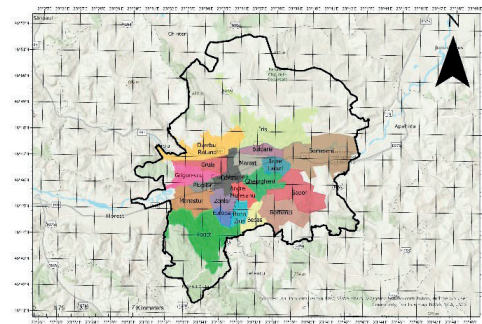


Figure 3. The Limitation of the Internal Neighbourhood

This source ensures high spatial coverage, open accessibility, and compatibility with standard GIS workflows. The building layer downloaded from GeoFabrik served as a fundamental dataset for delineating the built environment within the study area and for conducting subsequent spatial analyses related to urban structure, land use, and the quantification of built-up surfaces. The orthophotoplan presented in Figure 4 was generated based on an aerial survey flight conducted over the Municipality of Cluj-Napoca. Several photogrammetric cameras were mounted on the underside of the aircraft and were programmed to acquire successive images at predefined time intervals.

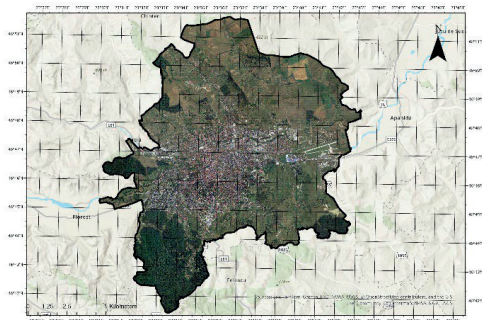


Figure 4. The orthophotoplan of Cluj-Napoca

The collected imagery was subsequently processed using specialized photogrammetric software, resulting in the final orthophoto product. The Iso Cluster Unsupervised Classification algorithm available in ArcGIS Pro performs an automated, data-driven segmentation of raster imagery based on the statistical similarity of pixel values (McGee, Parece, & John, 2023). A five-class configuration was selected because it provided the clearest visual separability between the main land-cover categories present in the urban orthophoto, namely impervious surfaces, bare or sparsely vegetated areas, vegetation, building rooftops, and surfaces with strong spectral contrast such as industrial roofs or construction areas. When applied to an orthophoto, the algorithm analyzes the spectral characteristics of each pixel, typically the red, green, and blue bands, and groups them into a predefined number of clusters that represent internally homogeneous and externally distinct spectral

classes. Iso Cluster uses an iterative procedure derived from the ISODATA (Iterative Self-Organizing Data Analysis) algorithm, which repeatedly calculates class means, assigns pixels to their closest spectral centroid, and updates these centroids until convergence is reached or a maximum number of iterations is achieved (Lemenkova P., 2021).

This process allows the algorithm to partition the orthophoto into clusters corresponding to visually or spectrally distinct land-cover types such as vegetation, built-up surfaces, bare soil, transportation infrastructure, or water bodies (Lemenkova P., 2021).

Because the method is unsupervised, no prior training data are required; instead, the classification emerges solely from the intrinsic spectral structure of the image. This makes Iso Cluster particularly useful for preliminary land-cover identification, exploratory spatial analysis, and automated extraction of thematic information from high-resolution orthophotography. However, as the resulting clusters are not semantically labeled, they require post-classification interpretation and validation against ground truth or auxiliary datasets (Jia, 2006). The left image illustrates the result of an unsupervised Iso Cluster classification applied in ArcGIS Pro to the 2018 orthophoto of the Municipality of Cluj-Napoca, constrained by the administrative boundary of the city.

The Iso Cluster algorithm automatically grouped image pixels into five spectral classes based on their statistical similarity across the RGB bands, without prior training samples. Each class represents a dominant land-cover or surface type characterized by similar reflectance properties. The numerical class labels shown in the legend (values 1-5) do not imply a hierarchical order but serve solely as identifiers for the resulting clusters: class 1 (gray) predominantly corresponds to impervious surfaces such as roads, paved areas, and dense built-up zones, where reflectance is relatively uniform and low in the visible spectrum; class 2 (yellow) is mainly associated with bare soil, sparsely vegetated areas, or dry surfaces exhibiting higher reflectance in the visible bands; class 3 (green) represents vegetated surfaces, including urban green spaces, parks, gardens, and other areas with active vegetation

cover, characterized by higher reflectance in the green band; class 4 (blue) corresponds largely to building rooftops and compact built structures, showing distinct spectral behavior compared to roads and vegetated areas, class 5 (red) highlights surfaces with strong spectral contrast, such as industrial roofs, construction sites, or mixed materials, often occurring as fragmented patches within the urban fabric.

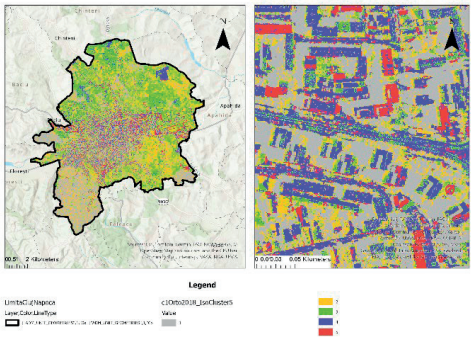


Figure 5. Iso Cluster classification applied in ArcGIS Pro

The spatial distribution of these classes reveals the heterogeneity of land cover across Cluj-Napoca, with a clear concentration of impervious and built-up classes in the central urban area and a higher proportion of vegetated and less disturbed surfaces toward the peripheral zones.

The right image presents a zoomed-in detail of a representative urban area, emphasizing the pixel-level structure of the Iso Cluster classification. At this scale, the discrete nature of the raster classification becomes evident, with individual pixels and small pixel groups clearly delineating buildings, streets, courtyards, and vegetated patches. This detailed view highlights the strengths and limitations of the Iso Cluster approach in high-resolution urban imagery, particularly the occurrence of mixed pixels and spectral confusion between adjacent land-cover types in dense urban environments (Coroian, et al., 2021).

Recent advances in geospatial automation and computer-aided design have significantly expanded the role of scripting languages in the control and customization of CAD environments. In this context, Python has emerged as a widely adopted solution for automating CAD-based workflows, owing to its

flexibility, readability, and extensive ecosystem of libraries for numerical computation, data processing, and spatial analysis. Programmatic control of CAD software is typically achieved through application programming interfaces (APIs) and COM (Component Object Model)–based architectures, which enable external scripts to interact directly with native CAD objects, layers, and drawing properties. Such approaches have been successfully applied in a variety of engineering and construction-related applications, including automated geometry handling, design verification, and the integration of CAD data with computational analysis pipelines. Previous studies have demonstrated that scripting-based CAD automation can substantially reduce manual intervention, enhance reproducibility, and improve efficiency in complex or repetitive design tasks (Moghadam, 2019).

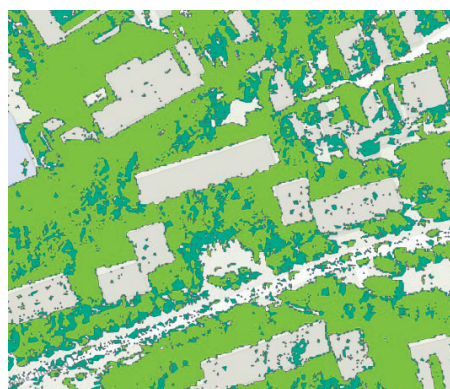
RESULTS AND DISCUSSIONS

Based on the Iso Cluster unsupervised classification, the generated spectral classes were analyzed in relation to their spatial distribution, vegetation cover, and potential implications for urban air quality in Cluj-Napoca. The classification results show a clear spatial contrast between the central urban area, dominated by impervious and built-up surfaces, and the peripheral or semi-peripheral areas, where vegetated surfaces are more frequent.

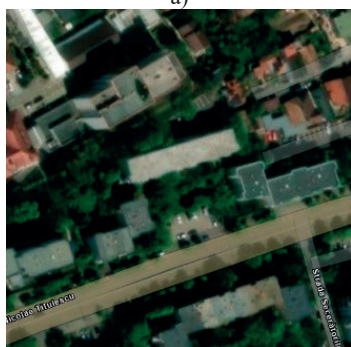
Following the assessment of the classified raster, Classes 2 and 3 were selected as representative of vegetation cover, as they consistently corresponded to urban green spaces, parks, gardens, tree-covered areas, and peri-urban vegetated zones. The presence and spatial continuity of these green areas are relevant for urban air-quality regulation because vegetation can contribute to the reduction of airborne pollutants through particle deposition on leaf surfaces, pollutant absorption, shading effects, and the mitigation of urban heat island intensity.

The selection was supported by visual interpretation of the classified raster in comparison with the original orthophoto, which confirmed the dominance of vegetative features within these classes. The remaining classes were associated primarily with impervious surfaces

and built-up areas and were therefore excluded from the vegetation analysis.



a)



b)

Figure 6. Iso Cluster classification applied in ArcGIS Pro

The left image illustrates the vegetation layer extracted from the Iso Cluster unsupervised classification, in which the selected spectral classes corresponding to vegetated surfaces have been isolated and displayed as discrete polygons. The right image presents the corresponding orthophoto of the same urban area, serving as a reference for visual validation (Horkaew, Puttinaovarat, & Paramate, 2022).

A comparative analysis of the two images reveals a strong spatial correspondence between the polygons derived from the Iso Cluster classification and the green areas visible in the orthophoto (Lemenkova P., 2202). In particular, the classified polygons accurately delineate tree canopies, lawns, and other vegetated patches, demonstrating that the unsupervised classification effectively captures the spatial extent and distribution of urban green spaces (Figure 6 a). This visual agreement supports the reliability of the Iso Cluster approach for

vegetation extraction from high-resolution orthophotography data (Figure 6b).

Following the initial processing of the datasets in ArcGIS Pro, the resulting spatial layers were subsequently imported into AutoCAD and processed using Python scripts developed in the PyCharm environment. The Python-based workflow included coordinate data handling, layer classification, surface and perimeter calculation, spatial overlay between vegetation classes and administrative boundaries, attribute extraction, and the generation of statistical summaries.

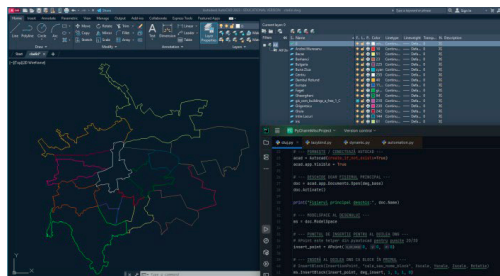


Figure 7. The administrative boundaries

These operations allowed the automated quantification of green areas within the analyzed urban sectors and improved the transparency and reproducibility of the geospatial processing workflow (Lemenkova P., 2015).

As a first step, the administrative boundaries of the city districts were incorporated, establishing the spatial framework for the analysis (Figure 7). The workflow was then extended by adding the building footprints, which enabled a more detailed representation of the urban fabric. In the final stage, the green space layer was integrated, allowing for the accurate delineation and spatial valuation of vegetated areas within the urban system (Figure 8).

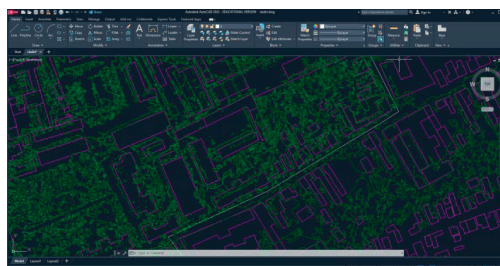


Figure 8. The green space layer and the building footprints

The district-level indicators were derived through a polygon-based spatial overlay procedure implemented in the PyCharm environment. First, the administrative boundaries of the districts were used as the reference spatial units. The building footprint layer was intersected with each district polygon in order to calculate the built-up area at the district level. Similarly, the vegetation polygons obtained from the Iso Cluster raster classification were overlaid with the district boundaries to determine the green-space area for each district. The computed spatial indicators were subsequently visualized and represented in AutoCAD, enabling a clear and structured cartographic interpretation of the results (Figure 9).

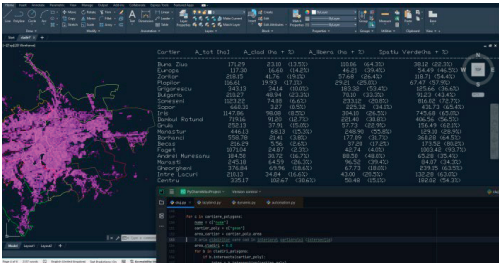


Figure 9. The building footprint area

The analysis reveals that the highest absolute values of green-space coverage are concentrated in Someșeni, Iris, and Sopor, all of which are characterized by low construction density and predominantly vegetated land use. Someșeni records the largest extent of green space, totaling 816.02 ha (72.7%) of the district surface, out of a total area of 1123.22 ha (Figure 10). The built-up footprint remains comparatively limited (74.08 ha; 6.6%), reflecting a semi-periurban spatial configuration dominated by open and vegetated land.

District	Total area (ha)	Built-up area (ha and %)	Open area (ha and %)	Green space (ha and %)
Buna Ziua	171.89	23.10 (13.5%)	118.66 (69.3%)	38.18 (22.3%)
Europe	117.38	15.60 (13.3%)	46.21 (39.4%)	54.49 (46.5%)
Zorilor	218.15	41.76 (19.1%)	57.68 (26.4%)	118.71 (54.4%)
Regina Elisabeta	114.41	19.93 (17.4%)	29.21 (25.5%)	64.27 (56.1%)
Grigorescu	343.13	34.14 (10.0%)	183.32 (53.4%)	125.66 (36.6%)
Bulgaria	218.27	48.94 (22.4%)	70.18 (32.1%)	91.23 (41.4%)
Comeni	1123.22	74.08 (6.6%)	233.12 (20.8%)	816.02 (72.7%)
Sopor	668.31	3.27 (0.5%)	225.36 (33.7%)	431.73 (64.4%)
Iris	1147.86	98.08 (8.6%)	384.80 (33.5%)	745.66 (65.0%)
Danub Rotund	119.15	9.20 (7.7%)	231.40 (19.4%)	48.55 (40.9%)
Griva	252.13	37.91 (15.0%)	57.73 (22.9%)	156.49 (62.1%)
Monstru	446.12	68.13 (15.3%)	248.90 (55.8%)	129.10 (28.9%)
Barbani	558.76	11.41 (2.0%)	177.09 (31.7%)	369.26 (66.3%)
Becas	216.29	5.56 (2.6%)	37.20 (17.2%)	173.52 (80.2%)
Eget	1071.04	24.87 (2.3%)	42.74 (4.0%)	1003.43 (93.7%)
Andrei Muresanu	184.88	38.72 (21.0%)	88.58 (48.0%)	65.58 (35.4%)
Marasti	245.18	64.59 (26.3%)	76.32 (31.1%)	104.27 (42.6%)
Decebal	376.84	69.56 (18.5%)	67.73 (18.0%)	239.55 (63.5%)
Intre Lacuri	210.13	34.84 (16.6%)	43.00 (20.5%)	132.28 (62.9%)
Centru	235.17	102.67 (43.7%)	50.48 (21.5%)	182.02 (77.8%)

Figure 10. District-level distribution of built-up areas, open areas, and green-space coverage in Cluj-Napoca

From the perspective of WHO guidance, the high proportion of continuous green surfaces provides favorable ecological support and air-quality mitigation potential. In this study, accessibility was interpreted through the spatial relationship between green-space distribution and the built-up urban fabric. The assessment focused on the continuity, proximity, and territorial availability of vegetated areas within each district, allowing the identification of urban sectors where green infrastructure is more spatially connected and those where it appears more fragmented or constrained by dense construction.

In Iris, green space accounts for 745.66 ha (65.0%) of the total 1147.86 ha, while the built-up area remains moderate (98.08 ha; 8.5%). Vegetation forms an extensive and relatively continuous matrix around the residential areas, indicating a balanced relationship between urbanized and natural land cover.

In this study, the interpretation of green-space distribution across neighbourhoods was carried out with reference to World Health Organization (WHO) guidance concerning the role of urban green infrastructure in supporting environmental quality and public health. The assessment focused on two main spatial indicators derived from the geospatial analysis: the total green-space area and the percentage of vegetation coverage within each district. These indicators were used to compare the territorial availability of green spaces and to identify districts with high or low vegetation coverage. Rather than considering only the absolute extent of vegetated areas, WHO emphasizes the importance of indicators related to spatial accessibility, per-capita availability, and the functional distribution of green spaces within the urban fabric, as these factors are more closely associated with population health outcomes and environmental well-being (WHO, 2017). On this basis, the comparative assessment of neighbourhoods in Cluj-Napoca integrates both land-cover characteristics and their potential alignment with WHO-oriented criteria for health-supportive urban green infrastructure.

In relation to WHO recommendations, the district demonstrates strong territorial green-space availability; however, full compliance can

only be assessed when considering per-capita indicators and spatial accessibility for residents. Sopor is characterized by a predominantly green landscape structure, with 431.73 ha (65.4%) of vegetated area out of a total of 660.31 ha, and an extremely low construction density (3.27 ha; 0.5%). This profile indicates an emerging development area where urbanization processes are still incipient. Under WHO principles, Sopor presents substantial potential for preserving and planning green infrastructure proactively; however, the functional utility of these areas will depend on future urban expansion dynamics and the integration of accessible recreational spaces. Overall, these districts represent the most vegetation-rich sectors of the city in absolute spatial terms. While their land-cover composition aligns with the ecological objectives promoted by the WHO, further assessment based on green space per inhabitant and spatial accessibility is required to evaluate compliance with public-health-oriented urban planning criteria.

At the opposite end of the spectrum, the lowest absolute values of green-space surface area are observed in Buna Ziua, Europa, and Plopilor. These districts display higher construction densities and more compact urban morphologies compared with the vegetation-rich peripheral districts.

Buna Ziua registers the smallest total green-space extent, with 38.12 ha (22.3%) out of a total surface of 171.29 ha, while the built-up footprint accounts for 23.10 ha (13.5%). The reduced share of vegetated land, in conjunction with ongoing residential densification, indicates a predominantly compact urban pattern. From the perspective of WHO guidance, this configuration may imply limited access to nearby recreational green areas and potential exposure to higher environmental stress factors, particularly in relation to heat-island and air-quality conditions.

In Europa, green space amounts to 54.49 ha (46.5%) of a total of 117.30 ha, whereas the building footprint represents 16.60 ha (14.2%). Although the relative proportion of vegetation is moderate, the absolute surface remains restricted due to the small spatial extent of the district. In the context of WHO recommendations, this situation suggests that the availability of accessible public green areas

may be constrained at the neighborhood level, especially in zones with higher residential concentration.

Plopilor includes 67.47 ha (57.9%) of green space within a total area of 116.61 ha, along with 19.93 ha (17.1%) of built-up surfaces. Despite a comparatively higher relative percentage of vegetation, the total green-space area remains limited, reflecting a compact urban structure with fragmented vegetated patches. In relation to WHO guidance, this configuration highlights the importance of improving spatial connectivity and the functional accessibility of existing green spaces to ensure equitable, health-related benefits for residents.

Taken together, these districts illustrate the areas of the city with the lowest absolute availability of green space, where urban densification processes may challenge the integration of green infrastructure. From a public-health perspective, they represent priority areas for targeted interventions aimed at increasing accessible, well-distributed urban green spaces in line with WHO recommendations. For a more focused interpretation, several representative residential districts were selected, namely Mănăştur, Gheorgheni, Zorilor, Andrei Mureşanu, Buna Ziua, and Grigorescu. These districts were chosen because they are important residential areas within Cluj-Napoca and present different degrees of urban density, development pressure, and green-space availability. Within the highly demanded residential districts of Cluj-Napoca, the largest absolute green-space surfaces are recorded in Gheorgheni, Mănăştur, and Grigorescu, which together concentrate the highest availability of urban green infrastructure within this subgroup of neighborhoods (Nap et al., 2020). Gheorgheni records the highest level of green-space availability among the analyzed districts, characterized by continuous and well-structured vegetated areas integrated within the residential fabric. Consistent with WHO guidance, the spatial configuration of the district suggests good short-distance accessibility and a favorable potential for per-capita green-space provision.

In Mănăştur, the absolute extent of green space is substantial; however, the relative proportion of vegetation is lower due to the compact, high-density urban structure. From a WHO perspective, the effectiveness of green spaces in

supporting public-health outcomes depends primarily on their internal distribution and accessibility within residential clusters.

Grigorescu combines moderate urbanization with structurally connected vegetated areas, particularly along major landscape corridors, which enhances recreational accessibility and contributes to environmental quality in line with WHO principles.

Among the remaining districts, Zorilor is characterized by a relatively favorable green-space profile; Andrei Mureșanu presents fragmented but functionally valuable vegetated areas, whereas Buna Ziua exhibits the lowest availability of green space, reflecting an increasingly dense and compact urban pattern.

Overall, Gheorgheni and Grigorescu display the strongest alignment with WHO environmental-health objectives, while Mănăştur requires greater attention to intra-neighborhood accessibility. Buna Ziua emerges as the most vulnerable case due to its limited and spatially constrained green-space resources.

CONCLUSIONS

The objective of this study was to analyze the spatial distribution and structural configuration of urban green infrastructure in Cluj-Napoca, using GIS-based classification, automated spatial processing, and CAD-based visualization. The study aimed to identify vegetated areas from high-resolution orthophotographic data, quantify green-space surfaces and built-up footprints at district level, and interpret the results in relation to environmental quality and potential air-quality mitigation.

This study aimed to assess the spatial distribution and structural configuration of urban green infrastructure in Cluj-Napoca by integrating GIS-based Iso Cluster classification, Python-based spatial processing, and CAD-based visualization. The main objectives were to identify vegetated areas from high-resolution orthophotographic data, quantify green-space surfaces and built-up footprints at district level, and interpret the spatial patterns of vegetation in relation to urban morphology, environmental quality, and potential air-quality mitigation.

At the opposite end, districts such as Buna Ziua, Europa, and Plopilor exhibit reduced absolute

green-space availability and more compact urban structures, reflecting ongoing densification processes and increased landscape fragmentation. From a WHO-oriented perspective, these neighbourhoods represent priority intervention areas where the integration of accessible and well-distributed green spaces is essential for mitigating environmental stress factors and supporting public-health outcomes.

Within the group of highly demanded residential districts, Gheorgheni and Grigorescu show the strongest alignment with WHO environmental-health objectives, owing to their structurally connected and functionally coherent green-space networks. By contrast, Mănăştur, despite holding a substantial territorial extent of green (Geo-Spatial, 2025) accessibility due to its compact and high-density urban fabric. Buna Ziua emerges as the most vulnerable case, with limited and spatially constrained vegetation resources that may restrict compliance with accessibility-based green-space targets under continued urban development. From a practical planning perspective, these results can support municipal decision-making by identifying districts where green-space interventions should be prioritized. Areas with limited or fragmented vegetation, such as Buna Ziua and compact residential sectors, should be considered priority zones for increasing green-space coverage, improving ecological connectivity, and integrating new vegetated corridors into future urban development plans.

Overall, the study highlights the value of integrating unsupervised classification techniques, Python-based automated spatial processing, and CAD-based visualization for the quantitative assessment of urban green infrastructure. The novelty of this workflow lies in the combination of raster-based vegetation extraction, vector-based spatial overlay, automated area computation, and cartographic representation within a single reproducible processing chain. Compared with conventional manual approaches, this integrated GIS–Python–CAD workflow reduces repetitive operations, improves consistency in district-level calculations, and facilitates the rapid generation of spatial indicators for urban green-space assessment. The results provide a spatially explicit basis for supporting urban planning strategies aimed at enhancing ecological

connectivity, improving environmental quality, and promoting equitable access to urban green spaces in Cluj-Napoca.

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USING GIS IN LANDSCAPING: A CASE STUDY IN CRAIOVA

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Abstract

The arrangement and management of urban green spaces require interdisciplinary approaches that combine ecological knowledge with modern spatial technologies. This study evaluates the applicability of Geographic Information Systems (GIS) for landscaping management through a case study conducted in Craiova, Romania. A database containing tree species, spatial coordinates, age categories, and health status was created using GPS-based field observations and processed in QGIS 3.40.1. Spatial analysis enabled the classification and visualization of dendrological material according to species type, age, and phytosanitary status. The results identified 69 deciduous trees and 11 conifers, as well as 10 trees that were simultaneously old and diseased, representing priority intervention areas. GIS-based mapping and buffer analysis improved the identification of maintenance zones and optimized decision-making regarding pruning, treatment, and replacement operations. The study demonstrates that GIS technologies can support more efficient and sustainable landscape management practices in urban areas.

Key words: Craiova, GIS, landscaping, study

INTRODUCTION

Since the beginning of civilization, humans have been concerned with beauty and the arrangement of the space in which they lived. The use of topographic knowledge for navigation is among the best early illustrations of GIS applications, albeit without the “digital” aspect.

A GIS is a computer-based system to aid in the collection, maintenance, storage, analysis, output, and distribution of spatial data and information (Figure 1).

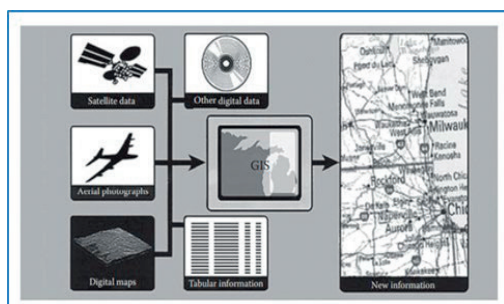


Figure 1. GIS system

Every GIS database must be referenced to a base map or base data layer. Ideally, the database should be referenced to a large scale, very accurate base map (Yousefi and Razdari, 2015).

The key element, or building block, of GIS is the geo-referenced, digital map. This element is the backbone to which we can integrate any data that we can collect or even imagine, as long as it has a spatial identifier. A variety of technical issues are tied to the digital map: map projection, a geo-rectification process to develop the digital map, and related issues that are nominally discussed in the metadata, which are data that thoroughly define/describe the aforementioned data. Another relevant background piece of knowledge is the nature of the data that comprise a GIS; these data can be points (e.g., well locations), lines (e.g., stream networks), and/or polygons (e.g., soil map units). Two distinct choices for the GIS data structures are raster (pixel based or rows and columns) or vector (coordinate based or lines that join to define a map unit).

Remote sensing technology linked with a GIS has been usefully implemented in the identification of multiple environmental characteristics, i.e., vegetation cover, urban sprawls, transitions in forests, and particularly variations in LULC changes for certain time periods (Helmer et al., 2000). It has been observed that remote sensing and GIS techniques offer more accurate and cost-efficient data evaluation in comparison to other traditional approaches and surveys (Jat et al., 2008).

GIS are regarded as essential for identifying the changes in remotely sensed images (Vibhute et al., 2013). It offers the ability to integrate data from multiple resources for the identification of change. The combined effect of the hydrological maps, soil and topographic maps, and classified images obtained through GIS may provide helpful information in extracting land use for a given area. In addition, it may show the tendencies of land use changes because of its ability to develop the model by using provided statistics and datasets. Moreover, GIS and remotely sensed images are widely utilized to recognize LULC changes (Rahman et al., 2012; Attri et al., 2015; Jiang et al., 2018). For tracking land cover changes, the combined use of GIS and remote sensing has been demonstrated to be a reliable and profitable technique (Hazarika et al., 2015; Lopez-Granados et al., 2013; Lambin et al., 2003; Serra et al., 2008).

In modern urban research, spatial data can be obtained from various sources, including satellite remote sensing data, geographic surveying and mapping data, traffic network data, census data, etc. (Zafar, 2024). GIS-based spatial analysis tools provide a new idea that can visually present massive urban data and evaluate landscape accessibility by analyzing the spatial relationship between urban elements (Chen et al., 2020).

Urban datasets often reflect socio-economic inequities, potentially introducing bias into the model's predictions (Aslam et al., 2024). For example, affluent areas may be overrepresented in datasets due to better data availability, while marginalized communities might be underrepresented (Boadi et al., 2025).

Practice has proved that the parametric design method of landscape architecture can take the basic spatial data provided by the field of surveying and mapping as the basic design input, and at the same time use ArcGIS software to complete the corresponding data analysis and processing, so as to accurately grasp the elements of landscape architecture and dynamically optimize the design scheme, which provides a brand-new design idea and technical path for the field of landscape architecture design (Ni Xiao, 2025).

Recent applications of integrating remote sensing-derived indices with machine learning algorithms have demonstrated improved

accuracy and efficiency (Mykola et al., 2025).

Therefore, the main objective of this study is to demonstrate how GIS-based spatial analysis can support urban landscape management in Craiova by identifying tree distribution patterns, health conditions, and priority intervention areas. The research also evaluates the practical role of QGIS in improving maintenance efficiency and decision-making in urban green spaces.

MATERIALS AND METHODS

This study was conducted in the Craiova area, located in Southwest Romania (44°20'N, 23°49'E). The methodological approach combined field data collection with GIS-based spatial analysis. The study did not aim only to inventory trees, but also to evaluate how GIS techniques can support decision-making in landscape management.

The following variables were recorded for each tree: species, latitude, longitude, age category (young/old), and health status (healthy/sick). Spatial coordinates were collected using the GPS Coordinates mobile application, while age and health status were determined through visual assessment based on trunk condition, canopy structure, and visible phytosanitary symptoms.

The collected data were organized in Microsoft Excel and converted into CSV format for processing in QGIS version 3.40.1 "Bratislava" (QGIS Association, 2025). Spatial analysis included thematic mapping, attribute classification, and buffer-zone generation around diseased trees to identify priority management areas. Additionally, spatial clustering of old and unhealthy trees was analyzed in order to optimize intervention routes and maintenance operations.

The study method does not aim to repeat previous documentation, but rather to synthesize existing elements and relate them to the concept of landscape, interpreting data to identify the distinctive competencies of the land in question through both objective - quantitative and subjective - perceptual analyses. The synthesis elements will be interpreted to structure recommendations and development directions that have as their main purpose the improvement

of landscape quality as an indicator of quality of life.

The following characteristics were chosen as variables:

- Species;
- Latitude;
- Longitude;
- Age with 2 graduations: young and old;
- Health status with 2 graduations: healthy and sick.

The spatial coordinates of the trees were determined using a mobile phone application (GPS coordinates). Age and health status were determined visually.

Tree age category was determined through non-invasive visual indicators commonly used in rapid dendrological assessments. Trees with small trunk diameters, smoother bark, and compact, symmetrical crowns were classified as young, while individuals with visibly thicker trunks, coarse or fissured bark, and expanded or irregular crowns were classified as old. These criteria ensured consistent categorization without requiring destructive sampling.

Health status was evaluated using visible phytosanitary indicators. Trees showing uniform foliage, intact bark, and no signs of pests or fungal infections were classified as healthy. Trees exhibiting canopy thinning, leaf discoloration, bark lesions, fungal bodies, or pest damage were classified as sick. These indicators follow standard urban tree assessment practices and allow rapid identification of risk trees.

The raw data were recorded in an Excel file. After converting it to a CSV file, it was imported and processed using the QGIS program. All mapping operations were performed in QGIS version 3.40.1 “Bratislava” (QGIS Association, 2025).

RESULTS AND DISCUSSIONS

Using the QGIS program, the data were processed to create presentation maps. In these maps, the program allows the introduction of all identification elements (north, scale, legend, etc.).

The classification of trees according to their type led to: 69 deciduous trees (86.25%) and 11 conifers (13.75%). Going further, trees can also

be classified according to botanical family to determine their degree of spread (Figure 2).

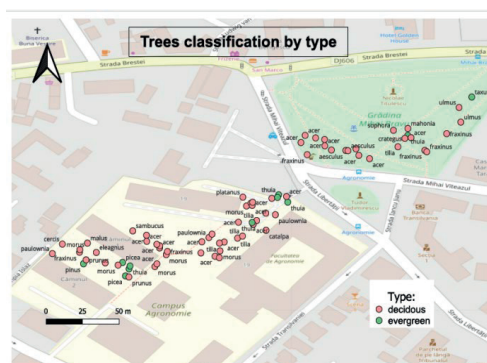


Figure 2. Trees classification by type

The age of the plants, another criterion taken into account, is very important because it provides information regarding future work and interventions on the plants in the near future. By classifying dendrological material by age, we can determine which trees require immediate intervention and we can create corridors to protect visitors from falling branches.

The program allows trees to be grouped so that any pruning interventions can be carried out as efficiently as possible, with minimal disruption to pedestrian traffic.

For example, in our study we found 35 old trees (43.75%) and 45 young trees (56.25%) (Figure 3).

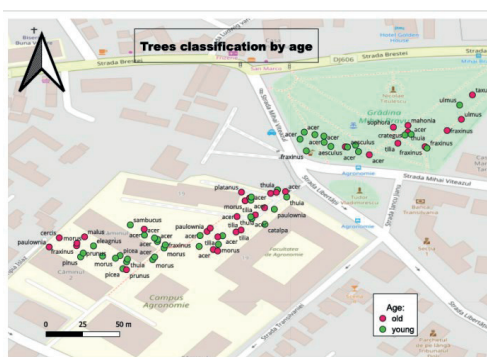


Figure 3. Trees classification by age

Classifying the dendrological material by health status, we have: 60 (75%) healthy and 20 (25%) diseased trees. The program allows the creation

of protective buffer zones around each diseased tree, depending on the type of disease or pest. This buffer zone helps us save time, labour and materials (substances) for the treatment of sick trees (Figure 4).

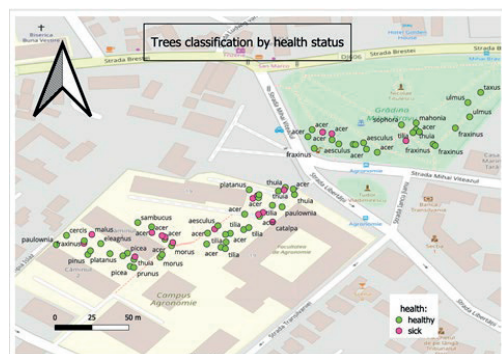


Figure 4. Trees classification by health status

Combining this information, we found a number of 10 (12.5%) old and diseased trees, grouped by action areas so that their replacement/treatment activity would be as efficient as possible.

Beyond the descriptive classification of trees, GIS analysis highlighted several important spatial patterns. Diseased trees were concentrated in specific sectors of the study area, indicating the possibility of localized environmental stress factors or pest propagation. Buffer zones created around affected trees allowed the identification of risk areas where monitoring and phytosanitary interventions should be prioritized.

Spatial clustering analysis also revealed that old trees were frequently located along the main pedestrian circulation corridors. This finding is important because it allows landscape managers to prioritize pruning and replacement works in areas with high public exposure. By integrating age and health status into the GIS database, intervention priorities could be established more efficiently.

The identification of 10 trees (12%) classified as both old and diseased demonstrates the practical utility of GIS for urban green-space management. These trees formed distinct intervention clusters that can reduce maintenance costs and improve work planning efficiency. In addition, the GIS-based approach facilitates future updates of the database and long-term monitoring of vegetation dynamics.

CONCLUSIONS

The present study demonstrates that GIS technologies represent an effective tool for urban landscape management and the monitoring of green spaces. By integrating field observations with spatial analysis in QGIS, it was possible to identify tree distribution patterns, health-risk areas, and priority intervention zones.

The results showed that GIS-based analysis can improve maintenance efficiency by supporting targeted pruning, treatment, and replacement operations. The creation of buffer zones around diseased trees and the identification of clusters of old and unhealthy trees provide practical decision-support tools for landscape managers.

Furthermore, the methodology used in this study can be replicated in other urban areas with similar environmental and landscape conditions. Future research may include advanced GIS techniques, remote sensing data, and machine-learning models to improve the monitoring and sustainable management of urban vegetation.

These technologies provide essential tools for monitoring, managing, and adapting practices to ensure sustainability and resilience against climatic variations.

Continued advancements in remote sensing and GIS will enhance their effectiveness in addressing the challenges posed by climate change in the future.

QGIS helps us be more efficient and save materials in our daily work.

While this study focuses on Craiova city, its methodology and findings could be adapted to other regions with similar landscape, geographical, and agricultural features.

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EDIBLE WOODY SPECIES IN SOME GREEN AREAS OF BUCHAREST

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Abstract

*Over time, urbanization has never excluded people's habit of growing edible plants near their homes. Despite the challenges of growing them in urban spaces, there is an increasing trend to have edible plants in cities around the world. Woody species from parks, private gardens, streets, and green areas near residential buildings were investigated for their edible qualities in Bucharest. The results showed a total number of 62 edible species of trees and shrubs present in the green areas studied. Edible species represented over 45% of the total species near residential buildings and private gardens. Most of the species were able to produce flowers/fruits under both climatic and soil conditions in Bucharest. A clear tendency of the population to plant near their homes new edible species for Romania, such as *Ficus carica*, *Punica granatum*, *Actinidia chinensis*, or *Aronia melanocarpa*, was remarked. Even though some of these do not produce suitable fruits for consumption, the increased interest of people in growing edible species suggests that landscape architects may consider these species and design more edible landscapes in the future.*

Key words: *edible ornamentals, fruiting trees and shrubs, gardens, parks, streets, temperate climate.*

INTRODUCTION

While UN statistics (2025) predict an increase in the urban population to 70% of the world's population in 2050, today there are concerns worldwide about maintaining the quality of life in cities by adopting practices and policies regarding the expansion and protection of green infrastructure in cities (Ashinze et al., 2024). Urbanization, which attracts progressively more people through the amenities offered, such as better access to well-paid jobs, healthcare, infrastructure, and education (Damon, 2011), tends to disconnect individuals from nature, which can have a negative impact on their health (Soga & Gaston, 2016; Sibthorpe & Brymer, 2020; Bashan et al., 2021). For this reason, many cities encourage community gardens, which not only reconnect people to nature, but also improve dietary habits (Twiss et al., 2003; Nova et al., 2018; Kelly & Brännlund, 2025), develop better relationships between neighbours (Glover et al., 2005; Firth et al., 2011; Bice et al., 2018) and facilitate cultural exchanges and traditions (Agustina & Beilin, 2012; Pearsall et al., 2017; Mejia et al., 2020). Furthermore, people involved in community gardens become more aware of urban ecological problems and are interested in adopting environmental protection

and resource-conservation practices (Janowska et al., 2022).

In large cities, such as Bucharest, community gardens are difficult to implement due to the lack of space. Compact building models and the need to expand parking lots result in small-sized common public green spaces, designed with ornamental plants. In these cases, edible landscaping can be an alternative to community gardens, by using more edible plants in public green space design projects. Implementing these edible plants in the urban landscape does not mean less attractiveness, but an intelligent selection of species with both aesthetic value and edible qualities, which can satisfy not only human needs but also flora and fauna (Rupprecht et al., 2023). Studies have shown that edible landscapes, compared with those consisting strictly of ornamental plants, generate benefits such as energy and water savings, better nutrition, improved food safety, and a community with a healthier lifestyle (Neal, 2011; Çelik, 2017; Karaca, 2019).

Converting the existing conventional landscape into an edible landscape can be challenging. Urban trees fulfil many functions and the selection of species for different green spaces is based on criteria of resilience and sustainability (Sjöman et al., 2017; Anisimova, 2023).

Therefore, it is essential to investigate edible plant species to determine whether they meet selection criteria to tolerate the growing conditions in urban cities. Furthermore, pollution can be restrictive for some edible plants. Von Hoffen and Säumel (2014) showed that fruits (walnut, hazel, ginkgo, blackberry, sea buckthorn, elder, apple, Mirabelle plum and plum) produced in green spaces in Central Berlin (Germany) have a significantly lower heavy metal content than vegetables, and their consumption does not pose a health risk as long as the placement of the fruit trees is considered. Various other researchers have reported that vegetables and fruits harvested from urban green spaces accumulate heavy metals differently (Samsøe-Petersen et al., 2002; Hough et al., 2004; Antisari et al., 2015; Khalilnezhad et al., 2024). Site-specific risk evaluation and selection of plants are key factors in minimizing contamination. Consequently, edible species cannot completely replace ornamental species in urban green spaces, due to their decontamination function, one of the most important for air and soil quality in cities, which only the latter can fulfil.

Even if pollution is not a concern, edible species should be evaluated for their impact on population comfort in terms of allergy risk, litter and fruit drop, slippery surfaces, and insects (Hajzeri & Kwadwo, 2019).

In this study, we investigate several green spaces in Bucharest, Romania, such as parks, private gardens, streets and green areas near residential buildings, to assess the diversity of woody species and to highlight edible plants. The results will complete the knowledge about edible species in Bucharest and the trends of its various green areas as they convert into edible landscapes.

MATERIALS AND METHODS

Investigations on edible woody species from different green spaces in Bucharest (44°24'N, 26°05'E) were carried out between 2023 and 2025. The city, which is located on a plain area in southern Romania, has a temperate climate, but dry and thermally unstable, with heatwaves in summer and frosts in winter.

Public parks, private gardens, street plantations, and green areas of residential buildings situated

in the city centre, District 1 and District 6 were selected for study (Figure 1).

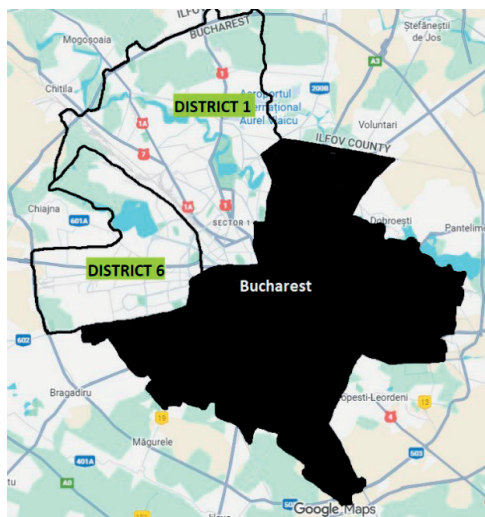


Figure 1. Map of Bucharest highlighting the two districts in which green spaces were studied (modified from Google Maps, 2026)

These two districts cover almost half (43.9%) of Bucharest's area and include various old and new green areas, both public and private, suitable for a study on edible species. District 1 is the greenest area of Bucharest, which includes forested areas, public parks, and private gardens, with significant importance for air quality and the urban microclimate. Old and new neighbourhoods, most of which have small buildings (2-4 floors) are accompanied by private or common public gardens (Figure 2).



Figure 2. Appearance of buildings in District 1

District 6 is densely built and has the smallest green area in Bucharest, represented by small

public parks and green areas of residential buildings, most of which have 10 floors (Figure 3).



Figure 3. Appearance of buildings in District 6

A total of 8 public parks, 90 private gardens, 12 streets, and 27 green areas of residential buildings were studied for edible woody species (Figure 4). All woody species with flowers, fruits, or seeds that can be eaten raw or prepared were considered. No herbaceous edible species were identified.

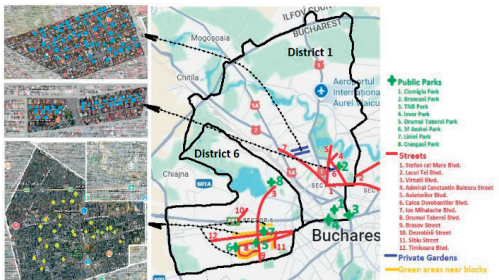


Figure 4. Studied areas (modified from Google Maps, 2026)

Chi-squared tests were applied to compare native versus non-native edible species in green areas, as well as the difference between the number of edible and non-edible species and the number of edible and non-edible woody plants.

RESULTS AND DISCUSSIONS

Edible species were found in all the green areas studied. The plant inventory revealed the existence of 60 species and 2 hybrids of edible trees and shrubs, from 24 botanical families in

the studied public parks, private gardens, streets, and green areas of residential buildings in Bucharest (Table 2). The largest group was represented by species of *Rosaceae* - 30.6% (Table 1). Edible species diversity was higher in green areas near residential buildings and private gardens.

Table 1. Distribution of edible species by botanical families

Botanical Family	Total no. of species	Share of total (%)	No. of species in different types of green areas			
			Public	Private	Street	Green
Actinidiaceae	1	1.61		1		
Berberidaceae	2	3.22	2	1		2
Betulaceae	2	3.22	2	1	1	2
Cannabaceae	2	3.22				2
Caprifoliaceae	1	1.61		1		
Cornaceae	1	1.61	1			1
Cupressaceae	3	4.83	2	1		1
Ebenaceae	1	1.61		1		
Elaeagnaceae	1	1.61	1			
Fabaceae	4	6.45	4	1	2	4
Fagaceae	1	1.61				1
Grossulariaceae	1	1.61		1		
Juglandaceae	2	3.22	1	1	1	2
Lamiaceae	2	3.22		2	1	2
Lythraceae	1	1.61		1		1
Malvaceae	3	4.83	3	3	3	3
Moraceae	3	4.83	2	2	2	3
Oleaceae	1	1.61	1	1	1	1
Pinaceae	5	8.06	4	2	5	4
Rosaceae	19	30.64	7	17	3	14
Salicaceae	3	4.83	2	1		2
Taxaceae	1	1.61	1			
Viburnaceae	1	1.61	1			1
Vitaceae	1	1.61		1		1
Total	62	100	34	39	21	48

Non-native species were more frequent than native ones in all the green spaces (Figure 5), but the differences were not significant ($\chi^2 = 3.061$; $p = 0.382$).

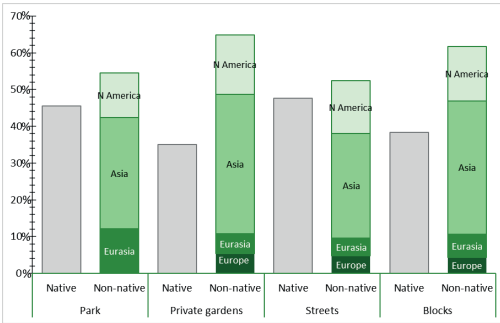


Figure 5. Distribution of edible species by their origin

It was remarked that most of the non-native species originate from Asia.

Table 1. Inventory of edible species and hybrids in some green areas of Bucharest

Scientific name*	Family	Origin	Edible part				Food uses**
			leaf	flower	fruit/seed	other	
<i>Abies alba</i>	Pinaceae	native	+				tea, syrup, as a flavouring
<i>Abies nordmanniana</i>	Pinaceae	Eurasia	+				tea, syrup
<i>Actinidia chinensis</i> var. <i>deliciosa</i>	Actinidiaceae	Asia			+		raw, juice, dried, syrup, jam, desserts, salads
<i>Amelanchier canadensis</i>	Rosaceae	N America			+		raw, dried, desserts, as a flavouring
<i>Aronia melanocarpa</i>	Rosaceae	N America			+		raw, juice, dried fruits, tea, syrup, jam, wine
<i>Berberis aquifolium</i>	Berberidaceae	N America		+	+		flower: salads, lemonade; fruits: raw, dried, jam, jelly, cakes, beverages
<i>Berberis bealei</i>	Berberidaceae	Asia			+		raw, cakes, jelly
<i>Betula pendula</i>	Betulaceae	native	+			sap	tea, salads; sap – raw, syrup
<i>Castanea sativa</i>	Fagaceae	Eurasia			+		roasted, dried, desserts, bread, soups, as a coffee substitute
<i>Celtis australis</i>	Cannabaceae	native			+		raw, desserts
<i>Celtis occidentalis</i>	Cannabaceae	N America			+		raw, jelly
<i>Cercis siliquastrum</i>	Fabaceae	Eurasia		+			raw, salads, tea
<i>Chaenomeles speciosa</i>	Rosaceae	Asia			+		raw, dried, tea, syrup, jam, jelly, as a flavouring
<i>Cornus mas</i>	Cornaceae	native			+		raw, juice, syrup, jam, alcoholic beverages, pickles
<i>Corylus colurna</i>	Betulaceae	native			+		raw, roasted, desserts, cakes
<i>Crataegus monogyna</i>	Rosaceae	native			+		tea, salads, jam, jelly, sauces
<i>Cydonia oblonga</i>	Rosaceae	Asia			+		raw, jam, desserts, dish, beverages
<i>Diospyros virginiana</i>	Ebenaceae	N America	+		+		leaf: tea; fruit: raw, dried, cakes, sauces
<i>Elaeagnus angustifolia</i>	Elaeagnaceae	Eurasia			+		raw, tea, soup, jelly
<i>Ficus carica</i>	Moraceae	Eurasia			+		raw, dried, jam, salad, desserts, cakes, dishes
<i>Gleditsia triacanthos</i>	Fabaceae	N America			+		raw, drinks
<i>Hibiscus syriacus</i>	Malvaceae	Asia		+			raw, salads, dried, tea, cakes, dishes
<i>Juglans nigra</i>	Juglandaceae	N America			+	sap	raw, roasted, cakes, desserts, dishes
<i>Juglans regia</i>	Juglandaceae	Asia			+		ras, drive, roadster, salade, desserts, cakes, jam, beurrages, dises
<i>Junipers haschischine</i>	Cupressaceae	Asia			+		as a flavouring
<i>Juniperus horizontalis</i>	Cupressaceae	N America			+		as a flavouring
<i>Juniperus virginiana</i>	Cupressaceae	N America			+		as a flavouring
<i>Lavandula angustifolia</i>	Lamiaceae	Europe		+			tea, cakes, desserts, as a flavouring
<i>Lonicea japonica</i>	Caprifoliaceae	Asia		+			tea, syrup
<i>Malus baccata</i>	Rosaceae	Asia			+		raw, jam, jelly, dried, dishes
<i>Malus domestica</i>	Rosaceae	Asia			+		ras, juive, drive, cakes, desserts, salade, beurrages, dises
<i>Malus x floribunda</i>	Rosaceae	hybrid			+		jam, jelly, sauces, beverages
<i>Horus galba</i>	Moraceae	Asia			+		ras, juive, drive, te, jam, Kelly, cakes
<i>Morus nigra</i>	Moraceae	Asia			+		raw, juice, dried, tea, jam, jelly, cakes, beverages
<i>Picea abies</i>	Pinaceae	native	+				tea, syrup, as a flavouring
<i>Pinus nigra</i>	Pinaceae	native	+				tea, syrup, as a flavouring
<i>Pinus sylvestris</i>	Pinaceae	native	+				tea, syrup, as a flavouring
<i>Prunus amygdales</i>	Rosaceae	Asia			+		ras, roadster, salade, fluor, mil, butter, desserts, cakes, dises,
<i>Prunes harmonica</i>	Rosaceae	Asia			+		raw, dried, juice, jam, jelly, desserts, cakes, dishes, beverages
<i>Prunus vivarium</i>	Rosaceae	native			+		raw, dried, juice, jam, jelly, desserts, cakes, dishes, beverages
<i>Prunus classifera</i>	Rosaceae	native			+		raw, jam, jelly, cakes, sauces, syrup, dishes, beverages
<i>Prunus crasseuse</i>	Rosaceae	Asia			+		raw, dried, juice, jam, jelly, desserts, cakes, dishes, beverages
<i>Prunus domestica</i>	Rosaceae	Asia			+		raw, dried, juice, jam, jelly, desserts, cakes, dishes, beverages
<i>Prunus persica</i>	Rosaceae	Asia			+		ras, drive, juive, jam, Kelly, desserts, cakes, salade
<i>Punica granatum</i>	Lythraceae	Asia			+		ras, juive, syrphie, te, salade, sauces, jam, beurrages, desserts, cakes, dises
<i>Pyracantha coccinea</i>	Rosaceae	Eurasia			+		Kelly, sauces, jam
<i>Parus communie</i>	Rosaceae	native			+		ras, juive, drive, jam, Kelly, cakes, desserts, salade, beurrages, dises
<i>Ribes nigrum</i>	Grossulariaceae	native			+		raw, juice, syrup, dried, sauces, cakes, desserts, beverages
<i>Robinia pseudoacacia</i>	Fabaceae	N America		+			raw, tea, fried, cakes, jam
<i>Rosa canina</i>	Rosaceae	native			+		raw, tea, syrup, jam, jelly, drinks, beverages
<i>Rosa hybrid a</i>	Rosaceae	hybrid		+			tea, syrup, dried, jam, jelly, desserts, cakes, dishes, sauces
<i>Salvia rosmarinus</i>	Lamiaceae	Europe	+				tea, as a flavouring
<i>Sambucus nigra</i>	Viburnaceae	native		+	+		flower: tea, lemonade, syrup, fried; fruit: jam, jelly, sauces, beverages
<i>Salix alba</i>	Salicaceae	native	+				tea
<i>Salix babylonica</i>	Salicaceae	Asia	+				tea
<i>Salix cinerea</i>	Salicaceae	native	+				salads, soups, stews, flour
<i>Styphnolobium japonicum</i>	Fabaceae	Asia		+			tea, dried, fried, salads, dishes
<i>Syringa vulgaris</i>	Oleaceae	native		+			tea, syrup, drinks, desserts, jelly
<i>Tilia platyphyllos</i>	Malvaceae	native	+	+	+	sap	leaf: salads, dishes; flower: tea, jam, jelly; sap: raw, syrup; fruit: chocolate substitute
<i>Tilia tomentosa</i>	Malvaceae	native	+	+			leaf: salads, dishes; flower: tea, desserts, drinks
<i>Torreya nucifera</i>	Taxaceae	Asia			+		raw, roasted
<i>Vitis vinifera</i>	Vitaceae	native	+		+		leaf: dishes; fruit: raw, dried, juice, desserts, drinks, beverages

*Source of data: POWO, 2026

**Source of data: PFAF, 2026

This result is not surprising, as most non-native species in Europe originate from Asia (Roques et al., 2020). Woody species from Asia have

been introduced over the years due to climatic similarities and low soil requirements.

A substantial part of the identified species (67.7%) has edible fruits, and most of them can be eaten raw (83.3%). Several species of trees and shrubs (12.9%) have multiple edible parts, such as: *Berberis aquifolium* - flowers and fruits, *Betula pendula* - young leaves and sap, *Diospyros virginiana* - leaves and fruits, *Juglans nigra* - seeds and sap, *Sambucus nigra* - flowers and fruits, *Tilia platyphyllos* - leaves, flowers, fruits, and sap, *Tilia tomentosa* - leaves and flowers, and *Vitis vinifera* - leaves, fruits, and seeds.

Significant differences ($\chi^2 = 12.316$; $p = 0.006$) between edible and non-edible species in different types of green area were observed (Figure 6).

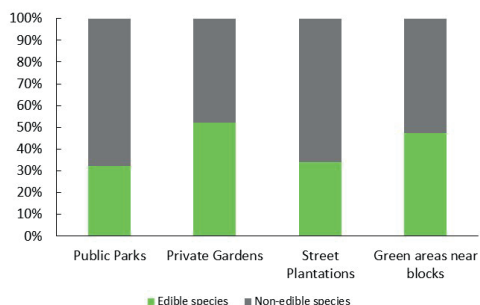


Figure 6. Share of edible species in different types of green areas in Bucharest

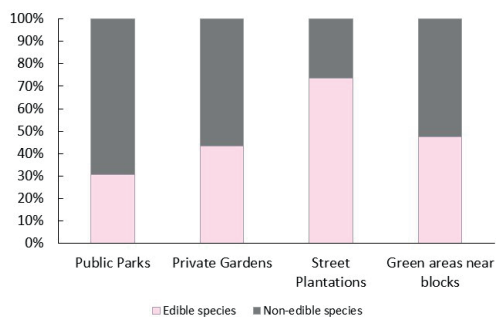


Figure 7. Share of edible trees and shrubs individuals in different types of green areas in Bucharest

Edible species represented over 45% of the total species in private gardens and green areas near blocks. Also, the difference between the number of edible and non-edible woody plants (Figure 7) was significant ($\chi^2 = 38.81$; $p = 0.00001$). The

lowest number of edible plants was observed in public parks and the highest in street plantations. On streets and boulevards, the edible part of most species was the flower or inflorescence (Figure 8).



Figure 8. Distribution by edible part of plants in different types of green areas in Bucharest

This result was expected because fruit-bearing species are usually avoided, or sometimes even prohibited, in city street plantations for reasons of cleanliness and safety. Therefore, tree species with flowers or decorative leaves are preferred. Fruitless cultivars can also be an alternative, as they have attractive flowers, such as *Malus* (Romer et al., 2003) or leaves, such as *Morus* (Rohela et al., 2025).

In public parks and private gardens, edible species were represented by a larger number of trees and shrubs with edible fruits (Figure 8). Some of these edible species, such as *Cydonia oblonga*, *Malus domestica*, *Prunus armeniaca*, *Prunus avium*, or *Prunus cerasus*, also have decorative leaves (Melinescu & Cosmulescu, 2025).

The species with edible fruits in private gardens were more varied, even if the number of plants was smaller. In contrast, public parks contained only a few species, such as *Prunus cerasifera*, *Berberis aquifolium*, *Pyracantha coccinea*, and *Cornus mas*, but in large numbers of individuals. Although all of these species produce fruits in the city's climatic and soil conditions, only *Prunus cerasifera* and *Cornus mas* are popular and consumed by Romanians. The explanation is that native, wild species, such as these, have been tested and experimented with for many years in Balkan cuisines (Nedelcheva, 2013).

Table 3. Distribution of edible species and hybrids by type of green areas

Scientific name*	Common name	No. individuals				Total plants
		Public Parks	Private Gardens	Street plantations	Green area near blocks	
<i>Abies alba</i>	Silver fir	27	12	1	5	45
<i>Abies nordmanniana</i>	Nordmann fir	11	-	6	-	17
<i>Actinidia chinensis</i> var. <i>deliciosa</i>	Kiwi	-	12	-	-	12
<i>Amelanchier Canadensis</i>	Canadian serviceberry	-	11	-	-	11
<i>Aronia melanocarpa</i>	Black chokeberry	-	7	-	2	9
<i>Berberis aquifolium</i>	Oregon grape	172	3	-	21	196
<i>Berberis bealei</i>	Leatherleaf mahonia	11	-	-	5	16
<i>Betula pendula</i>	Silver birch	108	24	14	17	163
<i>Castanea sativa</i>	Sweet chestnut	-	-	-	2	2
<i>Celtis australis</i>	European nettle tree	-	-	-	1	1
<i>Celtis occidentalis</i>	Common hackberry	-	-	-	2	2
<i>Cercis siliquastrum</i>	Judas tree	24	-	-	5	29
<i>Chaenomeles speciosa</i>	Flowering quince	-	13	-	4	17
<i>Cornus mas</i>	Cornelian cherry	116	-	-	2	118
<i>Corylus colurna</i>	Turkish hazel	14	-	-	3	17
<i>Crataegus monogyna</i>	Hawthorn	33	-	-	11	44
<i>Cydonia oblonga</i>	Quince	-	14	-	3	17
<i>Diospyros virginiana</i>	Common persimmon	-	3	-	-	3
<i>Elaeagnus angustifolia</i>	Russian olive	22	-	-	-	22
<i>Ficus carica</i>	Fig	-	18	-	9	27
<i>Gleditsia triacanthos</i>	Honey locust	-	-	5	16	21
<i>Hibiscus syriacus</i>	Rose of Sharon	51	12	32	17	112
<i>Juglans nigra</i>	Black walnut	-	-	-	4	4
<i>Juglans regia</i>	Walnut	14	6	2	18	40
<i>Juniperus chinensis</i>	Chinese juniper	8	-	3	-	11
<i>Juniperus horizontalis</i>	Creeping juniper	116	-	22	-	138
<i>Juniperus virginiana</i>	Eastern redcedar	27	3	-	1	31
<i>Lavandula angustifolia</i>	Lavender	-	68	4346	38	4452
<i>Lonicera japonica</i>	Japanese honeysuckle	-	15	-	20	35
<i>Malus baccata</i>	Siberian crab apple	3	-	-	7	10
<i>Malus domestica</i>	Apple	-	12	-	14	26
<i>Malus x floribunda</i>	Japanese flowering crabapple	1	3	-	-	4
<i>Morus alba</i>	White mulberry	8	-	5	2	15
<i>Morus nigra</i>	Black mulberry	20	3	2	12	37
<i>Picea abies</i>	Norway spruce	-	9	1	1	11
<i>Pinus nigra</i>	Black pine	198	-	26	24	248
<i>"Pinus sylvestris</i>	Scots pine	89	-	6	2	97
<i>Prunus amygdalus</i>	Almond	-	6	-	-	6
<i>Prunus armeniaca</i>	Apricot	-	9	-	5	14
<i>Prunus avium</i>	Sweet cherry	-	22	-	-	22
<i>Prunus cerasifera</i>	Cherry plum	201	18	51	38	308
<i>Prunus cerasus</i>	Sour cherry	-	15	1	4	20
<i>Prunus domestica</i>	Plum	-	5	-	1	6
<i>Prunus persica</i>	Peach	-	9	-	3	12
<i>Punica granatum</i>	Pomegranate	-	5	-	4	9
<i>Pyracantha coccinea</i>	Scarlet firethorn	159	16	-	-	175
<i>Pyrus communis</i>	Pear	8	12	-	2	22
<i>Ribes nigrum</i>	Black currant	-	9	-	-	9
<i>Robinia pseudoacacia</i>	Black locust	78	7	14	28	127
<i>Rosa canina</i>	Dog rose	7	2	7	2	18
<i>Rosa hybrida</i>	Garden rose	-	13	-	10	23
<i>Salvia rosmarinus</i>	Rosemary	-	8	-	2	10
<i>Sambucus nigra</i>	Elder	19	-	-	6	25
<i>Salix alba</i>	White willow	24	-	-	1	25
<i>Salix babylonica</i>	Weeping willow	46	-	-	12	58
<i>Salix cinerea</i>	Grey willow	-	3	-	-	3
<i>Styphnolobium japonicum</i>	Japanese pagoda tree	14	-	-	11	25
<i>Syringa vulgaris</i>	Common lilac	47	45	4	13	109
<i>Tilia platyphyllos</i>	Large-leaved linden	159	3	263	54	479
<i>Tilia tomentosa</i>	Silver linden	208	3	504	64	779
<i>Torreya nucifera</i>	Kaya	1	-	-	-	1
<i>Vitis vinifera</i>	Common grapevine"	-	21	-	15	36
Total		2044	479	5315	543	8381

Therefore, non-native ornamental species require time to become known and used as food. Eleven edible species were inventoried (Table 3) in all four categories of green areas studied: *Abies alba*, *Betula pendula*, *Hibiscus syriacus*, *Juglans regia*, *Morus nigra*, *Prunus cerasifera*, *Robinia pseudoacacia*, *Rosa canina*, *Syringa vulgaris*, *Tilia platyphyllos*, and *Tilia tomentosa*. All these species also have excellent qualities for urban conditions and can fulfil multiple functions (shading, masking unattractive views, windbreaks, reducing noise, or supporting wildlife).

However, the edible species that were present in over 50% each of these were: *Tilia tomentosa*, *Syringa vulgaris*, *Prunus cerasifera*, *Hibiscus syriacus*, and *Betula pendula* (Figure 9).

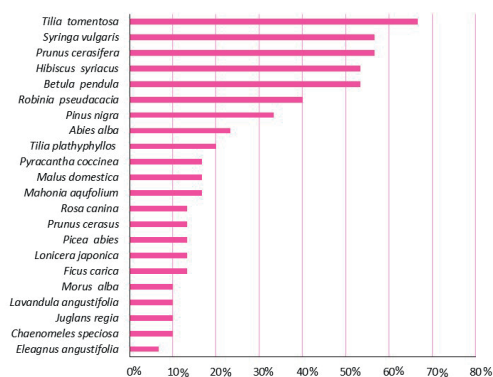


Figure 9. Frequency of edible species in the studied green areas

In each type of green areas, these species had a different number of individuals (Figure 10). In public parks, the dominant edible plants were *Tilia tomentosa*, *Prunus cerasifera*, and *Pinus nigra*; in private gardens – *Lavandula angustifolia*, *Syringa vulgaris*, and *Betula pendula*; on the streets – *Lavandula angustifolia*, *Tilia tomentosa*, and *Tilia platyphyllos*; in the green areas near blocks – *Tilia tomentosa*, *Tilia platyphyllos*, and *Lavandula angustifolia*.

Edible landscapes do not have an established percentage of edible plants to be called that. These landscapes combine ornamental characteristics of species with their usefulness as food, but not necessarily all of them have to be edible (Çelik, 2017; Rupprecht et al., 2023; Zheng & Chou, 2023; Ak & Güneş, 2023). In the

absence of areas with edible herbaceous plants, in the studied green areas, the dominance of edible woody plants was considered. Based on this criterion, none of the studied public parks had enough edible plants to be an edible landscape (Figure 11). Instead, some of the streets and boulevards contain a large number of edible plants. Certainly, public administration (which created these public green areas) selected the species mainly based on their resistance to urban conditions, rather than on their food qualities. Anyway, without intentions, many of the street plantations studied are edible landscapes. This is explained by the large number of lindens used in street alignments compared with other species (Table 3). Also, dense plantations of shrubs, such as lavender (*Lavandula angustifolia*) and rose of Sharon (*Hibiscus syriacus*) contribute to this high percentage of edible plants on the streets.

Linden flowers are highly appreciated in Romania, being used for tea. *Tilia tomentosa* and *Tilia platyphyllos* are native species in Romania, with high resistance to pollution. *Tilia tomentosa* has also proven to be an effective remover of toxic particles from urban air (Petrova, 2024). Furthermore, some other edible species used in street plantations, such as *Betula pendula* or *Morus nigra*, were reported as significantly effective in removing pollutants (Daud et al., 2011; Urošević et al., 2019). For this reason, although the number of edible plants identified on some streets and boulevards was high in our study, they are not recommended for consumption because of the high pollution in the area. On the streets, tree crowns are generally inserted high up on the trunk (for traffic safety reasons) and it is difficult to reach the flowers or fruits (Figure 12), but in the case of shrubs, the risk of consumption persists because their edible parts are much easier to pick. Therefore, it is important to have more edible species in less polluted green areas, so as to reduce the temptation to consume parts of the plants on the street.

Some private gardens (38.8%) and green areas near blocks (51.8%) have more than half of the species as edible trees and shrubs. These small green areas appear as spotted edible landscapes, but may be considered in the future for development as larger areas of edible landscapes.

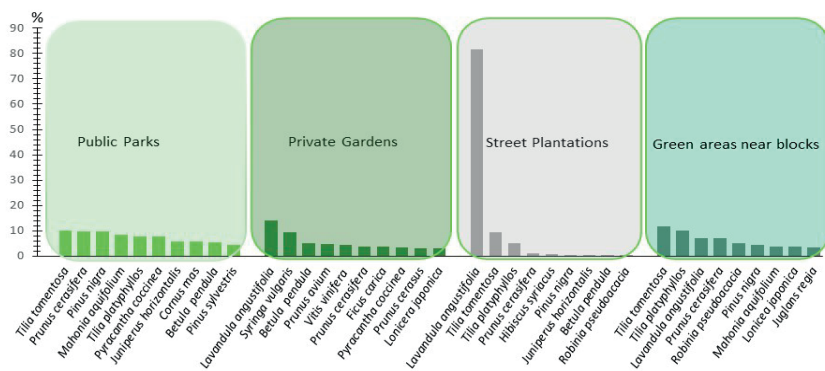


Figure 10. Dominant edible species as the number of individuals in each type of green area

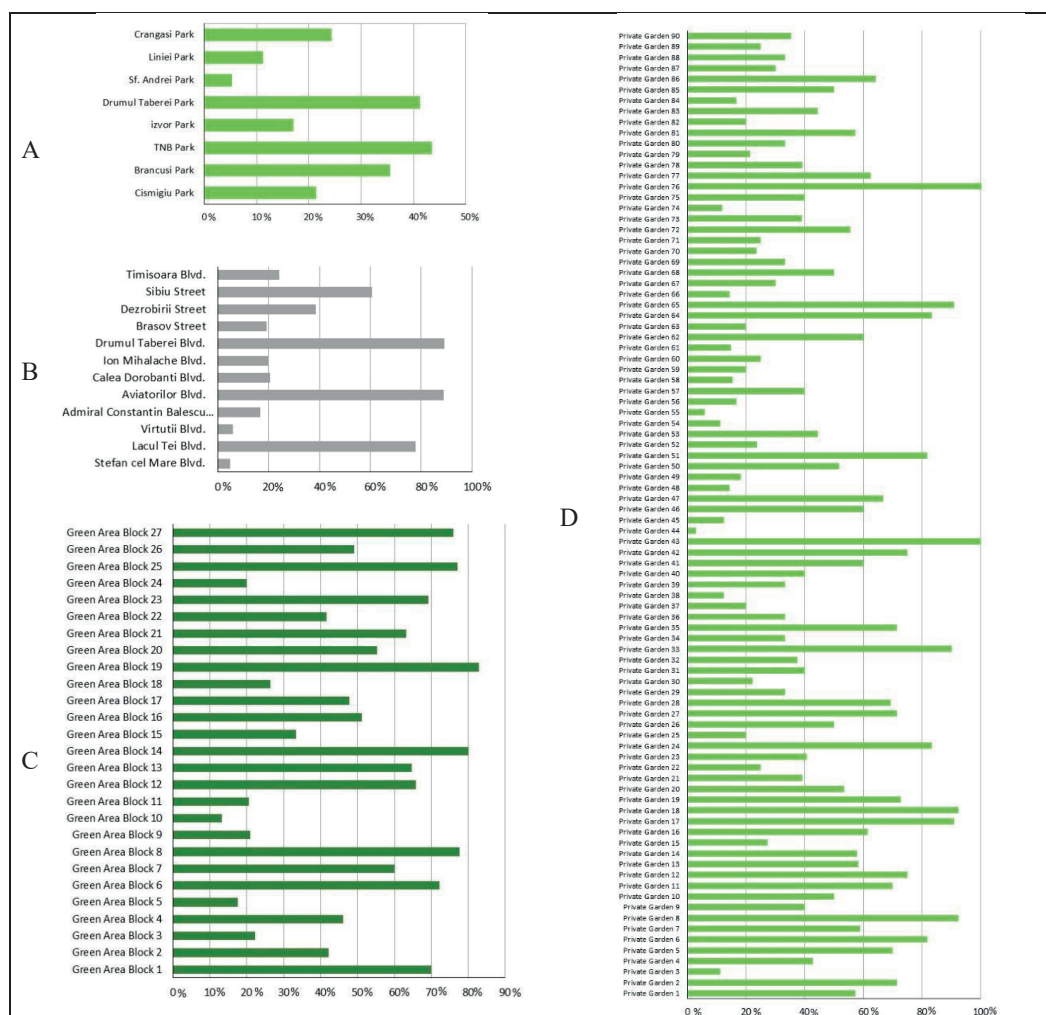


Figure 11. Share of edible plants: A. in public parks; B. on the streets; C. in the green area near residential buildings; D. in private gardens



Figure 12. Tree crowns level in a street plantation in District 6, Bucharest

It was observed a clear tendency of the urban population to cultivate edible species in their private gardens. In most of the private gardens studied, these species dominate. Moreover, the urban population living in apartment blocks has completed the existing plantations with edible species and, in some cases, these predominate. Most of the species were able to produce edible fruits/flowers in both climatic and soil conditions in Bucharest.



Figure 13. Fruits produced by plants grown near residential buildings. A. figs; B. pomegranate

Along the common fruit tree species (*Malus*, *Pyrus*, *Prunus*, *Juglans*, etc.), in recent years was noted a clear tendency of the population to

plant near their homes new edible species for Romania, such as: *Ficus carica*, *Punica granatum*, *Actinidia chinensis* var. *deliciosa* or *Aronia melanocarpa*. Although winters have become milder in southern Romania, as a consequence of climate change, some of these species do not produce suitable fruits for consumption due to their small size or fail to ripen (Figure 13).

Nevertheless, in Bucharest, the urban population seems to appreciate species diversity and be interested in new plants, even if they cannot enjoy their fruits. Researchers in other countries have observed that the urban population cultivates edible plants not only for food reasons, but also for the leisure activities they can provide (Sartison & Artmann, 2020; Janowska et al., 2022; Zheng & Chou, 2023).

CONCLUSIONS

Edible landscaping offers advantages that conventional landscaping cannot. It is closer to the principles of nature, providing food and facilitating relationships among various life forms, including humans. Urban conditions can be restrictive for many plant species, and their selection must consider not only their resilience, but also the functions they provide. However, in some green areas we studied, edible woody plants were dominant.

Diversity of edible species was higher in private gardens and green areas near residential buildings, as evidenced by urban population interest in these species.

Most species produced satisfactory quantities of flowers/fruits in the climate and soil conditions of Bucharest. Even without forming suitable edible parts, as observed in species such as *Ficus carica*, *Punica granatum*, or *Actinidia chinensis* var. *deliciosa*, people continue to cultivate new edible plants for interest and amusement. Also, it was also remarked that none of the studied public parks in Bucharest can be considered edible landscapes, due to the dominance of ornamental plants without food qualities. To avoid the consumption of leaves/flowers/fruits from street plants, where many edible plants can sometimes be found, landscape architects should design edible landscapes in less polluted areas of cities in the future.

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EVALUATION OF SOME GERANIUM CULTIVARS GROWN UNDER GREENHOUSE CONDITIONS

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Abstract

Geranium is one of the most valuable ornamental plants in commercial floriculture, appreciated all over the world, for its beautiful flowers in a wide variety of colours, decorative foliage, and abundant and continuous flowering from late spring to autumn. It is used in pots and planter boxes on patios, porches, or balconies, where it creates an impressive visual impact, as well as in flowerbeds and borders in gardens, but it can also be cultivated indoors. The research was conducted to evaluate the main growth and flowering characteristics of six geranium cultivars grown under greenhouse conditions. The results revealed significant variations between cultivars regarding the morphologically analysed traits. Among the studied cultivars, 'Morning Sun' recorded the maximum values of plant height (31.15 cm) and number of shoots per plant (5.33). 'Abelina' had the largest inflorescence diameter (10.83 cm), while the highest number of flowers in the inflorescence (39.56) was recorded in the 'Calais' cultivar.

Key words: *Pelargonium zonale*, growth, flowering, cultivar.

INTRODUCTION

Geranium (*Pelargonium zonale* (L.) L'Hér ex Aiton) is an ornamental perennial herbaceous plant belonging to the Geraniaceae family, 30-50 cm tall, with a compact, bushy growth habit and upright, branched, thick, fleshy stems. The leaves are long-petiolate, simple, alternate, velvety, slightly pubescent, round-reniform, with wavy edges and a narrow, distinctive dark green or brown band in the middle of the blade, more or less evident. The single, semi-double or double flowers grouped in large, dense, ball-shaped umbels on a long peduncle can be white or in various shades of pink, red, orange, and purple. Some varieties have bicoloured, particularly attractive flowers. Flowering usually occurs from spring to autumn. Geranium is an essential crop in commercial floriculture, providing significant economic benefits for growers and breeders (Rohith et al., 2024). New valuable cultivars are developed and introduced to the market every year, being appreciated for their various characteristics, such as the abundance and colour of flowers, attractive foliage, compactness, uniformity, long flowering period, high productivity, adaptability to both indoor and outdoor conditions, and ease of cultivation.

Geranium requires nutrient-rich, moist, well-drained soils and is sensitive to excessive humidity. Zonal geranium prefers slightly acidic soil (pH 5.8-6.5). Andrews & Hammer (2006) recommend a root medium pH of 6.4-6.5 for the greenhouse production of some geranium species. Kanwar et al. (2024) noticed that the best potting medium for producing high-quality zonal pelargonium plants is a combination of cocopeat + soil + farmyard manure (FYM)+leaf mould (2:1:1:1). Compost produced from various selected green waste or other agro-industrial by-products, as well as biochar, can be used in the preparation of growing media for geranium crops as a substitute for peat, which is the most common substrate used in floriculture, but is very expensive and non-renewable (Ribeiro et al., 2000; Conversa et al., 2015; López-Cuadrado et al., 2008; Massa et al., 2018; Gong et al., 2018; Zawadzińska & Salachna, 2018; Bonaguro et al., 2021). It grows and blooms best in full sun and can tolerate partial shade; however, prolonged periods without adequate sunlight may affect plant development, leading to elongated stems and reduced flowering. In temperate climates, it is grown as an annual plant or can be brought indoors over winter in a bright, sunny location, as it does not tolerate frost. Khattak et al. (2011) showed that light

quality, as well as temperature, has a substantial effect on the growth and flowering of geraniums.

Geranium can be easily propagated by seeds and vegetatively by cuttings taken in spring or late summer from healthy, vigorous plants and rooted in growing media such as peat moss, sand, or perlite. Unrooted *Pelargonium* cuttings can be stored in paper pots maintained in greenhouses at low temperatures for up to two weeks after delivery, without compromising rooting efficiency (Szewczyk-Taranek et al., 2025). Mamba & Wahome (2010) recommend sand and a mixture of garden soil, compost, and sand for the commercial propagation of geraniums. Mutui et al. (2010) revealed that ethylene significantly increased the rooting percentage of cuttings in the two cultivars of *Pelargonium zonale* studied ('Greco' and 'Surfing') but had no effect on total root length. Kutsher et al. (2022) reported that applying mild water stress to stock plants improved the rooting ability, quality, and chlorophyll content of cuttings in some *Pelargonium* cultivars. Khenizy (2016) showed that spraying rooted cuttings with GA₃ at 200 mg/L before planting and storing them for four days at 5°C in imperforated polyethylene bags improved vegetative growth, flowering, and root growth parameters of *Pelargonium zonale* cv. 'Belmonte Red'. Numerous studies have investigated the in vitro regeneration potential of some geranium species and cultivars using different combinations of growth regulators, media, and culture conditions, as well as various types of explants such as cotyledon, hypocotyl, and root (Qureshi & Saxena, 1992; Chang et al., 1996; Murthy et al., 1996; Haensch, 2004; Madden et al., 2005), leaf blade and petiole (Agarwal & Ranu, 2000; Hassanein & Dorion, 2005; Winkelmann et al., 2005), and bud (Alavijeh et al., 2025).

An adequate fertilization program for geraniums during commercial production and the growing season is extremely important for promoting vigorous plant growth and prolonged, abundant flowering. Gaber (2019) reported that the application of mineral fertiliser NPK (6 or 12 g/plant) monthly, combined with foliar application of ascorbic acid (200 or 400 mg/L), improved the vegetative and flowering growth characteristics

of *Pelargonium zonale* plants. Hamid & Imhmd (2024) showed that spraying 150 mgL⁻¹ salicylic acid (SA), combined with the monthly application of NPK fertiliser (3 g/plant), during the geranium growing season, led to a significant increase in most of the studied parameters. Nutrient solution concentration and the type of irrigation system used affect the growth and development of geraniums grown in closed soilless systems (Rouphael et al., 2008; Cardarelli et al., 2012). The typology of the soilless system significantly enhances the commercial characteristics of plants. Thus, by using a cultivation system with continuous fertigation on the substrate (peat) and drip irrigation, more attractive geranium plants for the market can be obtained (Brentari et al.; 2020). Morrison et al. (2024) showed that using timer-based controlled irrigation systems programmed to irrigate for 1 minute in the morning, with 6 g controlled-release fertiliser (Osmocote Plus), resulted in increased plant growth and flower production, as well as more efficient water utilisation in potted geraniums.

To obtain attractive potted ornamental plants with a compact, dense and bushy appearance, preferred by consumers, commercial growers use pinching and the application of chemical growth retardants. Tayama & Carver (1990) reported that effective control of geranium plant height, without affecting the number of inflorescences, was achieved by spray application of certain growth regulators, whereas drench application severely restricted growth and reduced the number of inflorescences. Hamza et al. (2007) concluded that foliar spraying of 75 ppm paclobutrazol applied twice may be recommended for obtaining compact geranium plants, with dark green leaves and earlier flowering. Pinching and spraying of *Pelargonium zonale* plants with 60 ppm paclobutrazol promoted branching and increased stem diameter, the number of leaves, and the number of flowers per plant (Abd El-Aal & Mohamed, 2017). Paclobutrazol combined with supplemental lighting enhanced compactness in geranium seedlings (Collado et al., 2022). El-Sayed et al. (2023) recommend the application of vermicompost at 10 g/pot as a soil amendment and foliar treatments with 1000 mg/L benzyladenine (BA) to improve rooting, vegetative growth, and flowering of *P.*

zonale plants. Exogenous treatments with plant growth regulators such as thidiazuron and gibberellic acid improved chlorophyll biosynthesis, increased net photosynthesis, and reduced leaf yellowing in potted geranium plants (Toscano et al., 2018).

Pelargonium zonale is cultivated in pots and window boxes, on balconies and terraces, and is appreciated due to its hardiness, abundant blooming, ease of cultivation, and availability in a wide range of cultivars with varied flower colours (Krzymińska et al., 2020). This versatile plant is also used in flowerbeds, borders, and along pathways, in combination with other annual or perennial ornamental plants, to create attractive and harmonious floral compositions in gardens (Nooh et al., 2015), and as an indoor plant or even as a cut flower. Geraniums contain active substances with biological and pharmacological activities, such as antibacterial and antioxidant (Iancu et al., 2023; Harzallah et al., 2022; Celi et al., 2024), antimicrobial (Koheil et al., 2012), antifungal and anticancer (Saraswathi et al., 2011; Lewtak et al., 2014), hemostatic (Páez & Hernández, 2003), anti-inflammatory, and many other properties (Matarrita Brenes et al., 2024). Numerous studies have indicated that *Pelargonium* leaf extracts, due to their various natural bioactive compounds, can be used for the synthesis of selenium, gold, cerium dioxide (CeO₂), zinc oxide (ZnO), silver nanoparticles by several physical, chemical, and biological methods (Jafarizad et al., 2015; Mohammadlou et al., 2017; Rivera-Rangel et al., 2018; Fardsadeh et al., 2019; Vahidi et al., 2019; Mahabadi et al., 2021; Abdelbaky et al., 2022; Lopez-Ayuso et al., 2023). Nanoparticles have unique physicochemical properties, which allow their use in various biomedical applications. The objective of this study was to evaluate several geranium cultivars under greenhouse conditions based on growth and flowering parameters.

MATERIALS AND METHODS

The research was conducted on six geranium cultivars with different flower colours, namely 'Clara' (white), 'Abelina' (dark red), 'Calais' (salmon pink), 'Spanish Wine Rose' (rose-pink), 'Morning Sun' (orange) and 'Ellen'

(violet), in the Floriculture Research Area of the Faculty of Horticulture, University of Craiova, in order to analyse their growth and flowering performance under greenhouse conditions. The plant material consisted of high-quality young plants obtained from cuttings and was purchased from the company Gruppo Padana (Italy). The plants were transplanted in February 2024 into plastic pots with a diameter of 12 cm (one plant per pot), using as a growing substrate a mixture of peat and perlite (2:1), and a slow-release fertiliser (Osmocote) was applied. The ambient temperature inside the greenhouse ranged from 20-22°C and the relative humidity was 50-60%. The experiment was carried out using a randomized complete block design with three replications. Observations were made at the full flowering stage on growth parameters (plant height, number of branches and leaves per plant, leaf length and width) and flowering parameters (number of inflorescences per plant, inflorescence diameter, number of flowers per inflorescence, flower diameter), which were measured and recorded for each cultivar. All experimental data were statistically analysed using one-way analysis of variance (ANOVA), followed by Duncan's Multiple Range Test at a significance level of $p \leq 0.05$, in order to determine significant differences among the studied cultivars.

RESULTS AND DISCUSSIONS

Vegetative growth parameters

The results regarding the growth performance of the six geranium cultivars under greenhouse conditions are presented in Table 1. According to the obtained data, there were significant differences among the analysed cultivars in terms of plant height. The maximum value of this parameter was recorded in 'Morning Sun' (31.15 cm) followed by 'Abelina' (29.56 cm), while 'Spanish Wine Rose' had the lowest value (23.78 cm). A significant variation was also observed in the number of branches per plant, which was highest in 'Morning Sun', with an average of 5.33 branches per plant, compared with the other cultivars. The lowest value was obtained in 'Calais', with an average of 2.71 branches per plant. Plant growth and development are influenced by a complex

interaction of multiple genetic and environmental factors (light, temperature, humidity, water and nutrient availability, soil quality, etc.) (Vagner, 2025). Variations in vegetative characteristics, such as plant height, leaf size, and shape, are due to the influence of these factors.

The number of leaves per plant and leaf morphological traits (length and width) differed significantly among the studied cultivars. The mean data indicate that the maximum number of leaves per plant was recorded in ‘Clara’

(38.75), while the minimum number of leaves was observed in ‘Calais’ (25.46). In terms of average leaf size, the cultivar ‘Abelina’ showed the highest values for both leaf length (7.25 cm) and leaf width (8.31 cm), whereas ‘Spanish Wine Rose’ had the lowest values for leaf length (5.00 cm) and leaf width (6.22 cm), respectively. Leaf colour ranged from light green in ‘Calais’ and ‘Morning Sun’ to dark green in ‘Abelina’, ‘Clara’, and ‘Spanish Wine Rose’ cultivars.

Table 1. Evaluation of vegetative parameters of geranium cultivars under greenhouse conditions

Cultivar	Plant height (cm)	Number of branches per plant	Number of leaves per plant	Leaf length (cm)	Leaf width (cm)
Abelina	29.56 ab	3.48 bc	32.33 bc	7.25 a	8.31 a
Calais	26.83 bc	2.71 c	25.46 e	5.92 bc	7.28 ab
Clara	24.61 c	4.12 b	38.75 a	6.33 ab	7.16 ab
Ellen	27.92 b	4.26 ab	34.51 b	5.76 bc	6.95 ab
Morning Sun	31.15 a	5.33 a	30.82 cd	6.14 b	7.43 ab
Spanish Wine Rose	23.78 c	3.25 bc	27.66 de	5.00 c	6.22 b
Mean	27.31	3.86	31.59	6.07	7.23

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 data recorded for flowering characteristics are shown in Table 2. Significant differences were found among the six cultivars for three parameters (number of inflorescences per plant, inflorescence diameter, and number of flowers per inflorescence).

The number of inflorescences per plant ranged from 3.14 to 6.33. The highest value for this parameter was observed in ‘Ellen’, while the ‘Abelina’ cultivar presented the lowest number of inflorescences per plant.

Regarding the average inflorescence diameter, the highest values were recorded in ‘Abelina’ (10.83 cm) and ‘Clara’ (10.66 cm) compared with the other cultivars. The minimum value was obtained in ‘Spanish Wine Rose’, whose inflorescences had an average diameter of 8.91 cm. The number of flowers per inflorescence varied depending on the cultivar. Statistical analysis revealed that there were no significant differences among cultivars with respect to flower diameter.

Table 2. Evaluation of flowering parameters of geranium cultivars under greenhouse conditions

Cultivar	Number of inflorescences per plant	Diameter of inflorescence (cm)	Number of flowers per inflorescence	Diameter of flower (cm)
Abelina	3.14 b	10.83 a	22.41 cd	4.76 a
Calais	3.45 b	10.31 ab	39.56 a	4.38 a
Clara	4.18 b	10.66 a	18.35 e	4.53 a
Ellen	6.33 a	9.12 b	17.68 e	4.16 a
Morning Sun	4.27 b	9.75 ab	33.16 b	4.22 a
Spanish Wine Rose	3.62 b	8.91 b	21.82 d	4.13 a
Mean	4.17	9.93	25.50	4.36

Means with the same letter(s) in a column are statistically non-significant at the 5% significance level according to Duncan’s multiple range test.

Flower colour is an important quality feature that directly influences the aesthetic and commercial value of ornamental plants. It is primarily determined by pigments such as flavonoids, carotenoids, and alkaloids and is influenced by several internal and external factors (Zhao & Tao, 2015). The wide range of flower colours is mainly due to differences in pigment composition and concentration. Tilney-Bassett et al. (1995) reported that the interaction of two complementary genes (R1/r1 and R2/r2) controls the inheritance of red flower colour in zonal pelargoniums. Flower colour is salmon (when R2 is present and R1 is absent in the genotype of a plant's genotype), rose-pink (when R1 is present and R2 is absent), and soft-pink (when both R1 and R2 are absent). Flower colour, along with other quantitative and qualitative morphological characters, is essential in the development of new cultivars in *Pelargonium*. The evaluated geranium cultivars exhibited various flower colours, ranging from white ('Clara'), salmon pink ('Calais'), rose-pink ('Spanish Wine Rose'), orange ('Morning Sun'), dark red ('Abelina') to violet ('Ellen').

CONCLUSIONS

The results revealed that the geranium cultivars showed significant variation in all analysed growth and flowering parameters, except for flower diameter. 'Morning Sun' recorded the highest values for plant height and number of branches per plant, 'Ellen' stood out for the greater number of inflorescences per plant, while 'Abelina' and 'Clara' had the largest inflorescence diameter. 'Calais' performed best in terms of number of flowers per inflorescence. Due to their special decorative qualities and abundant flowering, the studied cultivars can be used as indoor potted plants, as well as for decorating balconies, terraces, and windows in urban spaces.

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EARLY-STAGE EVALUATION OF INVASIVE PLANT IMPACTS ON HISTORIC MONUMENTS: CASE STUDY OF BELLU CEMETERY

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Abstract

*Urban cemeteries represent complex cultural landscapes where natural and anthropogenic factors interact, influencing both biodiversity and the preservation of built heritage. This preliminary study investigates the impact of invasive vegetation on the structural integrity and conservation status of historical monuments within Bellu Cemetery, Bucharest. Field observations and qualitative assessments were conducted to document invasive plant species, their distribution patterns, and the types of physical or biochemical deterioration observed on stone and metal monuments. Early findings indicate that woody species such as *Ailanthus altissima* exert mechanical stress through root penetration, while climbing taxa such as *Parthenocissus quinquefolia* accelerate surface decay and microclimatic changes that favour biodeterioration. The results highlight the need for integrated management strategies that balance ecological value with heritage conservation. This early-stage research will continue and contributes to the emerging field of biocultural heritage studies by providing baseline data for future quantitative analyses and restoration planning.*

Key words: Bellu Cemetery, biodeterioration, conservation, cultural heritage, historical monuments, invasive plant species.

INTRODUCTION

Urban green spaces such as cemeteries represent unique socio-ecological systems where natural processes and cultural values intersect (Francis et al., 2011; Kowarik et al., 2016). As multifunctional landscapes, they simultaneously serve as habitats for diverse species and as repositories of collective memory, art, and architectural heritage (Linden et al., 2020). Within these environments, the presence of invasive plant species has emerged as a growing concern, not only for biodiversity management but also for the conservation of historical monuments and funerary structures (Davis et al., 2019). The interaction between invasive vegetation and built heritage introduces complex ecological and physicochemical processes that accelerate material decay and compromise the integrity of monuments (Caneva et al., 2008).

Invasive plants are characterized by rapid growth, high reproductive capacity, and adaptability to disturbed habitats - traits that make them successful colonizers in urban ecosystems (Richardson & Pyšek, 2012). In cemeteries, where maintenance regimes may fluctuate and microhabitats are varied, these species often establish and expand rapidly (Löki

et al., 2019). Their physiological and morphological traits, such as deep root systems, climbing habits, and allelopathic interactions, can exert both mechanical and biochemical stress on monument materials (Warscheid & Braams, 2000). Mechanical stress arises from root penetration into cracks or joints, leading to bioexpansion and microfracturing, while biochemical stress results from organic acid exudation and moisture retention that enhance corrosion and biodeterioration (Caneva et al., 2008; Miller et al., 2020).

The degradation of stone, brick, and metal monuments by vegetation is well documented in conservation science (Caneva & Ceschin, 2009; Miller et al., 2020). Plants contribute to biodeterioration through a range of mechanisms including chemical alteration of substrates, microclimate modification, and facilitation of microbial colonization (Warscheid & Braams, 2000). These processes are amplified in the presence of invasive taxa whose growth dynamics differ markedly from native flora. Yet, despite increasing attention to plant-induced decay, the interdisciplinary dialogue between ecological research and heritage conservation remains limited (Lisci et al., 2003). Most studies address either the biological aspects of

invasiveness or the material science of deterioration, with few integrating both domains to provide a holistic understanding of how ecological succession influences heritage stability.

Certain plant communities possess significant naturalistic value and simultaneously contribute to the improvement of local microclimatic conditions (Zangari et al., 2023). Vegetation can play a dual role in heritage contexts: while some species offer protective and stabilizing effects, others pose severe threats to the conservation of historical structures (Motti et al., 2020, 2021b; Prieto et al., 2024). Higher plants, including invasive taxa, often colonize stone monuments, wall surfaces, and adjacent areas of historical sites. Their growth dynamics are influenced by human disturbance, microclimatic parameters such as temperature and humidity, and interactions with surrounding vegetation.

Increasing anthropogenic pressure in urban areas has often reduced biodiversity protection and altered the floristic composition of historical environments (Panitsa et al., 2022). Therefore, the primary objective of management interventions is not the complete eradication of vascular plants, but the regulation of vegetation growth to ecologically and aesthetically acceptable levels (Cozzolino et al., 2025). Such management should aim to evaluate the potential biodeterioration risks posed by vegetation and to develop preventive maintenance plans adapted to the site's ecological context.

Understanding the ecological role and mechanical impact of invasive plants within heritage contexts requires an integrative approach combining taxonomy, vegetation ecology, and conservation science. By comparing vegetation composition and structural patterns across one sector of Bellu Cemetery, this study aims to provide a preliminary assessment of how invasive flora contribute to monument deterioration and how their presence reflects the successional stage of the cemetery ecosystem.

MATERIALS AND METHODS

Popescu (2014) provides a comprehensive description of Bellu Cemetery in Bucharest that stands as a significant cultural landscape, reflecting the evolution of urban burial practices, funerary art, and national memory in Romania.

Until the mid-19th century, the dead of Bucharest were typically interred in churchyards or informal burial grounds within or at the margins of the city. In the early 1850s, new sanitary and urban planning concerns (including the risk of epidemics) motivated the municipal authorities to designate new cemetery grounds outside the densely populated centre. The estate owned by Baron Barbu Bellu (1825-1900) on Calea Șerban Vodă was chosen: he donated the land to the town in about 1853/1852. The landscaping and initial works commenced around 1852 under the direction of local authorities, including C. A. Rosetti. As the city expanded, and churchyard burials were gradually outlawed, many remains and family monuments were transferred to Bellu, increasing its prestige and spatial footprint. Over time, the cemetery expanded to cover around 28 hectares (or approximately 220,000 m²) and became home to a prodigious collection of funerary monuments, chapels, mausoleums, and sculptural works created by leading architects and artists of the era. The style mix in the funerary monuments spans Neo-Gothic, Art Deco, Neo-Romanian, and Beaux-Arts influences; the site is sometimes described as an “open-air museum” of funerary sculpture. Bellu became not merely a burial ground but a spatial archive of Romania's 19th- and 20th-century elite: writers, poets, scientists, politicians, and public figures. In recognition of its architectural and memorial value, it was included in the Association of Significant Cemeteries in Europe network and in the European Cemeteries Route. The cemetery's layout, monuments, and inscriptions function as tangible markers of collective memory and identity, linking the urban fabric of Bucharest with national heritage. Situated now within a largely built-up sector of Bucharest (Sector 4), the cemetery faces challenges typical of heritage sites: conservation of monuments, vegetation control, visitor management, and balancing its active role as a burial ground with its function as a heritage site. It continues to operate while being open to the public for walking, study, and contemplation. The “Șerban Vodă Cemetery - Bellu” ensemble is listed in the List of Historical Monuments 2010 - Municipality of Bucharest under no. 22438, LMI code B-IV-a-B-20118.

First step in our study was to inventory the plant species from a sector of a cemetery, about 200 square meters, highlighting the status (native or alien) of the registered plant species, their frequency and distribution on Romanian territory, and their general distribution in the world, according to Ciocârlan (2009).

The weathering patterns, following recommendations by Caneva et al. (2023), were established through direct observation, photographic documentation, and microscopic observation using a Bresciani portable microscope.

We analyze the invasiveness stage of *Ailanthus altissima*, *Hedera helix*, and *Parthenocissus quinquefolia*, and we tried to identify their weathering patterns on the graves and the surrounding walls.

In addition, it was analysed the growth pattern and spreading ways of *Ailanthus altissima*, as the main invasive species inside the cemetery. *Ailanthus altissima* is a dioecious tree (male ♂ and female ♀ flowers on separate plants) and has the ability to reproduce both by seeds and by sprouts, thus allowing tree of heaven to spread and quickly dominate disturbed areas.

On transects, 2 m in width, starting from one female individual toward the other female individuals considered as sources of the offspring, we measured, for every offspring, the distance from the source (m), density (number of individuals), canopy surface (m²), height (cm), trunk diameter (cm) [diameter at breast level – DBH]. Also, we made observations regarding the offspring: young (no ♀ and ♂ differentiation), seedlings possibly emerging from root sprouts and from cut stems – based on height and leaf shape.

Using a GPS Garmin E-Tres Summit, we recorded the GPS coordinates of female individuals – mother source of new shoots (offspring).

For mature trees, we registered a wealth of fruiting (poorly - richly).

RESULTS AND DISCUSSIONS

From an ecological perspective, the colonization of cultural heritage sites by invasive flora reflects broader processes of urban succession and habitat transformation (Kowarik, 2011).

Cemeteries, particularly those established in the nineteenth century, like Bellu Cemetery in Bucharest, were designed as landscaped envi-

ronments integrating ornamental vegetation with monumental architecture (Popescu, 2014). Over time, reduced maintenance, environmental changes, and species introductions have reshaped these landscapes, creating novel ecosystems that blur the boundaries between natural and artificial habitats (Francis et al., 2011). In this context, invasive woody species such as *Ailanthus altissima* (tree of heaven) are frequently documented as agents of ecological disturbance and structural degradation in heritage settings (Caneva et al., 2009; Papini et al., 2017).

The establishment and structure of plant communities depend on multiple factors, including spatial availability, soil characteristics, substrate disintegration, and both micro- and macroclimatic conditions. Persistent exposure to moisture, wind, and temperature fluctuations fosters the colonization of organisms that contribute to biodeterioration processes (Mascaro et al., 2021; Cozzolino et al., 2022). The vegetation surrounding a monument significantly influences the composition and diversity of local flora, thereby affecting colonization intensity.

For stone monuments, the potential of vascular plants to establish is closely related to the stone's chemical composition, degree of exposure, surface inclination, and prior interactions with other biodeteriorative agents (Cozzolino et al., 2025). Plants are considered deteriogenic agents because they induce erosion, cracking, structural instability, and material detachment through combined biophysical and biochemical mechanisms, which ultimately reduce the cohesion between mineral grains (Trotta et al., 2020; Mascaro et al., 2021). These processes involve both assimilatory mechanisms - through which plants extract nutrients from the substrate - and dissimilation, during which metabolic by-products react chemically with the stone matrix. Weathering often results from ion-exchange processes occurring at root tips, the action of colloidal particles along lyotropic gradients, or the release of root exudates that modify substrate pH (Elgohary et al., 2024). The mechanical pressure exerted by root expansion contributes further to damage, as the increasing root diameter and soil volume alteration lead to displacement and fragmentation of stone materials (Caneva et al., 2006). In addition to structural effects, vegetation growth can obstruct access to monuments, conceal

architectural features, and create an impression of neglect (Kanellou et al., 2017).

Abandonment or insufficient maintenance of monuments and historic sites frequently promotes the rapid colonization of invasive species (Caneva et al., 2023). Systematic risk assessment and related management measures are essential to prevent further structural damage. Fitzner (2004) proposed a general hazard index linking weathering processes to biological colonization, identifying 25 typologies of damage associated with biodeterioration. More recently, Caneva et al. (2023) defined a series of indicators describing hazards related to site

conditions and weathering patterns produced by higher plants, including soiling, superficial erosion, discoloration, fracturing, detachment, and collapse.

The plant species inventoried in Bellu Cemetery in summer 2025 (Table 1) are distributed very frequently and frequently in from the steppe zone of Romania to the beech level, most of them being characteristic to the sylvo-steppe zone (forest steppe ecoregion) (EEA, 2019) to which Bucharest metropolitan belongs. The adventive (invasive, alien) plant species (Sandlund et al., 2001) found here find the optimum for their survival and spread.

Table 1. The plant species inventoried from a segment of the cemetery in summer 2025

Species	Frequency in Romania					Distribution in Romania							General distribution	Status	
	very frequent	frequent	sporadic	rare	Very rare	steppe zone	Sylvo-steppe zone	oak forests zone	common oak level	beech level	boreal level (of spruce)	Subalpine level (of juniper)			alpine level (of alpine grasslands)
<i>Acer campestre</i> L.	1					1	1	1	1					Europe and West Asia	
<i>Acer negundo</i> L.	1					1	1	1	1					North America	
<i>Acer platanoides</i> L.	1						1	1	1					Europe and West Asia	
<i>Ailanthus altissima</i> (Mill.) Swingle	1													China	adventive
<i>Anisantha sterilis</i> (L.) Nevski (syn. <i>Bromus sterilis</i> L.)		1				1	1	1	1	1				Eurasia (sub-Mediterranean)	
<i>Aristolochia clematitis</i> L.			1				1	1	1	1				Europe	
<i>Ballota nigra</i> L.		1				1	1	1	1	1				Europe	
<i>Brachypodium sylvaticum</i> (Huds.) Beauv.		1					1	1	1	1				Eurasia (sub-Mediterranean)	
<i>Carex spicata</i> Huds.		1				1	1	1	1	1	1			Circumpolar	
<i>Celtis occidentalis</i> L.														North America	adventive
<i>Chelidonium majus</i> L.		1				1	1	1	1	1				Eurasia	
<i>Chenopodium album</i> L.		1				1	1	1	1					Eurasia	
<i>Cirsium arvense</i> (L.) Scop.		1				1	1	1	1	1	1			Eurasia	
<i>Convolvulus arvensis</i> L.														Mediterranean	
<i>Digitaria sanguinalis</i> (L.) Scop.		1				1	1	1	1					Cosmopolite	
<i>Erigeron canadensis</i> L. (syn. <i>Conyza canadensis</i> (L.) Cronquist)	1					1	1	1	1	1				North America	adventive
<i>Euphorbia</i> sp. (<i>Chamaesyce</i> group)		1					1	1	1	1				North America	adventive
<i>Fallopia dumetorum</i> (L.) Holub.			1											Circumpolar	
<i>Geum urbanum</i> L.		1				1	1	1	1	1				Mediterranean- Circumpolar	
<i>Hedera helix</i> L.		1						1	1	1				Atlantic-Mediterranean	
<i>Inula britannica</i> L.		1				1	1	1	1					Eurasia	
<i>Lactuca serriola</i> L.		1				1	1	1	1					Eurasia	
<i>Melissa officinalis</i> L.			1					1	1	1				Mediterranean	cultivated
<i>Oxalis stricta</i> auct. non L. (syn. <i>Oxalis fontana</i> Bunge)			1											North America	adventive
<i>Parthenocissus quinquefolia</i> Planch.			1											North America	adventive
<i>Pastinaca sativa</i> L.		1					1	1	1	1				Eurasia (sub-Mediterranean)	
<i>Phytolacca americana</i> L.			1											North America	adventive
<i>Plantago major</i> L.		1				1	1	1	1	1	1			Eurasia	
<i>Poa trivialis</i> L.		1						1	1	1	1	1		Eurasia	
<i>Polygonum aviculare</i> L. agg.		1				1	1	1	1	1	1			Cosmopolite	
<i>Portulaca oleracea</i> L.		1					1	1	1	1				Cosmopolite	
<i>Saponaria officinalis</i> L.		1					1	1	1	1	1			Eurasia	
<i>Sedum</i> spp.				1										-	adventive
<i>Setaria pumila</i> (Poir.) Roem. et Schutt.	1					1	1	1	1	1				Cosmopolite	
<i>Silene vulgaris</i> (Moench) Garcke	1					1	1	1	1	1	1			Eurasia	pioneer
<i>Solanum dulcamara</i> L.		1				1	1	1	1	1				Eurasia	
<i>Sonchus oleraceus</i> L.		1				1	1	1	1	1				Cosmopolite	
<i>Sorghum halepense</i> (L.) Pers.		1					1	1						Mediterranean	
<i>Verbascum blattaria</i> L.		1					1	1	1	1				Euras. (sub-Mediterranean)	
<i>Verbena officinalis</i> L.		1					1	1	1	1	1			Cosmopolite	
<i>Viola</i> sp. (either <i>V. odorata</i> L. or <i>V. alba</i> Besser)	1						1	1	1	1	1			Atlantic-Mediterranean	

Tree of heaven (*Ailanthus altissima* (Mill.) Swingle) is recognized as one of the most invasive woody species in temperate regions worldwide (Motti et al., 2021c; Soler & Izquierdo, 2024a). Its ecological success is attributed to a combination of high reproductive capacity, efficient dispersal mechanisms, and remarkable adaptability to disturbed environments. The diameter at breast height (DBH) serves as a reliable indicator of seed productivity, with mature female trees capable of producing up to 300,000 seeds annually (Soler & Izquierdo, 2024b). The seeds are primarily dispersed by wind, although many remain attached to the canopy throughout the winter, facilitating extended dispersal periods. The species exhibits both sexual and asexual modes of reproduction, enhancing its colonization potential. Wind dispersal typically allows seeds to spread up to 200 meters from the parent plant, although the final distance varies with wind velocity and local topography (Motti et al., 2021b).

Our studies revealed that in the cemetery environment, the highest density of the offspring is at about 5 meters from the female plant (Table 2). The female individuals, richly fruity, produce a large number of small offspring from seeds (offsprings - seedlings with heights between 5-40 cm). The young offspring (70-100-300 cm in height) may emerge from seeds (older age) or from root sprouts (faster growing). The structural parameters (density - number of individuals, canopy surface, height, trunk diameter at DBH) used to describe the physical characteristics show the diversity of individuals in different life stages. *Ailanthus altissima* also reproduces vegetatively through root suckers and stump shoots, allowing rapid regeneration after mechanical damage or pruning. The species lacks a true taproot and instead develops an extensive, asymmetrical root system that adapts to local soil conditions (Soler & Izquierdo, 2024a).

Table 2: The locations of investigated *Ailanthus* individuals, their life stage, structural parameters, and distance from the mother source

GPS coordinates		Type of life stage	Density	Canopy surface (m²)	Height (cm)	Trunk diameter (cm)	Distance from source (m)	Observations
N	E							
44.40356	26.09782	1 female ♀ (richly fruity)	50	900	60	0		
		male ♂	2	40	800	30	0,1	
		Os-s	7	0.2	30	0.5	1	
		Os-s	10	0.2	10	0.3	1	
		Os-s	4	0.2	30	1.5	3	
		Os-s	7	0.2	10	0.5	3	
		Os-s	1		40		4	
		Os-s	3		30		4	2 deteriorated
		Os-s	4		5-10		4	
		Os-y	1		300		5	
		Os-y	7		70 -100		5	
		Os-s	10		30		5	
		Os-s	15		10-20		5	
		Os-y	1		100		5	
		Os-s	1		60		5	
		Os-s	5		5-20		5	
		Os-s	1		30		6	
		Os-s	1		20		6	
		Os-s	1		20		13	
		male ♂	1		900	30	13	disturbed the wall
		dead	1				15	
		male ♂	1				17	disturbed the wall, lifted the soil
		young	1		500		27	
		Os-cs	2		30 - 50		27	
		Os-cs	2		10		27	
		Os-cs	2		5		27	
		Os-cs	1		5		27	
		Os-cs	3		5-15		27	
		young	1		500	10	27	
		Os-s	1		30		29	
		Os-s	1		5			

GPS coordinates		Type of life stage	Density	Canopy surface (m ²)	Height (cm)	Trunk diameter (cm)	Distance from source (m)	Observations
N	E							
44.40462	26.09702	1 female ♀ (richly fruity)		25	1000	70		
		male ♂	1		1000	50	0.1	
		male ♂	1		1000	60	0.2	20 x 20 m canopy surface together all 3
		male ♂	1		1000	50	0.3	♂
		Os-rs	3		150		1	
		Os-rs	3		200		1	1 x 1 m canopy surface together all 3
		Os-rs	1		250		1	small groups
		Os-rs	2		30		2	1 x 4 m canopy surface together all 2
		Os-rs	2		200		2	small groups
		Os-rs	1		100		2	
		Os-rs	1		70		2	
		Os-s	12		20		2	
		Os-s	1		30		5	
		Os-s	2		5		5	
		Os-s	2		10		5	
		young	1		500	10	5	
		young	2		450	5	5	
		Os-rs	6		300	3	5	
		Os-rs	4		100		5	
		Os-s	4		10		5	
		Os-rs	3		50		5	
		Os-s	3		30		6	
		Os-rs	2		100		7	
		Os-s	2		30		7	
		Os-s	14		5-10		10	
		Os-rs	1		50		10	developed leaves
		Os-rs	6		200-300		10	
		Of-cs	2				10	

Legend: Os-rs = offsprings - possible from root sprouts; Os-s = offsprings - seedlings; Os-cs = offspring - from cut stem; Os-y = offsprings - young

Most roots occur within the top 46 cm of soil, though vegetative sprouts have been recorded as far as 27 meters from the parent tree. When aerial parts of the plant are damaged or die, new shoots frequently emerge from the root network, reinforcing the plant's resilience and persistence. Our site comprises many individuals in different types of the life stages. Morphologically, *A. altissima* displays a degree of plasticity in leaf development. Leaf forms can vary depending on the origin of the shoot - ranging from unifoliate to penta-foliate in root sprouts, and often trifoliate in seedlings (Soler & Izquierdo, 2024b). This adaptability further enhances its ability to colonize diverse environments and regenerate following disturbance. Ecologically, *A. altissima* thrives in altered and degraded ecosystems, exhibiting strong associations with anthropogenic habitats such as railway embankments, freeways, urban walkways, and abandoned construction sites (Motti et al., 2021c). Its robust root system not only enables the plant to penetrate compacted substrates but also causes mechanical damage to infrastructure, pavements, and building

foundations. In rural landscapes, it commonly invades fencerows, woodland margins, and forest openings, where it outcompetes native vegetation and alters successional trajectories. The capacity of *A. altissima* to colonize and damage built structures makes it particularly concerning in cultural heritage contexts. In archaeological and historical sites, including cemeteries and monuments, the species contributes to structural degradation through root expansion, fissure enlargement, and substrate disintegration. Its presence in such areas is increasingly reported across southern and eastern Europe, where it threatens the preservation of both natural and cultural heritage assets (Motti et al., 2021a; Soler & Izquierdo, 2024a). Preliminary observations suggest that the colonization by *Ailanthus altissima*, *Acer negundo*, and several climbing species such as *Parthenocissus quinquefolia* correlates with visible physical damage, including surface disaggregation, mortar loss, and increased moisture retention on marble and limestone structures (Figure 1).

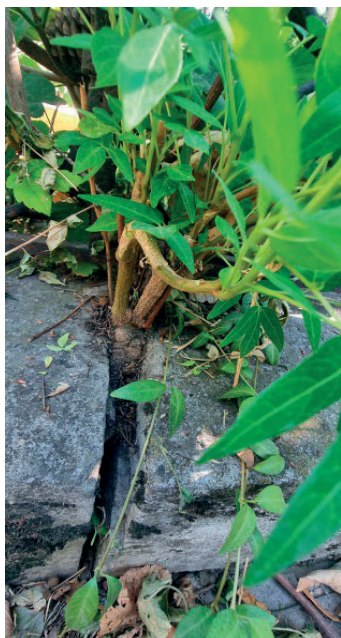


Figure 1. Fissures in the monument through disaggregation

A. altissima has a wide root system, and not all damages are always visible by direct observation. They could be at the foundation or found at a distance of 4 m from the walls, due to the fact that the roots of these trees develop for several meters laterally and vertically.

Ailanthus altissima damages graves and surrounding walls with roots, stems, and fruits (Figure 2).



Figure 2. Stems and roots development pushing and damaging the monument

Among invasive species, *Ailanthus altissima* (tree of heaven) is particularly noteworthy due to its high adaptability to diverse climatic and edaphic conditions and its resistance to eradication efforts. Its rapid growth, efficient vegetative reproduction, allelopathic root activity, and pathogen tolerance explain the ecological success of this aggressive invader (Sladonja et al., 2015). *A. altissima* has been documented on monuments, walls, paved surfaces, and roofs, demonstrating an alarming capacity for propagation.

In the studied fragment of the cemetery, *Parthenocissus quinquefolia* exhibits the same behaviour as *Hedera helix* (common ivy), another species frequently encountered on walls and stone monuments exhibits invasive behaviour as well, although generally to a lesser degree than *A. altissima* (Trotta et al., 2020).

The composition, abundance, and spatial distribution of native and invasive plant species hold considerable bioindicator value for assessing site conditions and guiding conservation management.

The main factors influencing colonization and biodeterioration are: the substrate, site exposure, the surrounding vegetation, and the degree of anthropogenic disturbance.

Taking into account substrate properties, the degree of anthropogenic disturbance, and the surrounding ecological context will be very important for identifying prioritizing maintenance interventions.

The maintenance activity should establish the interaction between invasive plants and substrate type, and thus the vulnerability of the colonized surface in the surrounding environment.

An adaptive management approach will contribute to the conservation of the "Șerban Vodă Cemetery - Bellu" by mitigating biodeterioration more effectively.

CONCLUSIONS

For our studies, Bellu Cemetery - founded in the mid-nineteenth century and now a historical monument of national importance - offers an exemplary context for investigating the impact of invasive plant species on historic monuments. It acts like an open-air laboratory for future more complex and inter-disciplinary studies, an active necropolis and cultural heritage site, exhibiting

a mosaic of ecological niches, ranging from shaded tree canopies to exposed masonry surfaces.

The methods of eradication of woody invasive plant species require a multidisciplinary approach to restoration practices based on strong collaboration between restorers, biologists, agronomists, and masonry experts.

Over the decades, fluctuating management intensity and urban environmental changes have facilitated the establishment of numerous invasive species, making the site a living laboratory for studying their dual impact on biodiversity and built heritage.

The Bellu Cemetery embodies the intersection of urban planning, funerary practice, artistic expression, and national remembrance. It serves as both a functioning necropolis and a cultural patrimony site, illustrating how burial spaces evolve from purely functional grounds to rich symbolic landscapes. Its continued preservation is crucial for understanding Bucharest's historical urban fabric and Romania's collective heritage.

Our studies will grow in space (more sections of the cemetery toward the entire surface) and complexity in the years to come, and the results will contribute to the development of interdisciplinary management strategies that reconcile biodiversity conservation with heritage preservation, ensuring that these cultural landscapes continue to function as both ecological refuges and memorial spaces.

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LANDSCAPE-LED DESIGN FOR RESILIENT PERI-URBAN COMMUNITIES: INTEGRATING GREEN INFRASTRUCTURE FOR REGENERATION

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Abstract

One of the major problems facing urban development today is the accelerated expansion of major urban centres, specifically the tendency to concentrate this development in the central areas of the city. To address this trend, an opportunity that is still not sufficiently developed can be found in the modernization and strategic expansion of peri-urban areas, which would offer viable possibilities to reduce the pressure on a congested urban core as well as valuable contributions to the development of the resilience of some micro-communities. This study brings into focus an example in which landscape design can be incorporated in a relevant way, presenting a small-scale residential complex and how the creation of an appropriate and carefully planned green infrastructure can bring numerous benefits-urbanistic, ecological, and on human health. The findings demonstrate that the synergy between a built environment and a green landscape designed based on current ecological needs is a catalyst for the regeneration of underutilized areas that can be transformed into functional and high-performance community assets. The integration of green infrastructure into real estate developments is an essential principle for the development of future-oriented peri-urban residential communities.

Key words: urban horticulture, plant-based, sustainability.

INTRODUCTION

Environmental policies are focused on a long-term vision of sustainability that integrates principles of the circular economy, green finance, digital transformation, and participatory governance (Pascu et al., 2022; Popek et al., 2024; Slobodnikova & Toth, 2025). Global trends, economic and environmental challenges, and crises all around lead to the need for a more sustainable way of existence. New environmental policy has to continue evolving as a dynamic governance instrument, able to manage uncertainty while ensuring that ecological security and human well-being remain central concerns for sustainable development (Janiszek & Krzysztofik, 2023; Macaione et al., 2024; Ozarchevici et al., 2024; Vladimir & Cojocariu, 2025).

In this context, there is a growing need to enable integrated initiatives to develop more participatory strategies to integrate solution-oriented approaches in green space planning and regeneration, including co-design, co-production, and co-management (Karrasch et al., 2017; Huba & Kascakova, 2023; Vladimir &

Cojocariu, 2024). This reflects the wider trend to support sustainability in other areas of society, as research, urban planning, management, human resources, and all parties involved in social cohesion (Cojocariu et al., 2023; Pascu et al., 2019; Ioannou et al., 2025). The restoration of green spaces must aim at restoring the earth's ability to self-heal. Thus, nature-based solutions represent an opportunity to transform current challenges into opportunities for urban regeneration (Kooijman et al., 2021; Pașcu et al., 2021; Zlati et al., 2024). Unlike traditional urban planning, where the shape of the city is imposed by the road network and underground infrastructure, landscape-led design places natural systems at the center of urban development. In this approach, elements such as watercourses, topography, vegetation, and ecological corridors become the primary framework upon which urban communities evolve, with resilience serving as a guiding principle. By prioritizing ecological processes and natural landscape structures, landscape-led planning promotes more adaptive and sustainable urban environments.

Green infrastructure manages climate risks much more efficiently and cheaply than "grey" engineering solutions (Vano et al., 2021; Chelariu et al., 2018).

The present study brings into focus an example of landscape-led design incorporated in a relevant way, presenting a small-scale residential complex and how the creation of appropriate and carefully planned green infrastructure can bring numerous benefits, as urbanistic, ecological, and wellbeing.

MATERIALS AND METHODS

The systematic framework for design decisions was imposed by the design objectives, which aimed to:

- ecological regeneration (species selection);
 - hydrological resilience;
 - social integration (community connection).
- Starting from the premise that a "landscape-led" approach implies that the analysis of the landscape dictates the urban form, not the other way around, the spatial criteria for the organization of the site were based on connectivity, accessibility, and functionality.

The zoning was based on the intensity of use (food forest area, buffer or connection area, relaxation area, conservation area).

The selection of species was made according to ecological criteria, the ability to adapt to thermal variations and water stress (specific to peri-urban areas).

Location. Cameo Residence is located at 6 Ion Nistor Street, 707410 Iași (Figure 1).

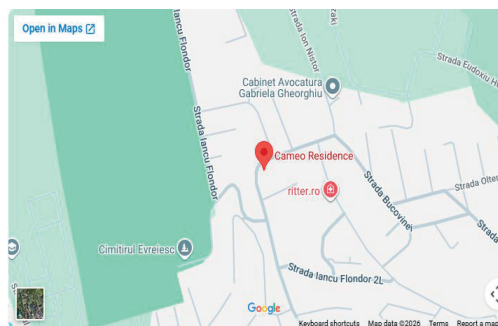


Figure 1. Site location
(<https://cameoresidence.ro/>)

Cameo Residence builds blocks to the NZEB (Nearly Zero-Energy Building) standard.

It assumes a building with very high energy performance, whose energy consumption is close to zero. The actual situation of the project is in progress (Figure 2).

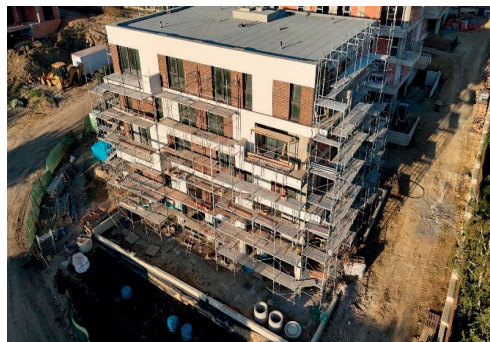


Figure 2. Site current stage

The planning proposal is in line with the concept of regeneration and urban resilience.

The central theme of integrating green infrastructure for regeneration is built around landscape-led design for resilient peri-urban communities. Cameo Residence consists of three blocks and the integrative green space around. Buildings have reduced-height regimes, designed for those who want a balanced, discreet, and modern lifestyle. With a limited number of apartments, the project emphasizes privacy, tranquility, and functional efficiency, without compromising safety and aesthetics. The optimization of the space aimed at maximizing the connectivity of the habitats.

Planting material

Species selection was not dictated mainly by aesthetic preferences, but by a three-step filtering protocol: compatibility with the soil type identified in the field phase (a low-fertility soil, specific to construction sites), adaptability and resistance to the urban heat island effect, and synergy between woody and herbaceous species to maximize stratified biodiversity.

Peri-urban communities often suffer from rapid concreting. Species selection directly influences the microclimate through the rate of evapotranspiration. Species with a large leaf surface and an active metabolism (*Acer*, *Liriodendron*, *Magnolia*, *Liquidambar*) pump water from the soil into the atmosphere, cooling the surrounding air.

Another important criterion was crown density. Species that provide dense shading reduce ground temperature by up to 10-15°C, protecting infrastructure and reducing energy consumption for cooling homes (Paşcu et al., 2019). Also, the proposed variety of species (Table 1) offers decor throughout the year through flowering plants that bring diverse color and fragrance, structure through the use of bark and leaves, or fruits that persist on branches even in the cold season (Negrea & Zlati, 2011; Bernardis et al., 2022, 2024; Zlati & Pascu, 2021).

Table 1. List of used woody species

Specie name
<i>Acer palmatum</i> "Atropurpureum"
<i>Acer platanoides</i> "Drummondii"
<i>Acer rubrum</i> "October Glory"
<i>Aronia melanocarpa</i>
<i>Berberis thunbergii</i> "Atropupurea", "Aurea"
<i>Ceanothus pallidus</i> "Marie Simon"
<i>Cornus sanguinea</i> "Midwinter Fire"
<i>Corylus avellana</i>
<i>Cotoneaster dammeri radicans</i>
<i>Crataegus</i> spp.
<i>Daphne mezereum</i> "Rubra"
<i>Deutzia gracilis</i>
<i>Euonymus alatus</i>
<i>Euonymus Aureomarginata</i> (espalier)
<i>Fagus sylvatica</i> "Darwick Purpurea"
<i>Fragaria</i> spp.
<i>Ginkgo biloba</i>
<i>Hedera helix</i>
<i>Hibiscus syriacus</i>
<i>Hydrangea macrophylla</i> "Endless Summer"
<i>Hydrangea</i> spp.
<i>Juniperus squamata</i> "Blue Carpet", "Blue Star"
<i>Juniperus virginiana</i> "Blue Arrow"
<i>Lavandula angustifolia</i>
<i>Liquidambar styraciflua</i>
<i>Liriodendron tulipifera</i>
<i>Magnolia x 'Cameo'</i>
<i>Ocimum basilicum</i>
<i>Photinia</i> "Red Robin" (espalier)
<i>Philadelphus hybrida</i> "Dame Blanche"
<i>Pinus mugo</i> "Carsten's Wintergold"
<i>Platanus acerifolia</i>
<i>Potentilla fruticosa</i> "Blink"
<i>Prunus laurocerasus</i>
<i>Prunus cerasifera</i> "Nigra"
<i>Pyracantha coccinea</i> "Red Cushion"
<i>Rhododendron</i> "Dr. H.C. Dresselhuys"
<i>Ribes</i> spp.
<i>Rubus fruticosus</i>
<i>Salvia officinalis</i>
<i>Spiraea japonica</i> "Golden Princess"
<i>Vaccinium corymbosum</i>
<i>Viburnum plicatum</i> "Wanabee"
<i>Viburnum opulus</i> "Compactum"
<i>Vitis vinifera</i>
<i>Weigela florida</i> "Victoria"

We used this wide range of species to contribute to a lively, fresh, and optimistic atmosphere that supports residents' quality of life, the ensemble's sense of identity of the ensemble and, nevertheless, the sustainability of the investment.

RESULTS AND DISCUSSIONS

Unlike conventional planning, where the landscape occupies the “leftover” spaces between buildings, the landscape-led approach allowed the use of topography as a structural skeleton. The result is not only aesthetic, the functional being a priority. Thus, the green infrastructure dictated the built density, ensuring that the “unproductive” areas remain unbuilt but serve as connection and restoration areas.

While the current model of peri-urban expansion fragments ecosystems through rigid street networks, the proposed design solution uses green corridors as development axes. This landscape-led approach ensures habitat continuity, an ecological benefit superior to standard parceled development.

The idea for the central theme of the landscape proposal was primarily seen as a change of perception, meaning that the landscape is no longer seen as a simple decoration, but as a form of connection between the built area and the human component, being at the same time the primary infrastructure (Figure 3).

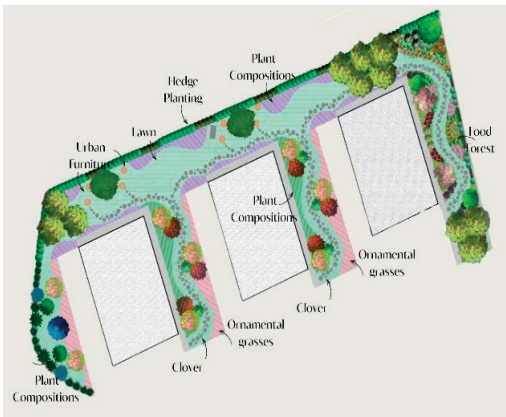


Figure 3. Proposed design plan

Table 2 presents a series of the proposed design benefits. The main functional benefit consists of the creation of a network of ecological corridors

that link the urban spaces, increasing the connectivity index of fragmented habitats. Through the proposed design, 90% of the residents in the area are less than 500 meters from a node of green infrastructure.

Table 2. The evaluation of the proposed design benefits

Resilience pillars	Design intervention	Arguments
Functional	Ecological corridors network that link the urban spaces	Increasing the connectivity index of fragmented habitats
Ecological	Hydric resilience	Used species reduces the rate of water runoff without expensive infrastructure
Social	Peri-urban community gardens	Creating local “micro-economies” and spaces for social interaction

The green space is designed as a contemporary, integrative landscape system based on principles inspired by the Food Forest concept in a modern composition (Figure 4). The proposed vegetation supports the quality of life of the residents, the identity of the ensemble, and the long-term value of the investment. Thus, beauty meets functionality and community becomes part of the garden. Floristic diversity, by increasing the number of native species introduced, has the effect of increasing ecosystem resilience in the face of pests.



Figure 4. Food forest integration within the landscape design

The integration of green infrastructures in the regeneration of urban areas is based on the idea

that green spaces have not only an aesthetic contribution but also a social one, by reconnecting isolated fragments of the habitat, allowing biodiversity to develop and circulate, and giving inhabitants access to recreational activities.

At the main entrance of the complex, a distinctive planting composition is introduced in order to create a recognizable and memorable landscape element that reflects the identity of the development. Due to the name of the residential complex's name, the entrance area is marked by specimens of *Magnolia “Cameo”*, which function as a symbolic and visually striking feature within the landscape design. Their seasonal flowering creates a strong visual accent and contributes to establishing a welcoming and representative image of the site (Figure 5).



Figure 5. *Magnolia “Cameo”* as a landmark element

The planting composition is further structured through a balanced combination of evergreen and deciduous species. Conifers provide year-round structure and visual continuity during the winter months, while deciduous species contribute seasonal variation through foliage color and flowering dynamics (Figure 6).



Figure 6. Entrance landscape composition

The transition from a drab landscape used in the past, dominated by species such as *Buxus* or *Thuja*, to a layered vegetation system (herbaceous, shrubby, arboreal) increases biological resilience by diversifying ecological niches. Together, these elements form a layered and dynamic landscape composition that enhances both the aesthetic quality and the ecological value of the entrance area.

Food Forest is a landscaping concept inspired by the natural structure of a forest, where the vegetation is organized on several complementary layers. Reinterpreted in the urban environment, this concept becomes a sustainable, valuable solution that combines edible and ornamental species in a pleasant, orderly, accessible, and highly aesthetic landscape.

The composition includes fruit tree species and shrubs, but also perennials and ornamental grasses. Edible species used are as follows: *Corylus avellana*, *Crataegus* spp., *Vitis vinifera*, *Rubus idaeus*, *Vaccinium corymbosum*, *Ribes* spp., *Aronia melanocarpa*, *Fragaria* spp., *Rubus fruticosus*, *Salvia officinalis*, *Ocimum basilicum* and *Lavandula angustifolia*, and are exemplified in Figure 7.



Figure 7. Examples of proposed plant species

Species we chose are easy to recognize, easy to maintain, and offer fruits, much appreciated by young people. Aromatic species add variety and special freshness to the garden, creating at the same time a pleasant environment, while low and cover plants help protect the soil, maintain moisture, and help control weeds.

Low, cover vegetation and ornamental grasses chosen to be placed between buildings create calm, green areas that filter the view, improve the microclimate, and complement the architecture in such a discreet and elegant way (Figure 8). For sloping land, ground cover plants work as a natural drainage system by reducing the rate of water runoff without expensive infrastructure.

Integrating water retention areas directly into public spaces transforms hydrological risk into a design element, demonstrating greater functional efficiency than 'grey' engineering solutions (pipes, channels), which have limited adaptability.



Figure 8. Compositions that include ornamental grasses

Relaxation areas, located in the shade of birch trees, complete the green space while offering a pleasant and protective natural setting, perfect for rest and daily use (Figure 9).



Figure 9. Relaxation area exemplification

Furniture elements for this project are specifically designed to support climbing plants. In addition, it provides additional shade while enhancing the decorative character of the space-exemplification in Figure 10.

We found that numerous research shows that green spaces help maintain the quality of life by reducing stress levels, supporting concentration, and promoting the development of emotional intelligence from a young age (Gradinariu et al., 2007; Negrea & Zlati, 2010; Zlati et al., 2022; Cojocariu et al., 2022). It is known that people who carry out their activities in a natural setting are calmer, more receptive, and show greater involvement in interpersonal relations. It is also

known that interacting with greenery, activities outdoor activities stimulates curiosity, supports exploratory learning, and contributes to the formation of a positive, lasting bond with the environment. Exactly in this context, the plan for Cameo Residence was conceived as a sustainable complex, where nature becomes an active, living partner for residents' well-being and the community.



Figure 10. Furniture elements for the resting

„Green concept” was further extended into the complex's interior spaces through the use of indoor species, clearly selected for this type of environment. These species are well adapted to low lighting conditions, a characteristic of interior spaces. Using indoor greens ensures both aesthetic continuity and long-term viability of the decor. Thus, the vegetation becomes an integrating element of the overall spatial experience (Figure 11).



Figure 11. Extending the greening concept into the interior of the complex.

Over the all, the proposed design facilitates social regeneration by creating points of interest based on natural elements, which encourage the community to approach the peri-urban territory, often perceived as a space of transit without identity.

CONCLUSIONS

The integration of green infrastructure in the plan of urban regeneration is based primarily on the idea that green spaces have not only an aesthetic contribution, but also a social one, aiming the reconnection of the isolated fragments of habitat, allowing biodiversity to develop and flourish, and providing inhabitants with access to recreational activities.

The green space surrounding Cameo Residence was designed as a contemporary, integrative landscape system based on principles inspired by the Food Forest concept in a modern composition. Species we chose are easy to recognize and easy to maintain. They offer fruits, much appreciated by children. Aromatic species also add variety and special freshness to the garden, creating at the same time a pleasant environment, while low and cover plants protect the soil, maintaining its moisture and helping in weed control.

The design idea we propose facilitates social regeneration by creating points of interest based on natural elements, which encourage the community connection to approach the peri-urban areas, often perceived as a space of transit with no clear identity.

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SUSTAINABLE URBAN PLAYGROUND RESTORATION THROUGH COMMUNITY CO-DESIGN AND EDIBLE PLANTINGS

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Abstract

Urban playgrounds are public spaces with a fundamental role in the growth and development of children, especially in today's times when younger generations are increasingly captivated by modern technologies. However, a worrying phenomenon of their degradation is observed, which affects their usability and sustainability. The presented case study analyzes the restoration of an urban playground through the use of natural materials, fruit trees, and a community co-design approach. The project has as final objectives the optimization of environmental performance and the stimulation of children's well-being through efficient, nature-based, urban design solutions. Based on field observations, the examination of international examples built on the same principles, and the formulation of design solutions, the aim is to highlight the importance of integrating built and vegetal elements that support biodiversity and can represent a catalyst for environmental education. The research illustrates how resilient landscapes are able to contribute to the transformation of urban playgrounds into sustainable environments. The study data add value to the broader debates about implementing participatory ecological design in urban spaces, creating more child-friendly cities, and developing strategic urban planning oriented on the long term.

Key words: urban green infrastructure, playground restoration, edible landscapes.

INTRODUCTION

Urban spaces intended for the public play a major role in social interactions and the level of comfort of the inhabitants of an urban center (Cojocariu et al., 2023; Istrate et al., 2025). In this context, playgrounds existing in most neighborhoods in a city become basic elements of the urban structure and involve carefully formulated and applied management measures. Within the urban fabric, these spaces represent indispensable elements that contribute to the physical, cognitive, and social formation of children; however, a significant percentage of them are in a state of progressive decline, both from a functional and identity perspective. The possibility of benefiting from and constantly using a green space for various activities is repeatedly correlated with benefits for children, such as increased physical activity levels, better understanding and management of the emotional spectrum, and a considerable increase in overall health (Tillmann et al., 2018; McCormick, 2017).

The increasingly accelerated urbanization process and the alarming decrease in biodiversity in urban centers considerably diminish the

opportunities that provide contact with the natural environment (Aronson et al., 2017; IPBES, 2019). Also, the amplification of the urban heat island phenomenon directly affects underage users of poorly shaded, or completely unshaded, play spaces, thus manifesting a further increase in the vulnerability of these spaces (Vanos, 2015; Bernardis, 2022). The current state of many playgrounds is largely due to the predominant presence of artificial surfaces and equipment, coupled with the lack of harmonious integration into the ecological structure of the environment, which also contributes to limiting the level of education offered, along with the decrease in initial performance. Therefore, the rehabilitation of these sites needs to be based on ecological interventions oriented towards resilience.

Current urban design strategies increasingly encourage the insertion of nature-based solutions (NBS) to enhance the resilience of the natural environment and increase human well-being. This typology of solutions provides ecosystem services such as microclimate regulation, responsible water management, and biodiversity protection (Raymond et al., 2017; Kabisch et al., 2017; Amisculesei et al., 2022).

At the same time, spending as much time as possible in nature enhances mental and psychological health (Bratman, 2019). In addition to this aspect, more and more recent research on the typology of these solutions demonstrates that an important direction is the integration of fruit species and community farming practices into urban planning, having a major role in strengthening social resilience and food literacy (Säumel et al., 2019; Artmann & Sartison, 2018). An additional benefit is that edible urban landscapes can facilitate community participation in their management, promoting inclusion and social interactions (Gradinariu, 2007; Zlati & Pașcu, 2021).

By applying the aforementioned principles and integrating natural materials and edible plants into play environments, these spaces become landscapes with diverse functionality that contribute to both ecological performance and environmental education. Therefore, the process of restoring urban playgrounds becomes simultaneously an environmental restoration intervention and a pedagogical act aligned with contemporary sustainability directions.

Currently, many studies address the concept of nature-based solutions and the possibilities of their application in urban planning (Negrea, 2011; Frantzeskaki, 2019; Bush & Doyon, 2019; Pașcu, 2021), yet there remains a significant gap in detailed implementation strategies specifically for children's play environments. This paper addresses this gap through a case study focused on the rehabilitation of an urban playground using natural materials, fruit species, and a community co-design approach. The primary aim of this research is to demonstrate the untapped potential of playgrounds as drivers for environmental restoration and child well-being. To achieve this objective, the study pursues the following specific research objectives:

- to evaluate the effectiveness of nature-based solutions (NBS) in mitigating the urban heat island effect and improving microclimatic comfort within play spaces;
- to design and implement a functional model for an edible urban landscape that integrates fruit species to promote food literacy and biodiversity;
- to establish a set of replicable ecological design principles that transition playgrounds from

artificial, high-maintenance structures to resilient, pedagogical natural environments.

MATERIALS AND METHODS

This research is based on a qualitative case study methodology, widely used in the field of landscape design for the analysis of complex interventions. In this case, the restoration of an urban playground is a process in which the effectiveness of solutions varies depending on the context of the site, local environmental conditions, and the needs of the beneficiaries in the area.

The site analyzed is located in the Alexandru cel Bun neighborhood of Iași city, one of the oldest and most populated residential neighborhoods in the urban structure. The development of these residential complexes, primarily a product of the communist era, is distinguished by multi-story apartment buildings organized around shared public spaces and circulation systems (Figure 1). Within these housing models, public areas are crucial in determining the daily quality of life and social dynamics within the communities.

The project was designed using Lumion and SketchUp software, and at the present time the project is in the implementation stage.

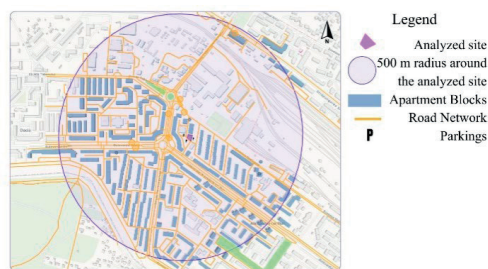


Figure 1. Site location and spatial analysis of the surrounding context

To achieve the research objectives, the study employs a mixed-methods approach.

Observational methods include systematic landscape audits and System for Observing Play and Recreation in Communities - SOPARC-based behavioral mapping to quantify user activity. Observations made at the site were used to document the existing conditions and highlight the challenges affecting the functionality of the site. This approach allows the documentation of the transformation process

from a degraded playground to a sustainable intervention.

The physical characteristics of the site were documented, such as the materials used, general location, accessibility to the site, shade conditions, and existing vegetation.

These observations were the basis for the identification of spatial deficiencies and landscape opportunities that can be implemented within the respective framework.

The study adopts a Collaborative Action Research (CAR) model, where the community is not merely a subject of study but a partner in the design process.



Figure 2. Community engagement activities

The community involvement is facilitated through co-design workshops and photovoice sessions, ensuring a participatory approach to edible landscape planning. Community was present from the first stage of the project by identifying community needs and local identity. Then, through collaborative workshops to select fruit species and the playground layout, involvement in organized community planting

days, participants fostered a sense of ownership (Figure 2).

RESULTS AND DISCUSSIONS

In particular, playgrounds within residential neighborhoods are positioned as accessible recreational environments that encourage physical activity, social interaction, and community involvement (Carmona, 2021).

Given that the site is surrounded by apartment buildings on all sides, thereby registering a high degree of urban density, the playground is positioned as the only outdoor recreational element, accessible to children and families living in the immediate vicinity. There were a limited number of children's structures, benches, or areas with vegetation on the playground. The configuration of the space and the materials used, such as plastic or iron, denote outdated design approaches, which were based on standardized equipment and artificial surfaces, such as concrete or rubber tiles (Figure 3). This way of organizing the space establishes a rigidity in the design that considerably reduces children's opportunities for exploration and limits children's acquisition of new experiences or the formation of responsible attitudes.



Figure 3. Assessment of the existing playground conditions

It was observed that one of the factors with a strong emphasis on amplifying environmental discomfort is the presence of parking areas that

border the site on three sides. This immediate proximity to traffic introduces several negative environmental factors, such as increased air pollution, noise pollution, and visual intrusion into a space that in theory is considered safe for children. The dominance of parking facilities at the site boundary induces even more insecurity for users of the space, while simultaneously reducing its ecological quality and limiting opportunities for expanding green infrastructure. The vegetation species found on the site are few in number and offer limited shade or ecological value. Their absence greatly reduces environmental comfort and the capacity of the space to support biodiversity. Consequently, the playground cannot provide protection against solar radiation, which significantly affects the use of the space, especially in the summer months, the period in which it is assumed to be most used (Figure 4).



Figure 4. Sunlight and shadow analysis – July 15 (9:00 AM / 5:00 PM)

The analysis of the solar study shows that throughout the day the space is exposed to strong sunlight, and this, together with the predominance of impermeable surfaces, contributes to the excessive accumulation of heat during the summer period. Hard layers absorb and retain solar radiation, creating inadequate thermal conditions for users of the space (Unal et al., 2021; Zhang et al., 2024; <https://www.wri.org>).

Also, the existing organization of the site does not offer children opportunities for learning based on the environment. The lack of natural materials, direct interaction with various textures and vegetation elements, limits children to learn about seasonal changes, biodiversity, and enrich their sensory experience of textures. Previous research has highlighted the value of introducing natural materials into the design of a playground, describing the numerous benefits

of implementing nature-based solutions within them (Vladimir & Cojocariu, 2024; Bernardis, 2024).

All the existing conditions analyzed in the site highlight the urgent need to implement a restoration strategy capable of generating an increase in both the level of environmental performance and the degree of use of the space from a social perspective.

In addition to sustainable design approaches, the project integrates participatory design strategies, focusing on community needs and potential users' proposals for the playground design.

The practice of participatory design is widely used in international urban planning with the aim of creating the most favorable conditions for users of public spaces and encouraging community participation in strategic planning decisions (Innes & Booher, 2010; Vladimir, 2025). Including children in the design process can also contribute to the development of a sense of belonging to both a physical space and a community with shared values and interests (Derr & Tarantini, 2016).

Following the analysis carried out, the study of specialized literature, and the consultation of the population, a concept for the restoration of the site was developed (Figure 5).



Figure 5. Proposed site redevelopment plan

The proposed design brings with it an improvement of the space from both an ecological and social point of view. It is recommended to introduce new trees to increase the shade area, replace impermeable materials,

and introduce natural materials both in the technical structure of the intended new equipment and as an independent use for free play. In order to expand the green area of the playground, the original site was expanded by incorporating the adjacent space located on the eastern side of the playground. This area was previously an undeveloped land area, used informally as a parking lot. Adding this area to the total area allows for the expansion of the vegetated area and the implementation of new interventions. A solution to the problem of surrounding the site with parking spaces was the introduction of a buffer zone of vegetation along the southwestern edge of the space (Figure 6). This green barrier will function as a protective space both visually, acoustically, and ecologically.



Figure 6. Proposed green barrier

On the northern side of the site, where there was previously an informal stone alley, the concrete and compacted surfaces were removed, and the soil was reconditioned to allow the creation of new vegetated areas (Figure 7).



Figure 7. Proposed new vegetated areas

The new modifications reorganize the space so that new distinct areas will appear, while

connected to the utilitarian function of the space, which will encourage children to dynamic play, movement, and socialization.

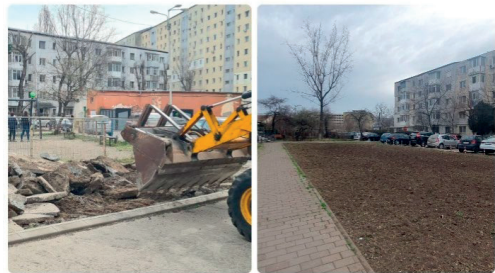


Figure 8. Soil rehabilitation measures

An important aspect of the planning was the inclusion of edible species. Previous research has concluded that their introduction into the landscape makes a significant contribution to improving knowledge about the environment, biodiversity, and responsibility. It also favors social inclusion and encourages the community to take part in management actions (Zlati et al, 2022). Such fruit species as *Corylus avellana*, *Crataegus monogyna*, and *Cornus mas*, proposed in the planning (Figure 9), can stimulate the population's interest in local food and the integration of species with potential, but little valued in their own consumption (Zlati et al, 2024).



Figure 9. Edible plant species in the landscape design

In addition to all the climatic benefits, this plant selection contributes to supporting pollinators, being also an organic way for children to learn about natural processes and about the creatures that can exist in the urban fauna. The integration of this plant typology is an approach also studied in the concept of peri-urban gardens that combine decorative and medicinal plant species

(Hangan et al., 2020) In the current project, this principle was applied by creating a small community garden with diverse plants selected for texture, shape, or scent. A visually attractive and ecologically functional composition was formed from species such as *Stachys lanata*, *Santolina chamaecyparissus*, *Rudbeckia hirta*, *Lavandula angustifolia*, and *Dianthus* spp.



Figure 10. Community garden section

The community garden element fits into larger strategies for reconfiguring degraded spaces. Similar methods have been used in other projects that aimed to revitalize public places by integrating a garden or areas that contribute to increasing the standard of living (Paşcu et al., 2019). In this case, similar principles are applied by incorporating landscape elements of plant infrastructure. The proposed intervention includes the use of the *Salix alba* species to create a natural fencing structure (Figure 11) and elements such as pergolas (Figure 12) or play structures (Figure 13).



Figure 11. Natural boundaries from *Salix* species

These structures fit into the general environment, not acting as rigid built boundaries.

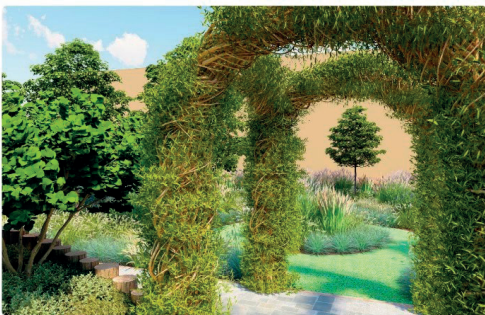


Figure 12. Living plant structures

Regarding the materials used – wood is the main one, used for the equipment proposed on the site as well as for benches or other relaxation spaces (Figure 13).



Figure 13. Wood as the main design material

It was chosen for its ecological properties, its ability to harmonize with the natural setting, and the tactile sensory experiences it offers.

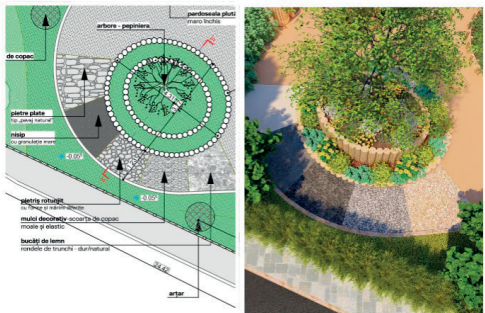


Figure 14. The sensory path

In addition to these wooden structures, it is proposed to arrange a small sensory trail designed to stimulate the tactile perception of children (Figure 14).

Materials such as – gravel of different sizes, sand, mulch will give children the opportunity to experience different textures, encouraging them to explore.

The first phase of the project began in the spring of 2025, through landscaping interventions that aimed to prepare the space and involve the community. These included planting tree species and installing furniture. The planting activities were organized by engaging several social actors, both community members and public figures, which aimed to develop a sense of shared responsibility for the future playground (Figure 15). Other initiatives and public workshops followed throughout the year, involving both adults and children from the community in the process of transforming the site into a playground that supports education and social interaction in the neighborhood.



Figure 15. Community engagement activities

It should be noted that the final implementation has not yet been completed. The second phase of the project, scheduled for 2026, will consist of the actual arrangement of the playground area, including equipment and related vegetation. Therefore, the study in question presents the initial steps of the project, the design and the conceptual solution.

Although the project and the redevelopment proposal have considerable potential, a number of limitations should be mentioned regarding the implementation process. Urban regeneration projects related to public space can often be delayed due to financing considerations, certain administrative procedures and regulations, or the lack of proper community involvement.

CONCLUSIONS

The study analyzed an urban playground, the rehabilitation of which was based on the integration of nature-based solutions and the community co-design approach.

The methods and actions proposed for implementation aimed to improve the challenges found on the site by introducing natural materials, appropriate vegetation, and edible species, transforming the space into an inviting one for users and offering multiple functions.

A significant component was the involvement of the community from the early consultation phases, thereby promoting a sense of belonging to the space and the responsibility assumed for its management.

The project presents opportunities to be replicated in other neighborhoods of the city, with a similar residential fabric and comparable challenges. In other neighborhoods, there are also aging, unkempt, and worn-out playground structures with limited green spaces and non-existent comfort.

Focusing restoration efforts on these spaces intended for children can significantly contribute to the development of more resilient and child-friendly cities.

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HIGH TEMPERATURE TOLERANCE IN FRAGRANT ROSE CULTIVARS

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Abstract

The rose, the most appreciated species in the world's floricultural industry, has become a prosperous business for those who cultivate and exploit this plant in our country, being widely used by landscapers due to its versatility and diversity of shapes, colors, and fragrance. This paper investigated the behavior and tolerance at high temperatures of nine cultivars of fragrant roses ('Acapella', 'Caprice de Meilland', 'Cristiana', 'Fisherman's Friend', 'Nobilia', 'Pasiune în mov', 'Romstar', 'Sandra' and 'Snow Owl'), existing in the UASVM - HRS Cluj-Napoca field collection, in the context of climate change. Biometric measurements highlight, in addition to the genetic characteristics of the cultivars, the significant negative influence of temperatures above 30°C, which affect the vegetative tips by 37% in the 'Fisherman's Friend' cultivar compared with the other cultivars. Chlorophyll biosynthesis occurs optimally at 26°C, while at temperatures below 16°C and above 32°C the photosynthesis process is affected. Among the alcoholic extracts, determined at 24 h from rose petals, the 'Fisherman's Friend' cultivar presents the most intense fragrance and the highest degree of persistence, followed by 'Cristiana'. As a result, in scented roses, high summer temperatures reduce the persistence of the fragrance while also affecting the overall health of the flowers.

Key words: morpho-biometrical measurements, chlorophyll, alcoholic extracts, rose scent.

INTRODUCTION

Roses were among the first ornamental plants used to decorate the environment. Their cultivation dates back millennia, with the earliest records appearing around 500 BC in Mediterranean countries, Persia, and China. In Romania, rose cultivation is a distant past; the plants were grown in gardens for their ornamental value by the Dacians (Wagner, 2002; Toma, 2009). Rose plants belong to the family Rosaceae, genus *Rosa*, which comprises more than 200 species and over 30,000 cultivars that continue to enhance and decorate lives worldwide (Salcă Roman et al., 2024).

The major rose-producing countries are the Netherlands, Colombia, Kenya, Israel, Italy, the United States, and Japan (Desta et al., 2022). In recent years, approximately 20 edible rose cultivars have been exploited in China. *Rosa* 'Crimson Glory' is one of the most important edible rose cultivars and is often referred to as

“the king of edible flowers” (Qiu et al., 2022; Xie et al., 2023). Roses are widely cultivated as cut flowers and hold significant economic value as sources of natural fragrance and flavoring (Xie et al., 2023).

Today in Romania, beyond their aesthetic importance, roses are also valued for their ecological, nutritional, therapeutic, and cosmetic uses, making rose cultivation a prosperous business for growers and processors (Chelariu et al., 2017; Șoltuzu, 2019).

The rose also carries numerous symbolic and spiritual connotations, frequently representing love, beauty, harmony, and elevated emotions (Gudin, 2000).

Recent studies have highlighted the potential of roses in functional foods containing biologically active compounds that may enhance human health or reduce the risk of disease, as well as in nutraceuticals with medicinal effects (Smulders et al., 2011). The best-documented effect of rosehips is their beneficial influence on

osteoarthritis; more recent research suggests that this effect may also be attributed to the fatty acids present in the seeds- α -linolenic and linoleic acids (Jäger et al., 2008). There is also evidence that rosehip extracts, combined with small amounts of blueberry, blackberry, and grapevine extracts, may help reduce the risk of cardiovascular diseases by lowering levels of inflammatory markers associated with early myocardial infarction (Kornman et al., 2007). Landscapers widely use roses because of their versatility, diversity of shapes and colors, fragrance, and especially their resistance to harsh environmental conditions and their adaptability to recent climate changes (Iliescu, 1998; Iuroaea et al., 2020).

Rose (*Rosa* L.) plants are among the most important ornamental flowers, used as cut flowers. They have been cultivated for centuries for their flowers and fragrance and serve as valuable sources of essential oil (Shalit et al., 2023; Rocchia et al., 2019).

The fragrance of roses has a long and rich history. Rose fragrance and rose oil were used throughout the Middle Ages for medicinal purposes and to mask unpleasant odors. In the 12th and 13th centuries, Crusaders brought Damask roses back from the Middle East because of their exceptional fragrance, and perfume was made from the petals. Numerous species and thousands of varieties possess fragrance; therefore, for growers, the selection of fragrant varieties that also tolerate high temperatures has considerable economic value (Salcă Roman et al., 2024).

Over time, many researchers have worked to develop fragrant varieties (Wagner, 2010; Salcă Roman et al., 2024). Many factors influence rose fragrance, including soil pH, water availability, humidity, time of day, season, and geographical origin (Elgar, 2024 –<https://rose.org/the-fragrance-of-roses-past-and-present/>).

Damask roses are renowned for their strong fragrance and are considered to have originated in Persia (present-day Iran). The most commercially significant Damask rose is the 30-petaled *R. damascena* ‘Trigintipetala’, which is cultivated for rose oil production. This oil is used by the perfumery industry in Bulgaria, Turkey, Iran, India, China, and North Africa (Smulders et al. 2011). Bulgaria has maintained its position as the world’s largest producer of

high-quality rose oil, accounting for approximately 70–75% of global production, with annual output ranging between 2.5 and 4.5 tonnes (2,500–4,500 kg). In 2025, Bulgarian rose oil production is expected to decline by 20–30% compared with previous years due to adverse weather conditions, including a cold May, frost, and hail (Bonchev, 2025). Bulgarian rose oil is exported to more than 30 countries, including the United States, China, Japan, South Korea, France, and the United Kingdom (<https://bnrnews.bg/en/post/114893/rose-oil-production-up-by-10-in-2024>).

Climatic factors play a critical role in the production of quality roses, influencing not only growth and yield but also flower quality. Among these factors, light, temperature, and relative humidity directly affect plant growth (Szekely-Varga, 2020). Light intensity is the most important factor influencing the growth and flowering of rose plants (Desta et al., 2022; Wagner, 2002). The timing of flowering flushes is influenced by both environmental conditions and plant health (Shinwari et al., 2003; Wagner, 2002; Wagner, 2010). The ideal temperature range for rose production is 20–25°C during the day and 13–16°C at night, combined with at least 8 hours of sunlight and relative humidity below 75% (Cui et al., 2022). Below 15°C, roses can still be grown, but the time between flowering flushes becomes longer, and bullheads (deformed, flat-topped, unmarketable buds caused by temperatures below 5°C) may form. At temperatures above 30°C, flowers of poor quality, often with fewer petals (Martin, 1980) are produced. Temperature directly affects key physiological processes such as photosynthesis, shoot formation, and plant growth. Climate change affects roses through elevated temperatures and greater thermal instability, often causing premature flowering (sometimes by as much as one month), heat stress, and increased water requirements. Large day/night temperature fluctuations (greater than 10°C) and early springs followed by late frosts disrupt normal plant development. High temperatures above 30°C (86°F) induce heat stress in roses, resulting in scorched leaves, wilted foliage, smaller or deformed blooms with fewer petals, reduced flowering, and, in severe cases, premature dormancy. Prolonged heat disrupts photosynthesis, degrades chlorophyll, and

causes cellular damage, leading to accelerated bloom cycles but overall poorer plant quality (Hatfield & Prueger, 2015).

High temperatures also favor the proliferation of pests and diseases. According to a study by the Research and Development Institute for Floriculture in Bucharest, the incidence of fungal diseases in urban-grown roses has increased by more than 17% over the last 10 years (www.icdf.ro). In Buzău County, during the summer of 2022, a collection of over 300 rose varieties lost 20% of its plants due to a prolonged heat wave followed by torrential rains (https://www.pasiuneaplantelor.ro/impactul-schimarilor-climatice-asupra-trandafirilor-prevenire-si-solutii-pentru-gradinari/).

Dobrotă (2013) noted that high temperatures also cause drying of plant parts containing secretory tissues-particularly the petals-due to intensified water evaporation.

In the context of global warming, the present research focused on identifying the effects of high temperatures on the photosynthetic process that supports plant growth and, indirectly, the synthesis of secondary metabolites responsible for the fragrance of rose petals.

The study also aimed to select and introduce rose cultivars specially adapted to high temperatures and to diseases associated with the changing climate.

MATERIALS AND METHODS

The research was conducted in the experimental field of the rose breeding laboratory and in the biochemistry laboratory at the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca – Horticultural Research Station Cluj-Napoca (USAMV-SCH Cluj-Napoca). The rose collection contains over 350 varieties from different groups. This genetic resource was developed over more than 70 years of research, with particular emphasis on the selection of parents for winter frost resistance and black spot tolerance (Salcă Roman et al., 2024).

The biological material consisted of nine rose cultivars: ‘Acapella’, ‘Caprice de Meilland’, ‘Cristiana’, ‘Fisherman’s Friend’, ‘Nobilia’, ‘Pasiune în mov’, ‘Romstar’, ‘Sandra’, and ‘Snow Owl’ (Figure 1).

These cultivars were selected considering their fragrance intensity, petal color, and potential use

in the cosmetics industry. According to the literature, all selected varieties are assumed to contain valuable biologically active compounds. Previous studies have shown a direct relationship between petal color intensity and fragrance intensity. Although the cultivars ‘Cristiana’ and ‘Snow Owl’ do not fully meet the color-intensity criteria, they were included because of the exceptional intensity and uniqueness of their fragrance.

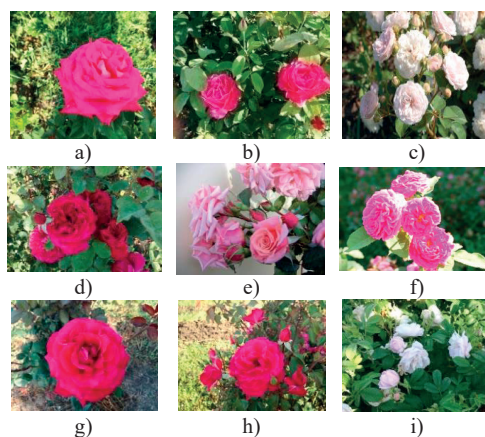


Figure 1. Rose cultivars: a) ‘Acapella’; b) ‘Caprice de Meilland’; c) ‘Cristiana’; d) ‘Fisherman’s Friend’; e) ‘Nobilia’; f) ‘Pasiune în mov’; g) ‘Romstar’; h) ‘Sandra’; i) ‘Snow Owl’

The purpose of research on the impact of high temperatures on roses (genus *Rosa*) is to understand how heat stress affects plant physiology, with a focus on the photosynthesis process and, consequently, on the biosynthesis of secondary metabolites responsible for flower fragrance.

The main objective was to establish the correlation between temperature and the synthesis of chlorophyll pigments and the development of photosynthesis processes in plants. The second objective was to obtain extracts from rose petals of selected fragrant varieties, extracts that can subsequently be tested for antibacterial and antifungal activity. The experiment was a multifactorial one; the independent variable was the temperature with three levels (16°C, 26°C, and 32°C) and the dependent variables were represented by biometric growth measurements, chlorophyll index, chlorophyll fluorescence, and the dry matter of leaves and petals.

Morphometric measurements of rose plant growth were recorded monthly from June to August 2022. All observations were conducted in the morning between 7:00 and 7:30 AM. The data presented represent the mean values derived from three consecutive replicates.

Chlorophyll content was estimated in situ and non-destructively using a CCM-200 chlorophyll content meter (Opti-Sciences). Measurements were made on the terminal leaflet of the third fully expanded leaf, from the tip of the woody branches. Three consecutive replicates were performed for each plant at different temperatures. Chlorophyll fluorescence was measured using a modulated fluorometer (OS1-FL, Opti-Sciences). The terminal leaflet of the third leaf from the tip of the woody branches was selected for analysis. Measurements were taken in series of three consecutive replicates per plant at different temperatures.

Dry matter content in leaves and petals was determined using fresh material manually harvested. The dry matter percentage was calculated as follows:

$$\text{Dry matter (\%)} = \frac{m_{\text{dry}}}{m_{\text{fresh}}} \times 100$$

The petals were harvested in July, during maximum temperatures of 26°C, as this is the time when photosynthesis is optimal for these rose cultivars.

For the preparation of the extracts, the flowers were harvested manually in July at a temperature of 23°C. The petals from the nine cultivars were separated from the other flower components, chopped, and weighed. A total of 200 g of petals was used for the aqueous extract (using an original method). For the alcoholic extract, petals from four cultivars ('Cristiana', 'Fisherman's Friend', 'Romstar', and 'Snow Owl') were used, with a total petal weight of 40 g. For the oily extracts from rose petals, a mixture of petals from the same four cultivars was used, with a total petal weight of 8 g. In all variants, the chopped petals were placed in 2000 ml Erlenmeyer flasks. Over the petals, the following were added: 1500 mL of distilled water for the aqueous extract, 80 mL of food-grade 96% ethyl alcohol (brand Croisiere) for the alcoholic extract, and for the oily extracts - in the first flask: linseed oil (Nature's Sense); in the second flask: cold-pressed olive oil (*Hypericum* Plant); in the third flask: jojoba oil (*Hypericum* Plant); and in the fourth flask:

coconut oil (Steaua Divina). The mixture of petals and water was placed in the refrigerator at +7°C. After seven days, the mixture was removed from the refrigerator and filtered into 100 mL Erlenmeyer flasks in fractions, which were sealed with parafilm and stored in the refrigerator at +7°C. The alcoholic compositions were kept at +24°C for maceration over 14 days, after which they were filtered and placed in Erlenmeyer flasks, covered with parafilm, and stored in the refrigerator at +7°C.

The containers with petals and oily extracts were completely covered with aluminum foil and kept at +24°C for 14 days to allow maceration. Afterward, they were ground in a mortar and pestle and then filtered first through funnels lined with cotton wool and a second time using filter paper. The oil extracts were subsequently placed in Erlenmeyer flasks, completely covered with aluminum foil, and stored in the refrigerator at +7°C. Using the volumetric values of the distillation fractions, the distillation yield was calculated with the following formula:

Yield (%) = (a/b) × 100 where:

a - is the total volume of distillate obtained;

b - is the initial volume of the filtrate.

RESULTS AND DISCUSSIONS

The results obtained from morpho-biometric growth measurements of rose cultivars at different temperatures are presented in Table 1. Analyzing Table 1, it can be observed that at 16°C, the cultivar showing the highest mean value of the biometric measurements is 'Caprice de Meilland' (165 cm), while the cultivars with the lowest value of 100 cm are 'Fisherman's Friend' and 'Romstar'.

Table 1. Morpho-biometric Growth Response of Rose Cultivars to Different Temperature Regimes

Cultivar	Plant height (cm)		
	at 16°C	at 26°C	at 32°C
Acapella	120	130	128
Caprice de Meilland	165	180	179
Cristiana	115	130	130
Fisherman's Friend	100	110	109
Nobilia	125	130	130
Pasiune in mov	120	120	120
Romstar	100	120	119
Sandra	135	140	138
Snow Owl	120	125	125

At 26°C and 32°C, the cultivar that continues to show the highest mean value of the biometric measurements is 'Caprice de Meilland', while the one with the lowest value is 'Fisherman's Friend'. The month of June was characterized by relatively low temperatures for that time of the year and abundant precipitation. August, however, was characterized by constant temperatures above 32°C and low humidity. As a result, the vegetative tips of the plants were affected, and their average height was lower than in the previous period at 26°C. Sensitivity to temperature manifests differently among cultivars, with the most sensitive ones being 'Fisherman's Friend', which suffered the most at 32°C, recording a 37% destruction of vegetative tips compared with the other cultivars.

Maximum flower yield, stem length, and flower quality have been reported in plants growing at 23.9°C day temperature (Holcomb & Tsinaraki, 1987). Studies conducted by Iliescu (1998) and Wagner (2010) show that, when it comes to ambient temperature, the behavior of roses varies; only a few cultivars can survive very low temperatures during winters. Regarding the tolerance of roses to positive temperatures, the upper limit at which vital functions can still occur under normal conditions is around 35°C. Above this threshold, the plant suffers because the losses caused by respiration are significantly higher than the gains from assimilation. Photosynthesis takes place with the help of energy provided by solar radiation and is conditioned by the presence of chlorophyll (Burzo et al., 1999; Dobrotă, 2010; Dobrotă & Roba, 2012). Photosensitive pigments are organic biomolecules specialized in receiving solar radiation (Burzo et al., 1999; Busuioc & Bălan, 2003; Dobrotă, 2010).

Light, temperature, oxygen, and minerals are environmental factors that influence the biosynthesis of pigments (Busuioc & Bălan, 2003; Dobrotă, 2010). The process of photosynthesis can occur in a wide temperature range, from -6°C to +47°C. For mesothermic plants, the optimal temperature for photosynthesis - that is, the temperature at which photosynthesis proceeds with the highest intensity - is between 20°C and 30°C (Burzo et al., 1999; Dobrotă, 2010). The results obtained from measuring the chlorophyll index at different temperatures are presented in Table 2.

Table 2. Effect of Temperature on Chlorophyll Index Measurements

Cultivar	The chlorophyll index		
	at 16°C	at 26°C	at 32°C
Acapella	37.03 ± 2.66	42.40 ± 5.22	10.10 ± 1.08
Caprice de Meilland	31.73 ± 2.05	83.30 ± 4.11	8.80 ± 0.46
Cristiana	23.90 ± 3.99	43.90 ± 3.22	11.37 ± 4.24
Fisherman's Friend	24.60 ± 1.04	59.20 ± 1.81	11.77 ± 3.24
Nobilia	20.17 ± 3.38	66.03 ± 5.83	13.33 ± 2.23
Pasiune în mov	18.40 ± 3.22	62.10 ± 2.79	5.53 ± 1.33
Romstar	53.60 ± 3.75	37.00 ± 4.87	14.47 ± 0.75
Sandra	40.47 ± 2.54	52.90 ± 2.00	11.33 ± 2.06
Snow Owl	26.57 ± 4.75	18.93 ± 1.50	8.97 ± 0.95

From the analysis of the data in Table 2, it can be observed that at 16°C, the 'Romstar' cultivar shows the highest chlorophyll index value (53.60), while the 'Pasiune în mov' cultivar shows the lowest chlorophyll index value (18.40). The percentage differences between the 'Romstar' cultivar and the cultivars 'Acapella', 'Caprice de Meilland', 'Cristiana', 'Fisherman's Friend', 'Nobilia', 'Pasiune în mov', 'Sandra', and 'Snow Owl' are 30.91%, 40.80%, 55.41%, 54.10%, 62.38%, 65.67%, 24.50%, and 50.44%, respectively. The largest difference is recorded compared to the 'Pasiune în mov' cultivar, with a difference of 65.67%.

At 26°C, the 'Caprice de Meilland' cultivar shows the highest chlorophyll index value (83.30), while the Snow Owl cultivar shows the lowest value (18.93). The percentage differences between the 'Caprice de Meilland' cultivar and the cultivars 'Acapella', 'Cristiana', 'Fisherman's Friend', 'Nobilia', 'Pasiune în mov', 'Romstar', 'Sandra', and 'Snow Owl' are 49.10%, 47.30%, 28.93%, 20.73%, 25.45%, 55.58%, 36.49%, and 77.27%, respectively. The largest difference is recorded compared to the 'Snow Owl' cultivar, with a difference of 77.27%.

Figure 2 highlights the differences between the chlorophyll indices of different cultivars at 26°C, the best temperature for the good development of the rose cultivars studied. Studies conducted by Shin et al. (2001) led to similar results, stating that the ideal temperature for rose production is 20-25°C during the day and 13-16°C at night, along with 8 hours of sunlight and <75% relative humidity.

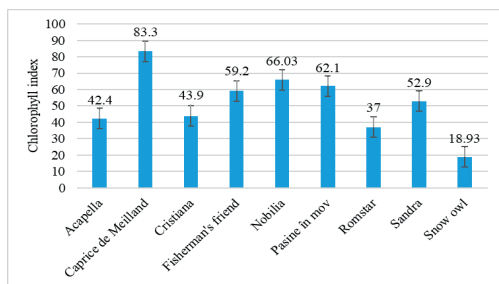


Figure 2. Chlorophyll index for rose cultivars at 26°C

At 32°C, the 'Romstar' cultivar shows the highest chlorophyll index value (14.47), while the 'Pasiune în mov' cultivar shows the lowest value (5.53). The percentage differences between the 'Romstar' cultivar and the cultivars 'Acapella', 'Caprice de Meilland', 'Cristiana', 'Fisherman's Friend', 'Nobilis', 'Pasiune în mov', 'Sandra', and 'Snow Owl' are 30.15%, 39.15%, 21.39%, 18.63%, 7.79%, 61.73%, 21.61%, and 37.99%, respectively. The largest difference is recorded compared to the 'Pasiune în mov' cultivar, with a difference of 61.73%.

Chlorophyll biosynthesis is affected by temperatures above 30°C and proceeds optimally between 16°C and 25°C. For low chlorophyll content values, there is a direct proportionality between this parameter and the progress of photosynthesis. Above average values, chlorophyll pigments accumulate in excess, and there is no longer a direct proportional relationship between them and photosynthesis. Below 15°C, roses can be grown, but the interval between flushes becomes longer, and bullheads (a physiological problem in some roses caused by low temperatures below 5°C, resulting in flat-topped, unmarketable buds instead of pointed tips) are produced.

At higher temperatures above 30°C, poor-quality flowers with fewer petals are produced (Martin, 1980; Lerner & Dana, 2003).

Photosynthesis is among the plant cell functions that is highly sensitive to high-temperature stress and is often inhibited before other cell functions are impaired (Sonal et al., 2014). Photosynthetic pigments, the two photosystems, the electron transport system, and CO₂ reduction pathways are a few important components of photosynthesis, and damage to any of them is enough to inhibit the overall photosynthetic mechanism of a plant (Alison et al., 1990).

The chlorophyll fluorescence measuring device displays four indices: Initial (basal) fluorescence (F_0) is given by the absorption of light by the pigment antennae. It indicates the degree of organization and operational efficiency of the pigments in the antenna complexes. The high value of this index indicates low efficiency of energy transfer to the pigments in the reaction center of photosystem II. Maximum fluorescence (F_m) indicates the maximum level of chlorophyll fluorescence when all plastoquinone molecules (a component of photosystem II) are saturated.

A low value of this index shows that the primary photochemical reactions within photosystem II are disturbed.

Variable fluorescence (F_v) is the fluorescence of dark-adapted leaves and represents the difference between maximum fluorescence and initial fluorescence. The ratio between variable fluorescence and maximum fluorescence reflects the maximum possible (potential) quantum yield of photosystem II, i.e., the extent to which the absorbed energy can be used by converting it into chemical energy. It usually ranges between 0.75 and 0.85, and a drop below the minimum value or an increase above the maximum value indicates inhibition of photochemical processes under the action of stress factors. The results obtained from measuring chlorophyll fluorescence at different temperatures are presented in Table 3.

Table 3. Chlorophyll fluorescence for rose cultivars at 16°C, 26°C and 32°C

Temp °C	Cv. Indices	Acapella	Caprice de Meilland	Cristiana	Fisherman's Friend	Nobilis	Pasiune în mov	Romstar	Sandra	Snow Owl
16	F_0	0.284	0.326	0.272	0.323	0.347	0.288	0.349	0.316	0.362
	F_m	0.689	1.032	0.940	0.940	1.171	0.803	1.073	0.857	1.124
	F_v	0.405	0.706	0.668	0.617	0.824	0.515	0.724	0.541	0.762
	F_v/F_m	0.588	0.684	0.711	0.656	0.704	0.641	0.675	0.631	0.678
26	F_0	0.425	0.349	0.309	0.434	0.434	0.473	0.396	0.365	0.376
	F_m	1.533	1.595	1.628	1.652	1.589	1.798	1.303	1.230	1.562
	F_v	1.108	1.246	1.319	1.218	1.155	1.325	0.907	0.865	1.186
	F_v/F_m	0.723	0.781	0.810	0.737	0.727	0.737	0.696	0.703	0.759

Temp °C	Cv. Indices	Acapella	Caprice de Meilland	Cristiana	Fisherman's Friend	Nobilia	Pasiune în mov	Romstar	Sandra	Snow Owl
32	F₀	0.409	0.389	0.358	0.372	0.367	0.387	0.416	0.425	0.369
	F_m	1.451	1.669	1.532	1.708	1.646	1.573	1.509	1.628	1.677
	F_v	1.042	1.280	1.174	1.336	1.279	1.186	1.093	1.203	1.308
	F_v/F_m	0.718	0.767	0.766	0.782	0.777	0.754	0.724	0.739	0.780

FO -Initial (basal) fluorescence; Fm-Maximum fluorescence; FV -Variable fluorescence.

From the data in Table 3 at 16°C, it can be observed that the basal fluorescence (F₀) value is maximum in the 'Snow Owl' cultivar (0.362) and minimum in the 'Cristiana' cultivar (0.272). The highest value of maximum fluorescence (F_m) is shown by the 'Snow Owl' cultivar (1.124), while the lowest value is shown by the 'Acapella' cultivar (0.689). The difference between maximum fluorescence (F_m) and basal fluorescence (F₀), i.e., variable fluorescence (F_v), is maximum in the 'Nobilia' cultivar (0.824) and minimum in the 'Acapella' cultivar (0.405). Due to variations in the fluorescence indices among the different cultivars, the value of the ratio between variable fluorescence (F_v) and maximum fluorescence (F_m) is maximum in the 'Cristiana' cultivar (0.711) and minimum in the 'Acapella' cultivar (0.588). The fact that these values fall between 0.75 and 0.85 indicates that the environmental conditions are optimal for photosynthesis.

Data from Table 3, indicates that a temperature of 16°C is sub-optimal for photosynthesis in rose plants, as all F_v/F_m ratio values recorded for the different cultivars are below the minimum value of 0.75.

At 26°C (Table 3), it can be observed that the basal fluorescence (F₀) value is maximum in the 'Pasiune în mov' cultivar (0.473) and minimum in the 'Cristiana' cultivar (0.309). The highest value of maximum fluorescence (F_m) is shown by the 'Pasiune în mov' cultivar (1.798), while the lowest value is shown by the 'Sandra' cultivar (1.230). Variable fluorescence (F_v) is maximum in the 'Pasiune în mov' cultivar (1.325) and minimum in the 'Sandra' cultivar (0.865). Due to variations in the fluorescence indices among the different cultivars, the value of the ratio between variable fluorescence (F_v) and maximum fluorescence (F_m) is maximum in the 'Cristiana' cultivar (0.810) and minimum in the 'Romstar' cultivar (0.696). The fact that three cultivars fall within the 0.75-0.85 range according to Table 3 indicates that the environ-

mental conditions are optimal for photosynthesis, exceeding the minimum value of 0.75.

At 32°C, it can be observed that the basal fluorescence (F₀) value is maximum in the 'Sandra' cultivar (0.425) and minimum in the 'Cristiana' cultivar (0.358). The highest value of maximum fluorescence (F_m) is shown by the 'Fisherman's Friend' cultivar (1.708), while the lowest value is shown by the 'Acapella' cultivar (1.451). Variable fluorescence (F_v) is maximum in the 'Fisherman's Friend' cultivar (1.336) and minimum in the 'Acapella' cultivar (1.042). Due to variations in the fluorescence indices among the different cultivars, the value of the ratio between variable fluorescence (F_v) and maximum fluorescence (F_m) is maximum in the 'Fisherman's Friend' cultivar (0.782) and minimum in the 'Acapella' cultivar (0.718).

The fact that most cultivars fall within the 0.75-0.85 range indicates that the environmental conditions are optimal for photosynthesis in most cultivars, with only three F_v/F_m ratio values falling below the minimum of 0.75 (Table 3). A temperature of 32°C represents a supra-optimal value for the photosynthesis process in the rose cultivars studied.

Studies conducted by Grodzinski & Jiao (1998) and Chimonidou (2003) show that temperature has a direct impact on processes such as photosynthesis, shoot formation, and plant growth.

Higher temperatures affect the formation of small flowers with fewer and smaller petals. Lower temperatures also affect the formation of "bullhead" flowers, which have a flattened appearance. Moldovan et al. (2023) reported in *Pelargonium zonale* that significant differences were observed between different fertilization strategies in terms of chlorophyll content and quantified nitrogen content.

Following the determination of the water content in the leaves and petals, the data presented in Table 4 were obtained.

From the data presented in Table 4, it can be observed that the leaves of the 'Romstar' and 'Sandra' cultivars (0.64%) show the highest

percentage of dry matter and the lowest percentage of water content (99.36%). On the other hand, the 'Acapella' cultivar shows the lowest percentage of dry matter (0.55%) and the highest percentage of water content (99.45%). From the analysis of the data, it can be noted that there are no significant differences between the cultivars analyzed regarding this parameter.

Table 4.The amount of water in the leaves and petals

Cultivar	Dry matter in fresh leaves (%)	Water in fresh leaves (%)	Dry matter in fresh petals (%)	Water in fresh petals (%)
Acapella	0.55	99.45	0.86	99.14
Caprice de Meilland	0.57	99.43	0.84	99.16
Cristiana	0.61	99.39	0.83	99.17
Fisherman's Friend	0.62	99.38	0.88	99.12
Nobilia	0.63	99.37	0.84	99.16
Pasiune în mov	0.58	99.42	0.86	99.14
Romstar	0.64	99.36	0.90	99.10
Sandra	0.64	99.36	0.86	99.14
Snow Owl	0.58	99.42	0.85	99.15

Analyzing the data presented in Table4, it is noted that the petals of the 'Romstar' cultivar show the highest percentage of dry matter (0.90%) and the lowest percentage of water content (99.10%). On the other hand, the 'Cristiana' cultivar shows the lowest percentage of dry matter (0.83%) and the highest percentage of water content (99.17%). From the analysis of the data, it can be noted that there are no significant differences between the cultivars analyzed regarding this parameter. Studies conducted by Moldovan et al. (2023) on the geranium cultivar 'Tango Dark Red' led to results similar to those obtained by us, with mean fresh mass values in leaves of 80.50% and 92.70% in flowers. The distillation yields calculated for the aqueous extracts from rose petals are 98.2%. Following the qualitative tests regarding the intensity and persistence of the fragrance of the aqueous extracts, determined at 24 h, it was found that the distillate of the extract presents an intense and persistent fragrance. As a result of the qualitative tests regarding the intensity and persistence of the fragrance of the alcoholic extracts, determined at 24 h, it was found that they decrease in the following order, depending on the cultivar: 'Fisherman's Friend', 'Cristiana', 'Snow Owl', and 'Romstar'. Yeon & Kim (2020) showed the negative impact of

short-term heat stress on the synthesis of pigments or scent compounds. After performing the qualitative tests regarding the intensity and persistence of the fragrance of the oily extracts, determined at 24 h, it was found that they decrease in the following order, depending on the oil used: jojoba oil, coconut oil, olive oil, and linseed oil.

CONCLUSIONS

Studying the behavior and tolerance of fragrant rose cultivars to high temperatures, within the context of climate change, can contribute to optimizing productivity, improving the quality of aromatic compounds, and selecting heat-stress resistant genotypes. The optimal temperature for rose production is 20-25°C. High temperatures (over 30°C) negatively affect production, being directly involved in photosynthesis. Rose plant height differs for each cultivar, being a genetic characteristic, but is also shown to be significantly influenced by temperature; temperatures over 30°C affect the vegetative tips of the plants, with significant differences recorded compared to measurements made at lower temperatures. Temperatures over 30°C also affect chlorophyll biosynthesis, which occurs optimally between 16°C and 25°C. Under certain limits, there is a direct correlation between chlorophyll content and the intensity of photosynthesis. Regarding chlorophyll fluorescence, the optimal photosynthesis temperature for the studied cultivars is 26°C, while 16°C and 32°C are suboptimal and respectively supra-optimal temperatures. Among the distilled aqueous extracts, the alcoholic extract from the petals of the 'Fisherman's Friend' cultivar shows the most intense fragrance and the highest degree of persistence at 24 hours. Among the oil extracts, jojoba oil demonstrated the best ability to extract and the best persistence of the scent. The research sought to elucidate how heat stress influences plant physiology, focusing on the process of photosynthesis - which supports growth - and the resulting biosynthesis of secondary metabolites, such as those responsible for the fragrance of rose petals. The information obtained through our research can be useful to rose breeders, especially in regions where hot and humid conditions prevail.

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LAND USE AND LAND COVER CHANGES IN THE COLENTINA SUB-BASIN DURING 2000-2018 USING LAND COPERNICUS DATA

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Abstract

This study analyses land use and land cover dynamics in the Colentina Sub-Basin between 2000 and 2018 using high-resolution Land Copernicus data. The results highlight consistent increases in artificial surfaces, particularly continuous and discontinuous urban fabric, indicating accelerated urban expansion within the watershed. In parallel, agricultural areas recorded a significant decline, suggesting ongoing land conversion processes driven by suburbanisation and infrastructural development. The semi-natural areas remained relatively stable, while wetlands and inland water bodies showed minor fluctuations. These spatial transformations are relevant, as changes in land cover influence ecosystem services, microclimate regulation, surface runoff, and exposure to environmental risks. Understanding these patterns supports integrated planning and provides evidence-based insights for managing green infrastructure, ecological connectivity, and the overall environmental health of the sub-basin.

Key words: land use, land cover, landscape change, landscape dynamics, Colentina.

INTRODUCTION

Land use and land cover changes represent some of the most important indicators of landscape transformation, influencing ecosystem services, hydrological processes, biodiversity conservation, and spatial development patterns (Foley et al., 2005; Lambin & Geist, 2006). In rapidly urbanizing regions, the conversion of agricultural and natural land into built-up areas may generate significant environmental pressures and alter the ecological functionality of landscapes (EEA, 2016).

The Bucharest Metropolitan Area has experienced substantial urban growth during the last decades, particularly within peri-urban localities situated along major transportation corridors. These processes have contributed to the expansion of residential, commercial, and infrastructural areas, often at the expense of agricultural land and semi-natural habitats.

Given its strategic position within the metropolitan area and the presence of important aquatic and green infrastructure elements, the Colentina sub-basin represents a relevant case study for assessing long-term territorial transformations.

The Colentina River sub-basin is located in the south-eastern part of Romania, within the

Romanian Plain. This region is characterized by lowland relief, with low altitudes and gentle slopes that favour slow water flow and the natural or artificial development of linear water bodies. From an administrative perspective, the Colentina sub-basin extends across two main counties, Ilfov and Dâmbovița, as well as marginal areas of Giurgiu and Călărași, while also covering a significant portion of the territory of Bucharest Municipality.

Within the capital, the river passes through densely urbanized areas and has a direct impact on the urban structure, the green space systems, and the infrastructure for managing stormwater and wastewater.

The Colentina sub-basin is an integral part of the Argeș river basin, one of the most important basins on the southern slope of Romania, which ultimately drains into the Danube River.

Although Colentina is a lower-order tributary, its role is strategic, especially within the Bucharest Metropolitan Area, where it contributes significantly to flow regulation, stormwater collection, and the support of valuable aquatic ecosystems. Its connection to the Argeș hydrographic network is established through the Dâmbovița River, into which Colentina discharges near the locality of Bălăceanca, southeast of the capital.



Figure 1. Positioning of the Colentina sub-basin in relation to the main: a. administrative units (counties); b. natural units (major river basins) (Boundary data sources: ANAR, INS)

The objectives of this study are to quantify changes in land-cover categories during the study period, identify the principal spatial trends related to urban expansion, agricultural land conversion, and assess the implications of these transformations for territorial planning and sustainable land management alongside the Colentina sub-basin.

MATERIALS AND METHODS

This study is based on the analysis of land use and land cover dynamics within the Colentina sub-basin, using data provided by the Copernicus Land Monitoring Service (Land Copernicus).

Land use refers to the set of social, economic, and cultural activities that determine how a piece of land is utilized. This includes, for example, agricultural land, residential areas, industrial zones, transportation networks, or recreational spaces.

According to the Food and Agriculture Organization, land use refers to the activities and interventions through which people manage a specific type of land cover in order to produce, modify, or maintain it (FAO, 1997).

Land cover, on the other hand, refers to the observable physical and biological layer of the Earth's surface, such as forests, grasslands, water bodies, built-up areas, or bare land.

The classification used is based on the CORINE Land Cover (CLC) nomenclature, which adopts a three-level hierarchical structure to describe land use and land cover across Europe.

- Level I includes the most general categories, five in total, representing the major land-use types;
- Level II provides intermediate thematic detail, structured into 15 classes;
- Level III is the most specific level, comprising 44 distinct classes that allow for an in-depth analysis of territorial characteristics.

The Coordination of Information on the Environment (CORINE) inventory provides harmonized land-cover datasets for Europe and includes change layers for four reference periods beginning in 1990. For this study, the CLC datasets for 2000-2018 were downloaded free of charge from the European Environment Agency (EEA, 2019). At the time the study was conducted, the 2018 dataset represented the most recent validated CORINE Land Cover product available for the study area, while the corresponding 2024 dataset had not yet been released. Therefore, 2018 was selected as the final reference year for the analysis.

Spatial data processing and analysis were carried out in QGIS Desktop 3.24, where the datasets were imported, clipped to the boundaries of the Colentina sub-basin, and analysed to assess temporal land use and land cover dynamics. Spatial overlay operations, attribute-based queries, and area calculations were applied to quantify the extent of individual CLC classes and identify transitions between land-cover categories over time.

The resulting thematic maps were produced using QGIS, whereas statistical calculations, summary tables, and charts were prepared in Microsoft Excel to facilitate the analysis and interpretation of landscape transformation patterns throughout the study period.

RESULTS AND DISCUSSIONS

The dynamics of land use and land cover indicate the pace and direction of transformations within the Colentina sub-basin, driven by both anthropogenic pressures and natural or climatic factors. Urban expansion, agricultural restructuring, and shifts in natural and semi-natural areas are clearly reflected in the temporal evolution of the CLC Level I categories. These spatial modifications show how the built environment has expanded, how agricultural land has diminished or changed in

function, and how green spaces have been reshaped under development pressure. Such patterns provide essential insights into ecosystem stress and the environmental risks associated with land-use change.

The interpretation of these trends contributes to a deeper understanding of regional planning challenges and the development of sustainable land-management policies. They also underline the need for integrated territorial management in rapidly urbanizing areas, where land-use decisions generate long-term ecological and socio-economic impacts.

Table 1. CLC Code Classification

CLC - I Code	CLC - II Code	CLC - III Code
1 - Artificial surfaces	11 - Urban fabric	111 - Continuous urban fabric
		112 - Discontinuous urban fabric
		121 - Industrial or commercial units
		122 - Road and rail networks and associated land
	12 - Industrial, commercial and transport units	124 - Airports
		133 - Construction sites
		141 - Green urban areas
		142 - Sport and leisure facilities
2 - Agricultural areas	21 - Arable land	211 - Non-irrigated arable land
	22 - Permanent crops	221 - Vineyards
		222 - Fruit trees and berry plantations
	23 - Pastures	231 - Pastures
	24 - Heterogeneous agricultural areas	242 - Complex cultivation patterns
		243 - Land principally occupied by agriculture, with significant areas of natural vegetation
3 - Forest and semi-natural areas	31 - Forests	311 - Broad-leaved forest
	32 - Scrub and/or herbaceous vegetation associations	321 - Natural grasslands
		324 - Transitional woodland-shrub
4 - Wetlands	41 - Inland wetlands	411 - Inland marshes
5 - Inland waters	51 - Inland waters	511 - Water courses
		512 - Water bodies

*Adapted from Bossard et al., 2000.

The analysis of land use and land cover dynamics according to the CLC Level I classification for the period 2000-2018 highlights significant transformations across the five major spatial categories. The most evident trend is the steady increase in artificial surfaces, which expanded from 10,854 hectares in 2000 to 13,469 hectares in 2018 (Table 2). This growth reflects accelerated urbanization and the continuous expansion of built-up areas, infrastructure, and anthropogenic functional spaces. The process is indicative of the pressure exerted by urban development on other land-use types, particularly on agricultural areas. Agricultural zones show a progressive decline throughout the entire period, decreasing from 20,770 hectares in 2000 to 17,752 hectares in 2018. This reduction may be associated, on one hand, with the conversion of agricultural land

into built-up areas and, on the other hand, with agricultural abandonment or the reconversion of land into other types of cover, such as natural vegetation or regenerating areas. A gradual loss of agricultural function is clearly visible, with direct implications for food security and local ecological balance.

The category “Forests and semi-natural areas” remained relatively stable, with a slight increase between 2000 and 2012, followed by stabilization in 2018. The surface area of this class fluctuated around 7,200-7,500 hectares, suggesting either natural regeneration in certain areas or the preservation of forest surfaces through conservation policies. Although the changes are not substantial, maintaining balance within this class is particularly important in the context of biodiversity conservation and the role of forests in mitigating climate change.

Although wetlands represent a small share of the total analysed area, they experienced a notable increase between 2000 and 2006, from 185 hectares to 284 hectares, reaching more than 308 hectares in 2018. This trend may be attributed to improved detection of these ecosystems through remote sensing. The maintenance of these surfaces at a relatively stable level between 2006 and 2018 indicates a positive stabilization of wetland ecosystems.

Inland waters recorded a slight increase, from 1,568 hectares in 2000 to 1,616 hectares in 2018. Although the variations are minor, they may reflect changes in the spatial delineation of water bodies, hydrotechnical interventions, or natural fluctuations in the area’s hydrological regime. Overall, a clear trend of agricultural land conversion into artificial surfaces can be observed (Figures 2 and 3).

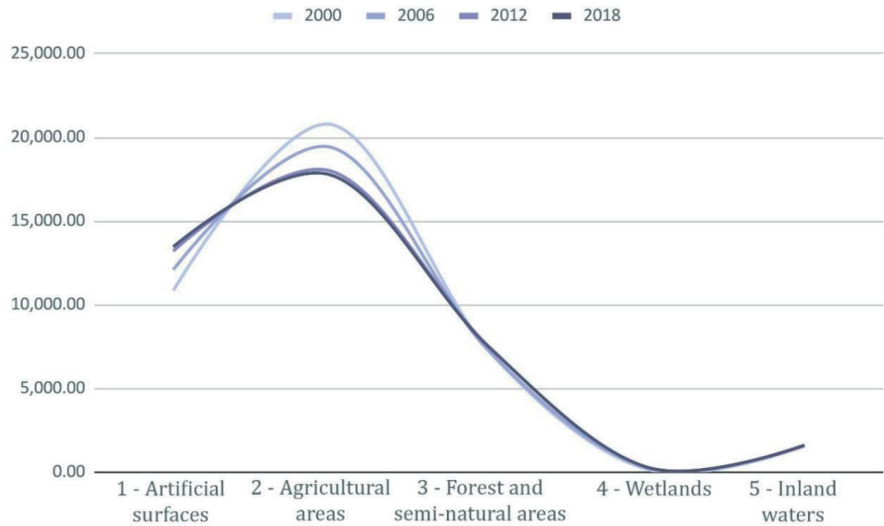
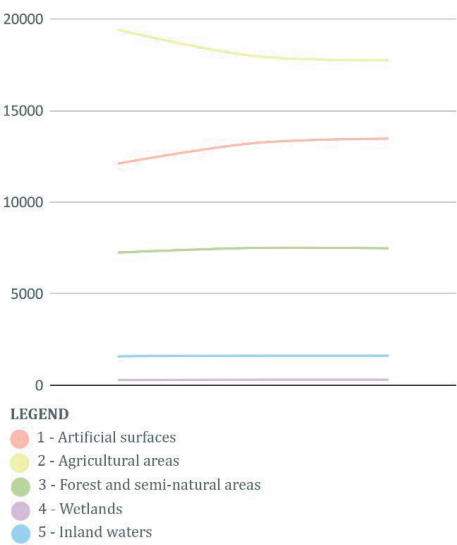


Figure 2. Evolution of the areas corresponding to the main CLC Level I categories during the period 2000-2018

Table 2. Areas resulting from the CLC Level I classification

CLC Code	Land-use category	Area in hectares (ha)/Year*			
		2000	2006	2012	2018
1	Artificial surfaces	10,854.06	12,101.55	13,218.77	13,469.18
2	Agricultural areas	20,770.74	19,410.29	17,984.46	17,752.52
3	Forest and semi-natural areas	7,248.74	7,249.04	7,499.95	7,481.46
4	Wetlands	185.70	284.71	308.39	308.39
5	Inland waters	1,568.72	1,582.40	1,616.47	1,616.47

*Areas in hectares resulting from the GIS analyses.

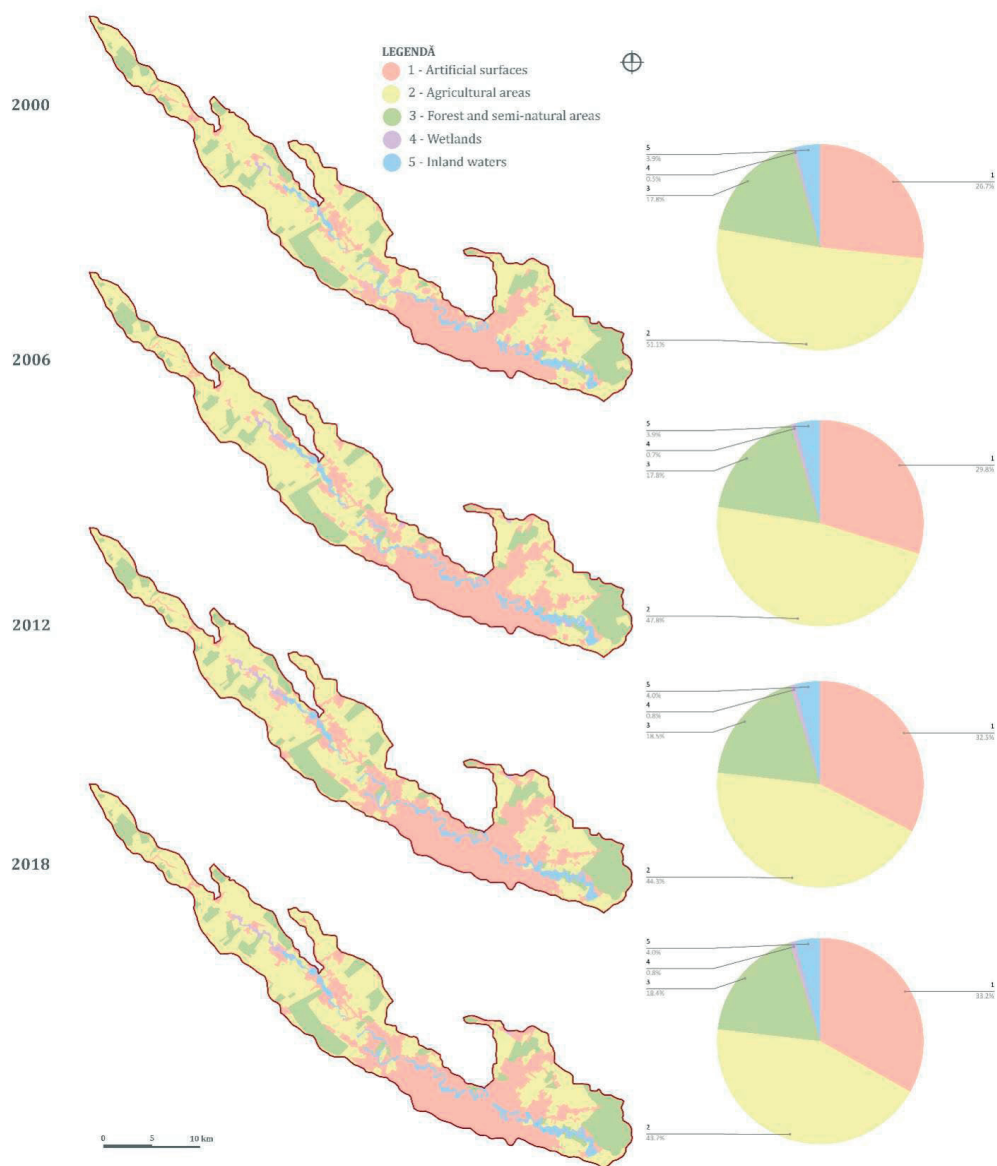


Figure 3. Land use according to CLC Level I codes for the years 2000-2018.
Data retrieved and interpreted from Land Copernicus

The period 2000-2018 is characterized by a clear process of territorial transformation (as shown in Table 3), in which agricultural land gradually loses its share in favour of artificial surfaces. The increase in continuous urban fabric and the expansion of construction sites reflects the pressure of urbanization, particularly in the vicinity of the Colentina River. At the same time, a gradual decrease in broad-leaved forests

is observed, accompanied by the emergence of areas undergoing ecological transition, indicating a structural modification of the vegetated landscape.

Thematic maps and associated pie charts support this spatial interpretation, providing a clear picture of the main land-use types and the changes that have occurred over the past two decades. These modifications reflect both socio-

economic processes, such as the expansion of residential and infrastructural areas, and spatial dynamics influenced by uneven urban development policies.

The reduction of agricultural land and the fragmentation of natural habitats may have consequences for the functionality of local ecosystems, biodiversity, and their capacity to adapt to climate change. Therefore, close monitoring of these trends becomes essential for informing sustainable territorial planning

measures and the conservation of natural resources.

The values in the table below confirm a progressive diversification of land-cover patterns, with increasingly complex interactions between urbanized areas, regenerating vegetation, and residual agricultural land, and they highlight the need for coordinated management strategies across the entire sub-basin.

Table 3. Areas resulting from the CLC Level III classification

CLC Code	Land-use category	Area in hectares (ha)/Year*			
		2000	2006	2012	2018
111	Continuous urban fabric	97.90	4,195.95	4,460.14	4,487.66
112	Discontinuous urban fabric	8,083.07	4,395.67	3,811.02	3,869.01
121	Industrial or commercial units	1,568.78	2,405.78	2,171.40	2,211.33
122	Road and rail networks and associated land	267.09	181.76	157.79	157.79
124	Airports	2.76	1.74	1.74	1.74
133	Construction sites		117.09	1,716.52	1,809.33
141	Green urban areas	622.82	511.01	704.59	704.59
142	Sport and leisure facilities	211.64	292.55	195.57	227.73
211	Non-irrigated arable land	18,318.42	16,910.71	12,996.69	13,181.13
221	Vineyards	34.48	34.48		
222	Fruit trees and berry plantations	606.80	808.34	231.32	231.32
231	Pastures	582.41	918.10	3,414.57	2,998.20
242	Complex cultivation patterns	1,164.41	484.18	1,113.51	1,113.50
243	Land principally occupied by agriculture, with significant areas of natural vegetation	64.22	254.48	228.37	228.37
311	Broad-leaved forest	7,196.40	6,910.06	6,587.67	6,304.97
321	Natural grasslands	52.34			
324	Transitional woodland-shrub		338.98	912.28	1,176.49
411	Inland marshes	185.70	284.71	308.39	308.39
511	Water courses	66.41	370.43	335.54	335.54
512	Water bodies	1,502.31	1,211.97	1,280.93	1,280.93

*Areas in hectares resulting from the GIS analyses.

The year **2000** (Figure 4) reflects a clear dominance of agricultural land, particularly non-irrigated arable land (code 211), which occupies more than 18,300 ha, representing approximately 45% of the analysed area. Discontinuous urban fabric (112) is the main form of artificial land use, exceeding 8,000 ha, while continuous urban fabric (111) is almost absent. Forested areas are significant, with broad-leaved forests (311) totalling over 7,100 ha. Wetland and aquatic areas (411, 511, 512) are present in a minor proportion.

This land-use structure reflects a predominantly rural spatial organization, with low levels of anthropogenic pressure and a still balanced presence of natural components.

The distribution of land-use types indicates limited landscape fragmentation, while the high share of agricultural land suggests an economy oriented toward primary activities. The small surface occupied by built-up areas and infrastructure points to an early stage of urban development, likely concentrated around transport nodes or existing traditional

settlements. The year 2000 can be considered a reference baseline for the initial state of the territory, prior to the onset of accelerated urbanization and the functional conversion processes observed in subsequent years.

By **2006** (Figure 5), a radical change can be observed in the occupation of the territory by artificial surfaces. Continuous urban fabric (111) shows explosive growth, reaching more than 4,100 ha, while discontinuous urban fabric (112) decreases significantly.

This pattern is mainly explained by the densification and consolidation of previously dispersed built-up areas, particularly within the Bucharest metropolitan fringe and the rapidly developing settlements of Ilfov County. The conversion of discontinuous urban fabric into continuous urban fabric reflects the expansion of residential developments, commercial areas, and supporting infrastructure, resulting in a more compact urban structure.

In addition, part of this change may be influenced by updates in the CORINE Land Cover classification methodology and improved interpretation of urban land-cover patterns between successive mapping campaigns.

Agricultural land loses more than 1,400 ha, particularly arable areas. These transformations signal the beginning of a new phase in the evolution of the territorial landscape, in which urban pressure becomes visible not only through the expansion of built-up surfaces but also through the progressive fragmentation of natural and agricultural areas. The emergence of class 133 (construction sites) serves as an indirect indicator of intensified functional conversion processes and changes in land use, especially near mobility corridors or highly accessible areas. At the same time, the appearance of transitional woodland-shrub (324) suggests an intermediate stage in the ecological reconfiguration of the territory.

This year marks a stage of imbalance between artificial development and the conservation of natural resources, with direct implications for the ecological and functional structure.

In **2012** (Figure 6), the trends already outlined in previous years continue: urban areas keep expanding, while agricultural surfaces continue

to decrease. A massive expansion of construction sites (133) is observed, exceeding 1,700 ha, a clear indicator of intensified real estate and infrastructural development.

The area of broad-leaved forests (311) decreases to below 6,600 ha, but an increase in class 324 (transitional woodland-shrub) is recorded, suggesting active vegetation regeneration processes.

This redistribution of land-cover categories reflects an intensification of spatial transformations, in which the expansion of built-up areas occurs simultaneously with reconfigurations in marginal or less productive lands. The increase in pastures (231) may indicate either a temporary functional reallocation of land removed from intensive agricultural use or the spontaneous development of vegetation in abandoned or less accessible areas. The decline in mature forests is partially offset by the emergence of regenerating surfaces (324), highlighting an ongoing ecological transition. Furthermore, the sustained development of built-up areas indicates a favourable context for continued urban expansion, while natural and agricultural spaces tend to become increasingly fragmented or subject to indirect pressures.

By **2018** (Figure 7), the urbanization process appears to continue at a steady yet more stabilized pace. Continuous urban fabric (111) reaches nearly 4,500 ha, while construction sites remain a significant component (over 1,800 ha). Arable land remains low compared to the year 2000, confirming the gradual conversion of these areas to other land uses. Broad-leaved forests (311) continue to decline slightly, while transitional woodland-shrub (324) reaches a historical maximum of over 1,100 ha.

The decline of non-irrigated arable land is primarily associated with the expansion of residential areas, industrial and commercial developments, transport infrastructure, and construction sites. These changes are particularly evident in the peri-urban areas surrounding Bucharest, where agricultural land has been progressively converted to urban functions as a consequence of suburbanization and increasing development pressure.

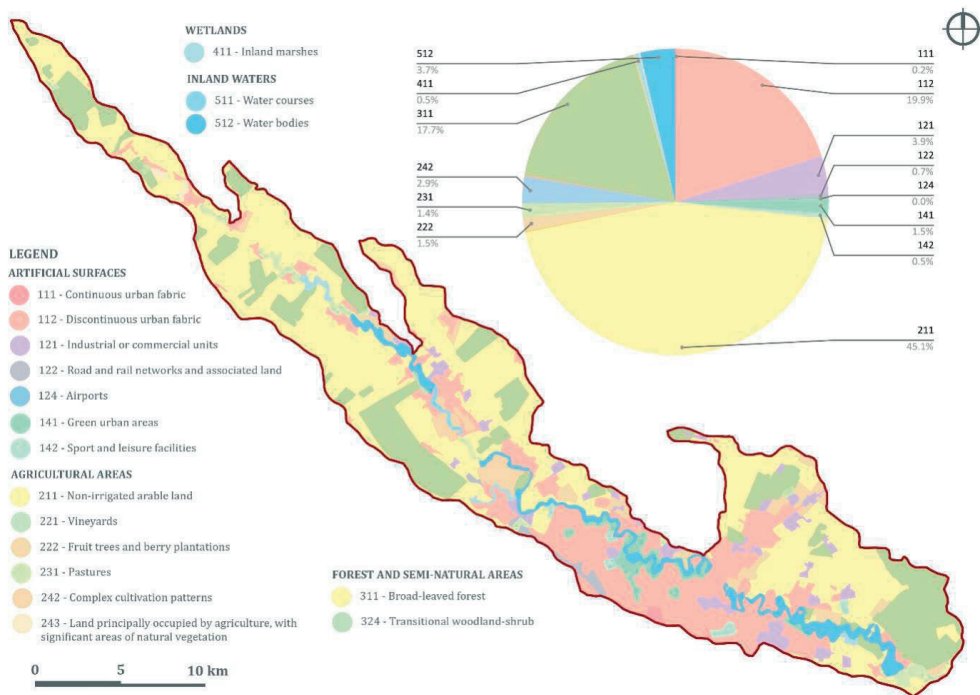


Figure 4. Land use - year 2000, according to the CLC Level III classification. Data retrieved and interpreted from Land Copernicus

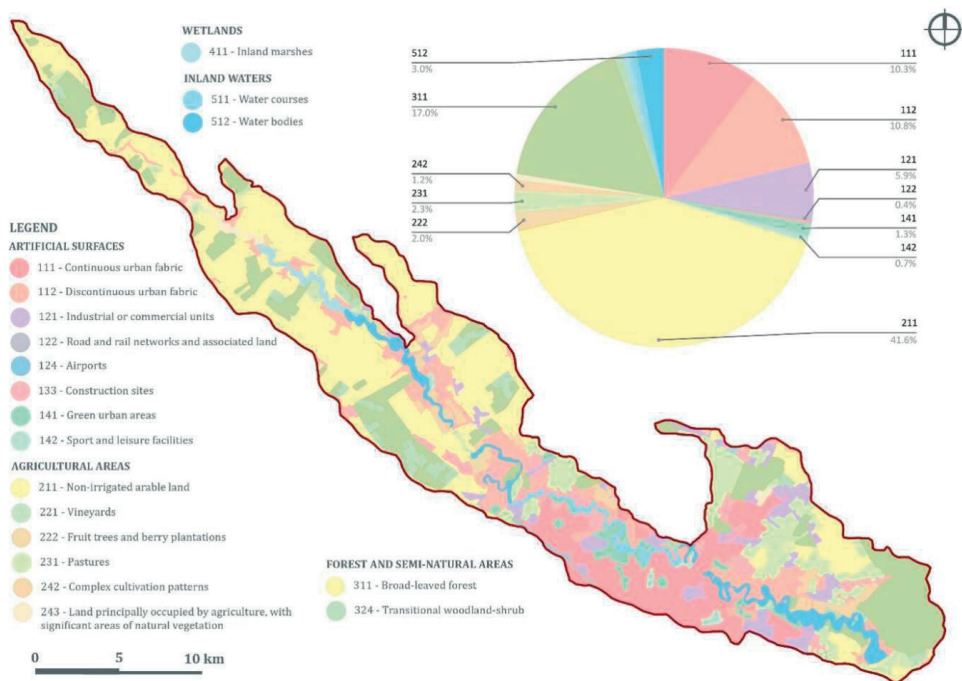


Figure 5. Land use - 2006, according to CLC Level III classification. Data were retrieved and interpreted from Copernicus Land Copernicus

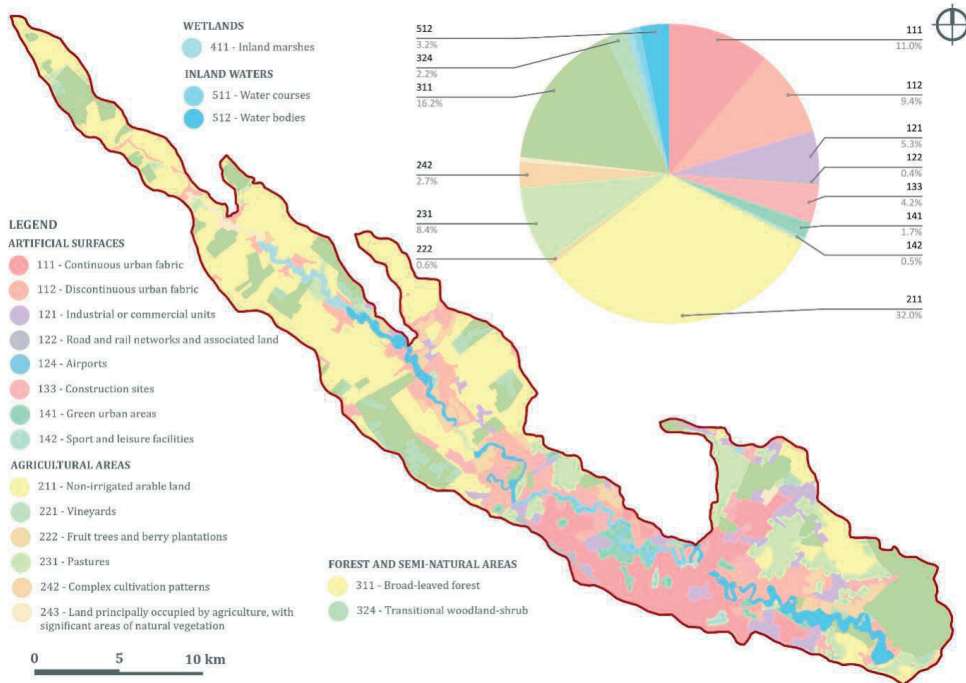


Figure 6. Land use - 2012, according to CLC Level III classification. Data retrieved and interpreted from Land Copernicus

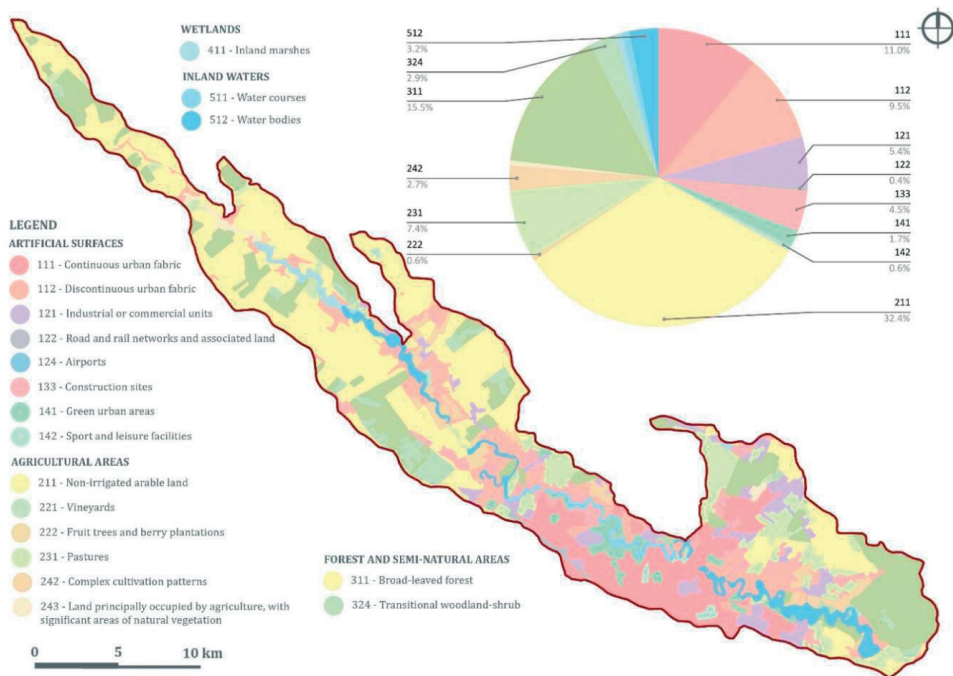


Figure 7. Land use - year 2018, according to CLC Level III classification. Data retrieved and interpreted from Land Copernicus

The persistence of construction sites indicates that urban expansion has not ceased but is in a phase of consolidation and localized extension. Likewise, the balance between the loss of mature forests and the emergence of transitional vegetation suggests a functional reconfiguration of ecosystems, possibly driven by planned interventions or uncontrolled natural processes. The year 2018 highlights a stage of maturation within the urbanization process, where transformations become more subtle but exert a significant cumulative impact on the landscape and on the territory's capacity to provide ecosystem services.

The percentage variation of land-cover categories between 2000 and 2018, presented in Table 4, illustrates the main territorial transformations within the study area.

The largest increases occur in continuous urban fabric and construction sites, indicating strong urban expansion. Growth in pastures and transitional woodland-shrub also reflects ecological regeneration or land abandonment. In contrast, notable decreases in discontinuous urban fabric and non-irrigated arable land suggest a continued decline in traditional agricultural uses.

Table 4. Percentage change for 2000-2018 in CLC Level III categories

CLC Code	Land-use category	Area in hectares (ha)/Year*		Percentage change (%)
		2000	2018	2000-2018
111	Continuous urban fabric	97.90	4,487.66	+4483.92%
112	Discontinuous urban fabric	8,083.07	3,869.01	-52.13%
121	Industrial or commercial units	1,568.78	2,211.33	+40.96%
122	Road and rail networks and associated land	267.09	157.79	-40.92%
124	Airports	2.76	1.74	-36.96%
133	Construction sites		1,809.33	n.a. **
141	Green urban areas	622.82	704.59	+13.13%
142	Sport and leisure facilities	211.64	227.73	+7.60%
211	Non-irrigated arable land	18,318.42	13,181.13	-28.04%
221	Vineyards	34.48		-100.00%
222	Fruit trees and berry plantations	606.80	231.32	-61.88%
231	Pastures	582.41	2,998.20	+414.79%
242	Complex cultivation patterns	1,164.41	1,113.50	-4.37%
243	Land principally occupied by agriculture, with significant areas of natural vegetation	64.22	228.37	+255.61%
311	Broad-leaved forest	7,196.40	6,304.97	-12.39%
321	Natural grasslands	52.34		-100.00%
324	Transitional woodland-shrub		1,176.49	n.a. **
411	Inland marshes	185.70	308.39	+66.07%
511	Water courses	66.41	335.54	+405.25%
512	Water bodies	1,502.31	1,280.93	-14.74%

*Areas in hectares resulting from the GIS analyses.

**Not applicable. Percentage change could not be calculated because the category was absent in the baseline year (2000).

CONCLUSIONS

The analysis of land use and land cover dynamics within the Colentina sub-basin for the period 2000-2018 reveals a significant transformation of the territorial structure. The most evident trend is the expansion of artificial surfaces, driven by rapid and persistent urbanization, which has reshaped the spatial

configuration of the area. At the same time, the continuous decline of non-irrigated arable land highlights the gradual reduction of traditional agricultural functions, largely replaced by residential, infrastructural, or transitional landscape uses.

Natural and semi-natural areas show a mixed evolution: while mature forests register a slight decrease, transitional woodland-shrub and

pastures increase considerably, indicating both ecological regeneration and possible land abandonment processes. These shifts contribute to a more fragmented landscape, marked by frequent transitions between built-up zones, regenerating vegetation, and residual agricultural parcels.

In summary, the changes observed during the 18-year interval underline the need for integrated territorial planning that balances urban development with the preservation of ecological functionality. Monitoring land-cover dynamics, especially in rapidly urbanizing regions, is essential for supporting sustainable land-management strategies and enhancing the resilience of ecosystems in the face of ongoing environmental and climatic pressures.

Beyond documenting the magnitude of land-use and land-cover changes, this study demonstrates the applicability of Copernicus Land Monitoring Service datasets and GIS-based analysis for assessing landscape dynamics at the sub-basin scale. The generated spatial database and quantitative indicators provide a replicable methodological framework that can support territorial planning, land-use monitoring, and environmental management in rapidly urbanizing peri-urban areas.

By identifying the main directions of landscape transformation, the results offer a scientific basis for future planning decisions aimed at limiting uncontrolled urban expansion and promoting sustainable land-use practices.

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IMPACT OF GIBB A3 ON GROWTH PERFORMANCE OF TWO *EUSTOMA GRANDIFLORUM* VARIETIES

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Abstract

Eustoma grandiflorum (Raf.) Shinn. has a significant role in the cultivation of ornamental plants, appearing as cut or sometimes potted ornamental plants. Commercial growers of ornamental plants incorporate plant growth regulators into their cultural practices. These regulators significantly affect both vegetative growth and flower yield in flowering crops. Notably, gibberellic acid is essential for improving flower production, quality, and yield in these crops. The aim of the study was to determine whether different concentrations of growth stimulators, notably GIBB A3, have a positive or negative effect on the production of cut flowers of two *Lisianthus* (*Eustoma grandiflorum* 'Croma lavender' and *E. grandiflorum* 'Rosita yellow'). 'Croma lavender' has thick, strong petals and characteristic extra-double flowers, and 'Rosita yellow' has double-flowered inflorescences, thick petals, and strong stems. GIBB A3 was applied to the plants as a foliar fertilizer. During the experiment, the following treatments were used: control (seedlings did not receive any treatment), 25, 50, 100 and 150 mg/L of GIBB A3. Each treatment was carried out in four replicates, with each replication containing 10 individual plants. The growth of the two varieties, including the length and number of shoots, stem diameter, bush diameter, number of flowers, and flower diameter, were measured and recorded during the experiment. Results indicated that the plants treated with 150 mg/L concentration of GIBB A3 reached the expected outcomes. Further more, when comparing the control to the treated plants, statistically significant differences were determined for all the measured parameters. Also, significant differences were observed between the two studied *Lisianthus* varieties. From the results, it could be concluded that the GIBB A3 treatments positively influenced the flowering and development of the two *Lisianthus* varieties.

Key words: cut flowers, growth hormones, GIBB A3, *Lisianthus*.

INTRODUCTION

One of the most significant segments of the horticulture industry is undoubtedly floriculture, which produces a wide variety of products (Traversari et al., 2021), such as bedding plants, garden plants, indoor and outdoor plants, and cut flowers and greens (Wani et al., 2018). The floriculture industry is one of the most inventive segments of the plant cultivation industry, and its inventions are frequently applied to other horticultural fields (Volckaert et al., 2010).

The *Eustoma grandiflorum* (Raf.) Shinn. is generally a new floriculture crop on the global market, which quickly ranked among the top ten cut flowers due to its excellent post-harvest life (Harbaugh, 2007; Castillo-González et al., 2018), which generally can range between 12-25 days (Jamal Uddin et al., 2013), other authors are mentioning approximatively 15 days when stems are properly handled (Chuang & Chang,

2013). Its origin is from North America, but it was bred in Japan, and since then it has become more and more popular worldwide (Pow). According to Stock et al. (2022) the *Lisianthus* blooms from June through October with multiple branching stems. Worldwide, 48 colors of flower variety can be found (white, pink, violet, white pink, lavender, etc.), moreover there can be found both single and double flower (Uddin et al., 2015; Fatima et al., 2022). Growth, yield, and flowering can be enhanced by the use of growth hormones; however, in the case of the quality of flower production, the concentration of hormones and the time of application are of utmost importance, or else it will lead to an undesirable effect (Javid et al., 2022). However, it is also stated that growth hormones can have a positive influence on the quality and production of cut flowers (Rademacher, 2015). Campos-Rivero et al. (2017), reported that plant hormones such as

GA, abscisic acid, jasmonic acid, and auxins have crucial effects on flowering. Plant hormones are involved in the regulation of flower senescence and could also have a noticeable influence on the vase life of cut flowers (Sui et al., 2015). It is also reported that GA₃ has a delaying effect on wilting and is associated with senescence (Eason, 2002). Application of GA₃ also promote branch growth, flower bud development, and improve flower quality (Guan et al., 2019). In another study, it was reported that the application of plant hormones on *Gladiolus* plants, flowering parameters and also vase life were highly influenced (Orpa et al., 2023). GA₃ is also used as a postharvest treatment to increase the vase life of alstroemeria (Yeat et al., 2012). Also, it was shown that salicylic acid at 120 mg/L influenced it a positive way the number of leaves, the number of inflorescences, stem diameter in *Tagetes* (Basit et al., 2018).

Plant hormones are substances naturally created by plants that manage plant functions and development, such as root and plant development and fruit ripening (Mirihağalla & Fernando, 2020; Anfang & Shani, 2021). Moreover, they are significant and vital elements required to regulate plant development throughout the life cycle, from embryogenesis to reproductive development (Sajjad et al., 2017; Toungos, 2018; Mendel et al., 2020).

According to research, the use of growth regulators such as gibberellic acid (GA₃) and benzylaminopurine (BAP) in the cultivation of *Lisianthus* is limited because there are only a few reports indicating that gibberellins promote budding in rosette plants (Castillo-González et al., 2018). The growth stimulator GIBB A3 is intended for use in a number of agricultural and horticultural crops: vegetables, cereals, sunflowers, potatoes, fruit trees and shrubs, alfalfa and clover, vines. The application of the growth stimulator GIBB A3 determines increases in production, larger and firmer fruits, with good shelf life and quality. The growth stimulator GIBB A3 contains 20% gibberellic acid GA₃ (gibberellin) which is not toxic or dangerous in the doses recommended for use (SemintePlante).

The *Eustoma grandiflorum* (*Lisianthus*) is a member of the Gentianaceae family and is an herbaceous flowering plant (World Flora

Online), it has been cultivated since the 1960s (Ohkawa et al., 1999).

The Croma variety group from within the species was used for the 'Croma lavender' a) variety in the experiment. The thick, strong petals and extra-double flowers characteristic of the Croma variety make it stand out among the premium varieties. Due to the strength of the flowers and stems, it is very suitable for cold chain transportation. Rosette formation may occur in high temperature conditions. Its height varies between 70 and 90 cm (Takki.eu).

Within the Rosita variety group, we have chosen the variety 'Rosita yellow' for the experiment. The Rosita cultivar group is characterized by double-flowered inflorescences, thick petals, and strong stems, so it is easy to grow and the flowers are easy to transport. Good and in the vase long-lasting color also delight consumers. Their height is between 75 and 85 cm (Sakataornamentals.com).

Since *Lisianthus* in recent years had become a very popular plant worldwide and there is an ever-increasing demand for it, we wanted to test whether it can be successfully grown under our climatic conditions (Romania, Braşov). *E. grandiflorum* has an important role in the ornamental market, as it can be found both as a cut or potted flowering ornamental plant. The aim of our research was to determine whether different concentrations of GIBB A3 have a positive or negative effect on the production of cut flowers of the two selected varieties (*E. grandiflorum* 'Croma lavender' and *E. grandiflorum* 'Rosita yellow'). Our motivation for choosing the GIBB A3 treatment was that, during the past few years, flower production (potted and cut flowers) has reached a significantly higher demand on the market; hence, ornamental plant producers need to increase both the quality and also the cultivated plants. Furthermore, since plant growth regulators have gained higher attention in the previous year's horticulture section, GA₃ is also classified as one of these hormones and can have different positive effects on the cultivated plants; however, the price of GA₃ is quite expensive. Because of this fact, we have tried to use a cheaper version in which the GA₃ percentage is only 20%, still could have a satisfactory influence on the selected plants.

MATERIALS AND METHODS

Experimental Site and Plant Material

The study was conducted in 2022, in Crizbav, Braşov County (45°48'59"N 25°28'27"E) at a commercial greenhouse. The rooted plantlets were obtained from Qualipant SRL, Corunca, Mureş County.

The experimental site was plowed and leveled, and then divided into two large plots: one for *Eustoma grandiflorum* 'Croma lavender' (Figure 1a), and the other for *E. grandiflorum* 'Rosita yellow' (Figure 1b). Within each of these two large plots, five smaller plots were created for the hormone treatments. After the distribution of the plots was ready, the irrigation system was put in place. Irrigation was done by means of drip tapes. As a defense against weeds, a mulch barrier was added in each row. The plantlets were then planted at a distance of 10 x 15 cm. 40 plants were placed in each small plot, for a total of 400 plants of both varieties. Irrigation was done twice a day, for 10 minutes in the morning and in the evening for 15 minutes.



Figure 1. The two selected *Eustoma grandiflorum* varieties: 'Croma lavender' (a), and 'Rosita yellow' (b)

Experimental Design

The treatments were started 8 weeks after planting. The first treatment was made on the 16th July and then repeated every two weeks, so the treatments were made on the following dates: 30th of July, 13th of August, 27th of August, the last one being made on the 10th of September. Treatments were applied as foliar fertilizers using a normal sprayer (the appropriate dose for each plot). In the case of both *Eustoma*'s, the first plot was the control part (C), which was only treated with clean water; the following plots (A1, B1) received 25 mg/L GIBB A3. The hormone dose increased with each plot, so the next 40 plants (A2, B2)

received 50 mg/L, the 4 plots (A3, B3) 100 mg/L, and the last one received 150 mg/L (Table 1). During the treatments, a plastic separator was used so that the spray would not transfer to the other treatments (Castillo-González et al., 2018; Kwon & Heo, 2024; Pal, 2019).

Table 1. Experimental processing settings

Treatment	Rosita yellow	Croma lavender
Control (clean water)	CA	CB
25 mg/L GIBB A3	A1	B1
50 mg/L GIBB A3	A2	B2
100 mg/L GIBB A3	A3	B3
150 mg/L GIBB A3	A4	B4

Data Evaluation

The measurements were made every two weeks before spraying. The following data were recorded on the plants: growth (cm), shoot number (pcs), shoot length (cm), stem thickness (mm), diameter of the plant (cm), number of flowers (pcs), flower diameter (cm).

Initially, no differences were observed between the varieties or the different treatments.

Statistical Analysis

The significance of the differences between the treatments was tested by applying a two-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).

Heatmaps and dendrograms were created by employing Euclidean distance to indicate the similarities and differences in the development of two *Lisianthus* varieties using different concentrations of GIBB A3. The packages used included clusterProfiler, dendextend, and ggplot from the R database (version 4.05).

RESULTS AND DISCUSSIONS

Growth

The treatments affected the plants in different ways (Figure 2). On the one hand, when the two *Lisianthus* varieties were compared, no significant differences were found. On the other hand, the growth of the plants was significantly affected by the GIBB A3 treatments. As the concentration increased, the height of the plants also changed. The control plants were the lowest ('Rosita yellow' - 46.31 cm and 'Croma lavender' - 46.23 cm), in contrast, the 150 mg/L

GIBB A3 treatment achieved the highest growth, which was 60.29 cm for 'Rosita yellow', whereas for 'Croma lavender' it was 60.45 cm.

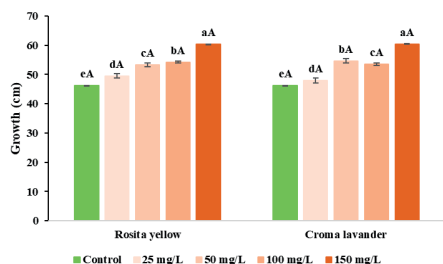


Figure 2. Effect of different concentrations of plant hormones on the growth of selected Lisianthus varieties ('Rosita yellow' and 'Croma lavender'). Bars represent the means $\pm$ SE ($n = 40$). Different lowercase letters indicate a significant difference between treatments, and uppercase letters indicate a significant difference between the two varieties ($p < 0.05$)

Stem Thickness

When comparing the Rosita yellow and Croma lavender Lisianthus varieties, it was found that significant differences appeared in stem thickness (Figure 3). According to the data, by increasing the concentration of GIBB A3, the treatments significantly affected the stem thickness compared to the control. The thinnest stems were measured in the control plants ('Rosita yellow' - 7.6 mm and 'Croma lavender' - 7.26 mm), followed by the treatments with concentrations of 50, 100 and 150 mg/L. The greatest stem thickness was recorded with the 25 mg/L GIBB A3 treatment, where it was 11.13 mm for 'Rosita yellow' and 11.5 mm for 'Croma lavender'.

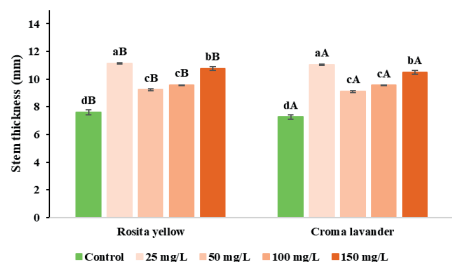


Figure 3. Effect of different concentrations of plant hormones on the stem thickness of selected Lisianthus varieties ('Rosita yellow' and 'Croma lavender'). Bars represent the means $\pm$ SE ($n = 40$). Different lowercase letters indicate a significant difference between treatments, and uppercase letters indicate a significant difference between the two varieties ($p < 0.05$)

Plants Diameter

The plant diameter of the 'Croma lavender' variety significantly exceeded the 'Rosita yellow' variety, except in the case of the 100 mg/L treatment (Figure 4). Furthermore, compared with the control plants, the diameter of the plants increased significantly in parallel with the increase in concentration. It can be deduced from the collected data that those of the control plants at 'Rosita yellow' reached 7.98 cm and at 'Croma lavender' reached 9 cm. The greatest plant diameter was observed in the 150 mg/L GIBB A3 treatment, where it was 25.75 cm for 'Rosita yellow' and 26.73 cm for 'Croma lavender'. Interestingly, in the case of the 'Croma lavender' variety, no significant differences were found between the control and the 25 mg/L treatment.

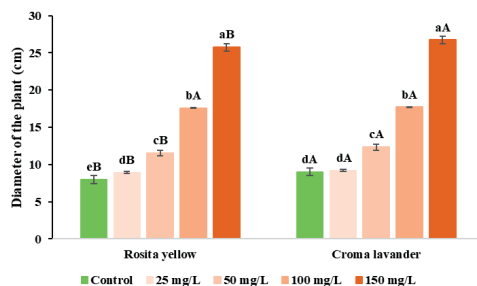


Figure 4. Effect of different concentrations of plant hormones on the diameter of plants from the selected Lisianthus varieties ('Rosita yellow' and 'Croma lavender'). Bars represent the means $\pm$ SE ($n = 40$). Different lowercase letters indicate a significant difference between treatments, and uppercase letters indicate a significant difference between the two varieties ($p < 0.05$)

Number of Shoots

Considering the number of shoots, no significant differences were found among the three higher dose treatments (50, 100, and 150 mg/L) when comparing the two varieties (Figure 5). However, in the case of the control and the 25 mg/L treatments, significant differences were recorded when comparing the two selected Lisianthus. The treatments significantly affected the number of shoots at both varieties, with the exception of the 25 mg/L treatment for the 'Croma lavender' variety, where no significant difference was found compared with the control.

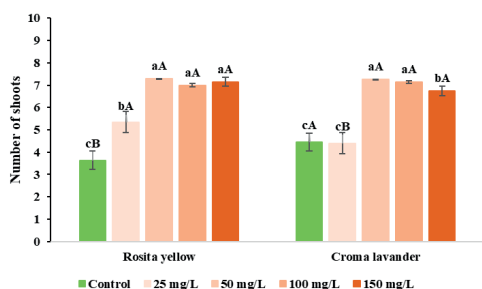


Figure 5. Effect different concentrations of plant hormones on the shoot number of the selected of *Lisianthus* varieties ('Rosita yellow' and 'Croma lavender'). Bars represent the means $\pm$ SE ($n = 40$). Different lowercase letters indicate a significant difference between treatments, and uppercase letters indicate a significant difference between the two varieties ($p < 0.05$)

Length of Shoots

Significant differences were found between the control and the treatments when comparing shoot length in the case of both *Lisianthus* varieties (Figure 6). The longest shoot length was obtained at the 150 mg/L treatment for the Rosita yellow variety, which was 38.09 cm, and for the Croma lavender variety, it was also observed in the treatment with the longest shoots, where we measured 35.58 cm. When we compared the two varieties, we also determined significant differences, with the exception of 25 and 50 mg/L treatments.

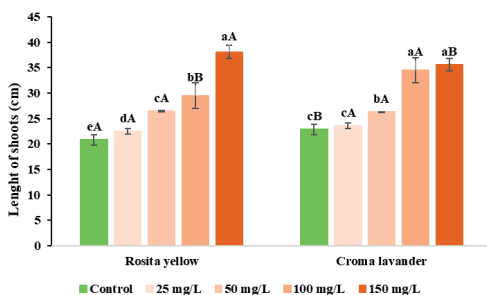


Figure 6. Effect of different concentrations of plant hormones on shoot length of the selected *Lisianthus* varieties ('Rosita yellow' and 'Croma lavender'). Bars represent the means $\pm$ SE ($n = 40$). Different lowercase letters indicate a significant difference between treatments, and uppercase letters indicate a significant difference between the two varieties ($p < 0.05$)

Diameter of Flowers

In the case of flower diameter, a significant increase was found in the 25, 50 and 100 mg/L

treatments compared to the control (Figure 7). Interestingly, we found a significant difference between the control and 150 mg/L for Rosita yellow, but not for the other variety (Croma lavender) subjected to the same treatment. In the case of the comparison between the two varieties, no significant differences were found in the 25, 50 and 100 mg/L treatments, in contrast, significant differences were found between the control and 150 mg/L treatments.

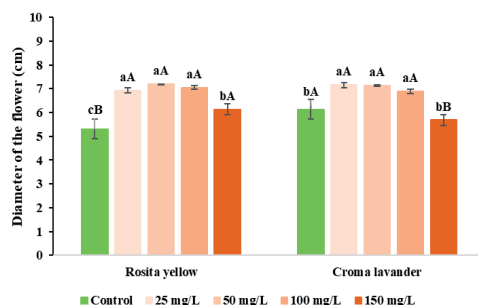


Figure 7. Effect of different concentrations of plant hormones on the flower diameter of the selected *Lisianthus* varieties ('Rosita yellow' and 'Croma lavender'). Bars represent the means $\pm$ SE ($n = 40$). Different lowercase letters indicate a significant difference between treatments, and uppercase letters indicate a significant difference between the two varieties ($p < 0.05$)

Number of flowers

Regarding the number of flowers, the best results were obtained with the 150 mg/L GIBB A3 treatment. At Rosita yellow, a significant difference can be seen between the control plants and the treatments (Figure 8).

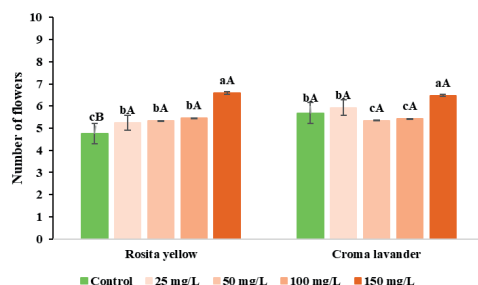


Figure 8. Effect of different concentrations of plant hormones on the number of the selected *Lisianthus* varieties ('Rosita yellow' and 'Croma lavender'). Bars represent the means $\pm$ SE ($n = 40$). Different lowercase letters indicate a significant difference between treatments, and uppercase letters indicate a significant difference between the two varieties ($p < 0.05$)

For Croma lavender, there is no significant difference between the control and the 25 mg/L treatment, but when comparing the other treatments to the control, a significant difference can be observed. Furthermore, a decrease in the number of flowers was recorded in the Croma lavender treatment at 50 and 100 mg/L. Moreover, a significant difference was reported between the two *Lisianthus* varieties only in the case of the control.

Hierarchical Clustering and Heatmap Visualization of the Plant Development Characteristics

The distinct cluster positions of the *Lisianthus* Croma lavender variety made comprehensible the influence on growth and development imposed by the used GIBB A3 concentrations (Figure 9). The primary cluster positioned the highest used concentration (i.e., 150 mg/L) which proved to be clearly distinct compared with the other used solutions, clearly discriminated by growth, plant diameter, and flower number. Although a positive influence in terms of plant and flower development using the 150 mg/L GIBB A3 concentration is obvious, a significant reduction in the number of inflorescences has been observed, which is commonly reported when using these biostimulants. Next, the second cluster divided the groups into two sub-clusters according to the used GIBB A3 solutions. Therefore, both the 50 and 100 mg/L concentrations presented similarities in terms of flower diameter, number of shoots, and growth. As compared to the 150 mg/L concentrations, in this respect the use of lower concentrations led to a higher flower diameter although the number of flowers decreased significantly. The subsequent sub-cluster grouped the control and the lowest GIBB A3 concentration, which imposed the lowest effect on the *Lisianthus* plants as observed by similarities in plant diameter, but also regarding the shoot length and number. Overall, the results suggest that lower concentrations of GIBB A3 may prove to be more beneficial in terms of promoting flower diameter, while higher concentrations may lead to a decrease in flower number. The control and lowest GIBB A3 concentration group exhibited similar effects on plant growth parameters, indicating minimal impact on plant development.

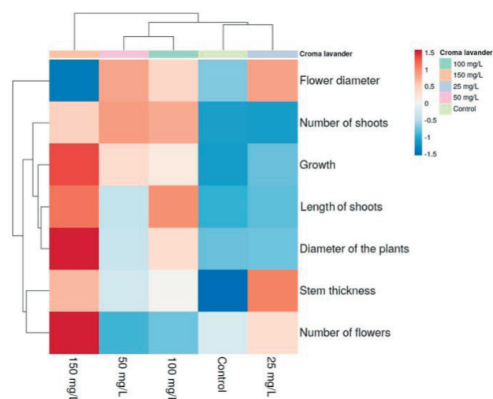


Figure 9. Hierarchical clustering and heatmap visualization of the plant development characteristics in Croma lavender as influenced by different concentrations of growth parameters

Regarding the Rosita yellow *Lisianthus* variety, the cluster placements made it possible to observe whether the employed GIBB A3 concentrations positively impacted growth and development (Figure 10). The primary cluster revealed the control group, which was clearly anticipated by the plants' slow development, as evidenced by the significantly smaller growth, plant diameter, number of shoots, and flowers. The subsequent group highlighted the 150 mg/L solution, which positively impacted the plants' growth, diameter, but also the length, as shoots and number of flowers, as was also observed in the other variety (i.e., Croma lavender). The following sub-cluster revealed the concentration of 25 mg/L GIBB A3 which showed a positive influence mainly on stem thickness and flower diameter; conversely, a negative effect has been observed in the plants' diameter and shoot length. The last sub-cluster highlighted similarities in terms of flower diameter and number of shoots using both the 50 and 100 mg/L concentrations. Discrepancies in terms of overall growth, plant diameter, and shoot length have been observed with regard to the 100 mg/L solutions. Overall, the results suggest that the concentration of GIBB A3 has a significant impact on certain aspects of plant growth and development in both Rosita yellow and Croma lavender varieties. It is important to further investigate the specific effects of different concentrations of GIBB A3 on various parameters to optimize plant growth and flower production.

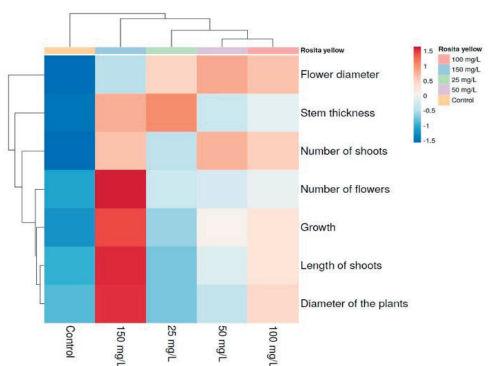


Figure 10. Hierarchical clustering and heatmap visualization of the plant development characteristics in Rosita yellow as influenced by different concentrations of growth parameters.

Plant hormones have an important role in controlling plant development (Davière & Achard, 2013; Claeys et al., 2014) and also in signalling plant responses to abiotic and biotic stresses (Bari & Jones, 2009; Wu et al., 2021). Plants can also produce a diverse variety of hormones, which include gibberellins, auxins, cytokinins, abscisic acid, and salicylic acid (Gomez-Roldan et al., 2008; Umehara et al., 2008). Bari and Jones (2009) mention that gibberellins (GA) promote plant growth by promoting the breakdown of negative regulators of growth called DELLA proteins. According to Hedden and Sponsel (2015) gibberellins are a family of plant growth regulators produced naturally. Gibberellins are also produced by fungi and bacteria, not only by higher plants (MacMillan, 2001). GA also has effects on germination, stem elongation, flowering, seed dormancy, leaf and fruit senescence, and enzyme induction (MacMillan, 2001; Pacifici et al., 2015; Bagale et al., 2022). In 2002 Spielmeyer et al. (2002) report that GA is a key element of the Green Revolution, it is also mentioned that there is a continuously increasing interest in gibberellins research because of their current and possible applications in crop improvement (Castro-Camba et al., 2022). Moreover, Shtin et al., (2022) mention that gibberellins are a key regulator for the root meristem development, and this phytohormone can activate as a molecular clock for the root development. From our results, it can be concluded that the plant development of both studied varieties was

significantly increased by the treatments compared to the control plants. The smallest amount of gibberellin (25 mg/L) used as foliar fertilization already increased the growth of the plants compared to the control. However, the highest significant differences were reported at the biggest amount of gibberellin (150 mg/L) used in the experiment. The stem height is not an important factor in the case of the potted *Eustoma* but, on the contrary, it is highly important for the cut Lisianthus. It is one of the most popular flowers in flower arrangements, it is therefore advisable to have as long a stem as possible for it will last longer in the vase. At the same time, the stems are cut back on a daily basis in order to increase the longevity of the plants, so there is a danger that if the stem remains too short, the plant loses its decorative value (Nagh Shiband Hassani et al., 2020). In previous research it was reported that application of GA₃ and Rhizobium that significantly increased the growth, and also the nutrient content of *Cicer arietinum* L. (Rafique et al., 2021). Moreover, in the case of *Beta vulgaris* L. a proper concentration of 160 mg/L of GA₃ sprayed 60 days after planting is the most recommended to increase growth (Castro-Camba et al., 2022). Moreover, it was also reported that GA₃ can improve the flowering, vase life, and growth of lilium by increasing water uptake (Leilah & Khan, 2021).

Regarding the stem thickness of the subjected *Eustoma* varieties, in both cases the treatments with GIBB A3 influenced it in a positive way; however, the thickest stem was recorded in plants treated with 25 mg/L. This could be explained by the fact that the other treatment concentrations were too high. In addition, the thickness of the stem is an important factor in the cut flower industry. Regarding the thickness of the stem, it is important that the stem is as thick as possible because otherwise it can break easily. Another aspect regarding stem thickness is important when selling the plant: the thicker the stem of the plant, the higher its market value, because for florists it is important to have a more durable stem, and generally a thicker stem diameter means a longer vase life (Hojatipour & Hassanpour Asil, 2022). In previous research, it was reported that a concentration of 300 ppm of GA₃ foliar application highly enhanced the stem diameter compared to the other treatments

(Poorani et al., 2024). In another study, *Lisianthus* seeds were treated with GA₃ and it was reported that a concentration of 1000 ppm resulted in the highest mean value of stem thickness (Shah et al., 2018).

Here again, the plant diameter was clearly influenced in a positive way by GIBB A3 treatments; yet, in this case, the highest diameter was determined at 150 mg/L. Furthermore, when comparing the two varieties, the Croma lavender reached a higher diameter than the Rosita yellow.

Under our experimental conditions, the number of shoots reported significantly different results under the effect of the treatments compared to the control in the Rosita yellow variety. On the other hand, in the Croma lavender variety, no difference was observed under the 25 mg/L treatment compared to the control, however, in the case of the 50, 100, and 150 mg/L GIBB A3 treatments, the number of shoots increased compared to the control. In an earlier study, it was reported that the highest number of side shoots and number of leaves per plant were determined at double pinching and 150 ppm of GA₃ (Lakshmaiah et al., 2019).

Considering the length of shoots, it can be clearly observed that the higher the concentration of the treatment is, the higher the length of the shoots. At *Eustoma grandiflorum* Rosita yellow, the greatest shoot length was reported at 150 mg/L GIBB A3 treatment. On the other hand, at *E. grandiflorum* Croma lavender variety, the 100 and 150 mg/L GIBB A3 treatments reported the highest significant differences.

From our results, it can be concluded that, on the one hand, the application of GIBB A3 increased the diameter of the flowers; however, the highest concentration (150 mg/L) reported smaller diameters compared to the other treatments. On the other hand, when comparing the number of flowers of both *Eustoma grandiflorum* varieties, the 150 mg/L GIBB A3 treatment reported the highest number. Interestingly in the case of the Croma lavender variety, an inhibition of flower number was observed under the influence of 50 and 100 mg/L of GIBB A3. In a study, it was reported that 10 and 50 mg-l of GA₃ increased the flower diameter of Asiatic lily (Othman et al., 2021). In another experiment, the authors determined that gibberellin treatment caused a

large number of flowers; unfortunately, with wilting and senescence symptoms (Calaboni et al., 2023) which reduces the post-harvest quality of *Lisianthus*. Furthermore, it was also reported that in the case of the *Gladiolus hybridus* 'Black velvet' cultivar, the number of developing flowers was affected only by a concentration of 100 mg/dm³ in the second year of the research (Janowska et al., 2018). Other authors reported, in the case of *Tagetes erecta*, that the maximum number of flowers per plant was obtained at a concentration of 250 ppm. Moreover, 300 ppm of gibberellin acid resulted in a longer duration of flowering, up to 58 days (Acharya et al., 2021). At *Chrysanthemum* cv. Thai Chen Queen, was determined that a higher concentration of GA₃ (400 mg/L) increased the vegetative and reproductive growth of the selected plants (Aparna et al., 2018). In the floriculture section is used different plant growth regulators to enhance the production of quality flowers. Additionally, growth regulators facilitate responses to both biotic and abiotic stresses (Chandel et al., 2023). Gibberellin could be one of these beneficial products, which positively promotes flowering and accelerates the flowering process (Pradeepkumar et al., 2020; Panwar et al., 2022). However, it could also have a negative effect on ornamental plants because of the elongation of the internodes (Al-Rawi, 2021), and thus the treated plants could be classified as lower quality.

CONCLUSIONS

During the experiment, the plants fertilized with clean water (control) were the least developed. The tallest plants were obtained at the GIBB A3 concentration of 150 mg/L.

In the case of stem thickness, the best results were obtained with 25 mg/L GIBB A3. Regarding plant diameter, the best data were obtained with the 150 mg/L GIBB A3 treatment. In terms of shoot length, 150 mg/L also reported the best results. From the point of view of the flowers, the flowering of the two varieties did not occur at the same time: the Rosita yellow bloomed earlier, while the Croma lavender variety began to bloom 3 weeks later. According to the observations, the diameters of the flower branches of Rosita yellow were smaller because it has smaller types of inflorescences than

Croma lavender, but their lifespan was much longer. In the case of flower diameter, the best results were measured for the 25, 50 and 100 mg/L treatments, and for the number of flowers for the 150 mg/L treatment.

According to the data from the present experiment, in most cases the best results were achieved at the treatment with a concentration of 150 mg/L. In our opinion, it is even worth trying a higher concentration treatment in the near future.

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LANDSCAPE AS NEW TOWNS INFRASTRUCTURE. FROM CANVAS TO URBAN IMAGE

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Abstract

The article presents the results of the New Towns/New Narratives international network project, led by the International New Town Institute, examining the landscape as a multi-dimensional reality in the context of the construction, in the 20th century, of Europe's New Towns. The project was developed under the 'Telling the story' theme as the backbone of the programme and the spin-off of other ongoing topics, such as green practices or the green transition. Starting from the creation, with the (pre)existing territory as a (considered) white canvas, to the green space as the critical infrastructure of the new towns, the green spaces were a central part of urban policy at the time. In the aftermath of the modernist movement, green infrastructure became the central topic in the survival and revival of the New Towns, as a resource for well-being. The article addresses the transformations of the landscape along the rise, the decay, and the (potential for) revitalisation of the New Towns, based on the study cases of the 16 new towns from 13 European countries that were part of the project.

Key words: New Towns, modernist/socialist urbanism, green infrastructure, landscape transformations.

INTRODUCTION

Europe is the land of old cities with deep and rich history and strong identities. These pre-modern cities of continuous temporality are specific spatial figures that reconcile a re-centred space with a reordered time. They are symbolised spaces (places) as opposed to non-symbolised spaces (non-places) (Augé 1995). Beside the historical cities, an entire network of more than 200 New Towns overlaps the entire continent. These New Towns, built in the aftermath of the 19th century industrialisation and of the WWII, are regarded as non-places, devoid of history, identity, and specific urban culture. However, these New Towns have, by now, a 50-70 years (hi)story that defines their particular identities. While the historic cities and city-centres in Europe are regarded as the places of culture and history, the New Towns are often seen as dull cultural wastelands (INTI, 2024a). The New Towns were, at the time of their planning, the (scientific) response to all the diseases of the City. The medical analogies abounded and, in this paradigm, the clean slate to work upon was the dream of planners, unhindered by old and obsolete buildings, attachments, ideas and people (White, 1968).

They are the embodiment of modern planning theories varying from the Garden City to CIAM functional city theory, from game theory to environmental planning, and (in eastern bloc) from Stalin's monumentality to later soviet *microraiou*. Today they stand as proofs of concept, or proof of the incapacity of abstract, technical concepts to plan life and communities (Figure 1).



Figure 1. Zoetermeer - First phase of the new town, Driemaspolder neighbourhood (INTI, 2024a)

Regarded from this perspective, the New Towns are not only places of their own history and (community and/or individual) stories, but also places of the history of urban thinking, heritage of modernist utopian dreams.

MATERIALS AND METHODS

It is around these stories and histories that the International New Town Institute (INTI) was formed in 2009, as a platform for research, education, and knowledge. The spin-off New Towns/ New Narratives international network is looking for transformations of these cities and is analysing their histories as the basis for these transformations and the construction of specific identities. The present network, established in 2023, comprises 15 New Towns from 13 different countries:

- Dimitrovgrad (Bulgaria),
- Drumul Taberei, Bucharest (Romania),
- Dunaujvaros (Hungary),
- Evry-Senart, Grand Paris Sud (France),
- Fritz Heckert Gebeit, Chemnitz (Germany),
- Hellersdorf, Berlin (Germany),
- Ilijaš (Bosnia-Herzegovina),
- Kohtla Järve (Estonia),
- Milton Keynes (United Kingdom),
- Nova Gorica (Slovenia),
- Nowa Huta (Poland),
- San Polo, Brescia (Italy),
- Spijkenisse, Nissewaard (Netherlands),
- Visaginas (Lithuania),
- Zoetermeer (Netherlands).

Beside those, Rotterdam was included as a mentor-town alongside Milton Keynes. The network involved 23 partners: local NGOs, local administrations, universities, cultural and educational centres, and institutions.

The preparatory online meetings framed the urgent issues that the New Towns are confronting now, including: “the health and wellbeing of vulnerable citizens’ groups, obesity and loneliness, the gap between local government and residents, the accessibility of the housing market for all, the deterioration of nature, the safety and accessibility of public space, the untapped potential of open space and green areas, the unheard voices of youth and seniors, and the unseen qualities of the cities’ history or the vitality of its social structures”. (Provoost &

Axinte, 2025). The summarising ‘umbrella’ theme, as came out from the majority of voices, was decided to be ‘Telling the Story’, which became the title of the resulting volume, published in 2025.

During the two-year project, six workshops, organised around six main themes, were held in Spikenisse (*Transformation of identity*), Evry-Senart (*Just and Inclusive Green Transition*), Drumul Taberei, Bucharest (*Informal Green Practices*), Chemnitz (*Telling the Story of Everyone*), Nowa Huta (*The Social Redesign of Space*), Nova Gorica (*The Unplanned Histories of Planned Towns*). The workshops were inclusive, involving local residents as a key point in ‘Telling the Story’, as a compelling story can only be created together with the population, not being imposed top-down, as the initial planning of the New Towns was (Provoost & Axinte, 2025).

Visual and written materials were prepared in advance of each workshop and presented and discussed during the meetings, synthesising cross-information about the history of the town and the present dynamics related to the subsequent research themes.

RESULTS AND DISCUSSIONS

One of the main ideas that resulted from the many exchanges that took place during the two years of research is the important role that open space and greenery play in residents’ use of neighbourhoods and collective life as part of ‘Telling the Story’ (Provoost & Axinte, 2025). Planned from scratch, the New Towns share many similarities, despite the political split in the post-war period. As William White (White, 1968) put it, “philosophically as well as physically, the new town is to be a complete community – so complete that it can exist independent of the old city, and, quite literally, help cut it down to size.” The New towns were supposed to be self-contained, with no need to commute to the (old) city nor to be visited from outside. However, as history showed us, most of them became planned suburbia that only function in the shadow of the central (historical) cities that they support, as dormitory neighbourhoods. As an ironical turnout, many New Towns are still described (and describing themselves) as ‘self-contained satellites’.

All New Towns were invented as finite, finished realms on a drawing board, showing existing and necessary infrastructures (mainly transport and industry), on a white canvas (Figure 2). Scientific Masterplans imposed new visions of ‘perfect worlds’ based on nearly abstract models, neat, rational, logical, and fixed (Kaplan, in White, 1968).



Figure 2. Scale model of Drumul Taberei district, Arhitectura 1/1972 (courtesy of Union of Architects in Romania - in Provoost & Axinte, 2025)

The original landscapes

Despite their early origins in Ebenezer Howard’s Garden City model, supposed to combine the countryside and city qualities and avoid all their shortcomings, all New Town Masterplans erased the rural and/or agricultural past. The modernist approach of the city as a *tabula rasa*, beginning with the striking erasure of the Marais neighbourhood in Paris by Le Corbusier’s Plan Voisin, was the dominating model of thinking when installing the New Towns.

As many New Towns were developed on old rural territories (Dimitrovgrad, Evry-Senart, Hellersdorf, Nova Gorica, Nowa Huta, Spikenisse, Zoetermeer), one cannot find many traces of it.



Figure 3. La Grande Borne district, Grigny in Évry (INTI, 2024b)

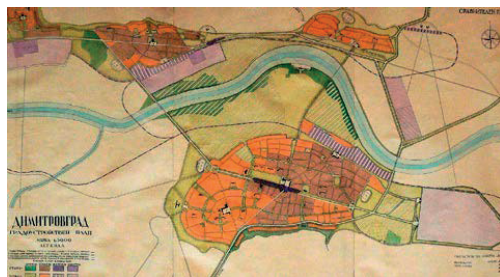


Figure 4. Masterplan for Dimitrovgrad by Luben Tonev, 1947-1948 (Atrium Archive in Provoost & Axinte, 2025)

In general, the rural heritage and landscapes tend to be radically transformed, if not obliterated, in order to make way for abstract, geometrical, scientifically urban compositions (Figures 3, 4). In other cases, like the one of Ilijaš, in Bosnia-Herzegovina, the rural past is kept as an architectural reference, while the original landscape of the rural settlement agglomeration and forest expanses, and the rural life style is drastically transformed. If the agricultural practices and economy are mostly replaced by industrial activities, the new, modern architecture seeks its roots in a critical regionalism spirit, borrowing elements from the traditional vernacular one (Figure 5).



Figure 5. Worker house in Ilijaš, built in 1939 by Juraj Neidhardt, as an expression of modern regionalism; photo: Elša Turkušić Jurić (Provoost & Axinte, 2025)

However, in Ilijaš’s case, the rural way of life made a strong comeback, as after the 1990s the ancient rural cores developed into rural sprawls of the urban-industrial centre (Figure 6). “As such, Ilijaš assumes the character of an urbanised local node with clear elements of centrality, toward which surrounding rural settlements gravitate, albeit without clear transitions or harmonised narratives.” (Elša Turkušić Jurić, Irfan Salihagić, Maja Plićanić in Provoost & Axinte, 2025).



Figure 6. The town of Ilijaš and its surrounding context (photo: Anto Baskarad in Provoost & Axinte, 2025)

At the opposite extreme, Nova Gorica completely replaced the natural meadow and the villages around it, part of the Italian city of Gorizia's hinterland. The New Town, a kind of modernist abstract and *masterly, correct and magnificent play of volumes brought together in light*, as Le Corbusier saw the new architecture, was a *middle of nowhere* composition, completely ignoring the previous landscape features (Figure 7).



Figure 7. Model of Nova Gorica original design, with the going-nowhere axis, photo: Ioana Tudora

The central axis, starting from and leading to vanishing points without territorial connection, was only running parallel to the new frontier. No connection with the past was integrated into the masterplan. The New Town was the perfect image of the new man in a new society, in a new geopolitical landscape (Figure 8). But the meadow and its rural occupation were no longer there. The New Town was 'connected' by a cut from its landscape.



Figure 8. Still from the video essay 'Town on the Meadow' by directors Anja Medved and Nadja Velušček, 2004 (Provoost and Axinte, 2025)

In other cases, such as the one of Visaginas, Lithuania, the New Town built in the Soviet era and known as the Atomic City (Atomgrad), the Masterplan (Figure 9) on the natural setting, replacing the forested area on the shores of Visaginas Lake. Nevertheless, in the case, due to its high status among Soviet New Town (*Atomgrads* and *Naukograds* - for science cities - having a special prestige) and the complexity of the terrain, part of the natural setting was integrated into the fabric of the city (Figure 10).



Figure 9. A model of the Visaginas masterplan, photo: Oksana Denisenko (Provoost and Axinte, 2025)



Figure 10. Pine forest in a courtyard of Visaginas, photo: Olga Rožkova (Provoost and Axinte, 2025)

In the case of Nowa Huta, planned to be an anti-Kraków, opposing new values to the old ones; some of the pre-existing agricultural and natural settings stood at the fringes of the city. The Meadows are both a reminder of a pastoral past in the area and a resource for the present and future of the city, already central to its social life (Figure 11).



Figure 11. The Nowa Huta Meadows, photo I. Tudora

In most cases, the New Towns were conceived as new urban implants squeezed in between existing rural settlements, replacing agricultural land but keeping the villages' cores, thus transforming the entire territory's way of life, as in the case of the new implants in Paris-Sud (Évry, Sénart) or the insertion of Fritz Heckert Gebeit in Chemnitz (INTI, 2024b, 2025a).

As a demonstration of an alternative vision, in the exceptional case of Milton Keynes, the existing towns and villages were incorporated into the Masterplan of 1970, seeking to maintain their individual identity and strengthening their role (CNT, 1992). Even the name of the new planned city was that of one of the engulfed villages. Today, the traces of the old settlements are scattered and trapped among precast terraces, business parks, and large car-oriented boulevards (Sudjic, 2017). All of these fragments of city are connected and yet separated by a comprehensive green network (Figure 12). Although the entire public-designed masterplan was developed around the distinctive green grid system, much of its parkland is managed not by the municipality but by The Parks Trust, a charitable body set up by the outgoing Development Corporation in 1992 (Provoost and Axinte, 2025).



Figure 12. The network of parks and green spaces in Milton Keynes (Provoost and Axinte, 2025)

This brings us to the paradox of the New Towns. While dislocating and replacing agricultural land and / or natural areas, all New Town projects were looking to create a green urban realm, having in the centre of the planning schemes generous and elaborated green infrastructures.

The Planned Landscapes

Starting with Frederick Law Olmstead's projects for the new Riverside suburb of Chicago (1868) and the park system of Boston (1878), the image of the new, modern city was connected with the idea of greenery. Green spaces were the main feature of the Garden Cities built at the beginning of 20th century, as well as of the New Towns built after WWII. Green was the panacea for all urban diseases. If the first suburban garden cities were laid out around tamed parkways and public gardens with individual houses (with private gardens) sagely ranged along the leafy streets, the modernistic perspective, expressed by the Charter of Athens, brought a vision of unleashed nature under suspended highways and collective buildings. In the UK, the New Town Act of 1946, revised several times until 1969, gave the means and the power to create a new utopian vision of tomorrow's dwelling. The landscape became a tool for connecting and separating the planned areas of the city. As Sir Frederick Gibberd wrote about the Harlow development he mastered (1947 - Figure 13): "The Plan was concerned with three arts: Architecture, Road Design and Landscape Architecture. These three arts are fused to become town scenes or townscapes, the making of which is the art of Town Design"

(Gibberd Architects). The proposed landscape scheme separates the built-up areas and extends the New Town into the surrounding countryside, with an irregular boundary, produced by the reorganisation of field patterns from surrounding farms and the use of natural features. The layout of building groups was arranged to preserve woods and tree belts. In the centre, Henry Moore's *Family group* was sitting as the symbol of the new life beginning. A new model of living was set.



Figure 13. Harlow Master Plan - <https://www.gibberd.com/projects/harlow-master-plan>

In the frame of most New Towns' masterplans, The Green was tackled in a multiscale way, solving problems of territorial connections and those of daily bases dwelling. As the Rotterdam plan (Figure 14) shows, while great corridors and green infrastructures are (re)waving the new, modern scattered city, connecting the centre with its suburbia, most of the peripheral neighbourhoods, conceived as autonomous New Towns, were planned as Garden Cities, putting the green spaces in the centre of modern housing/inhabiting.

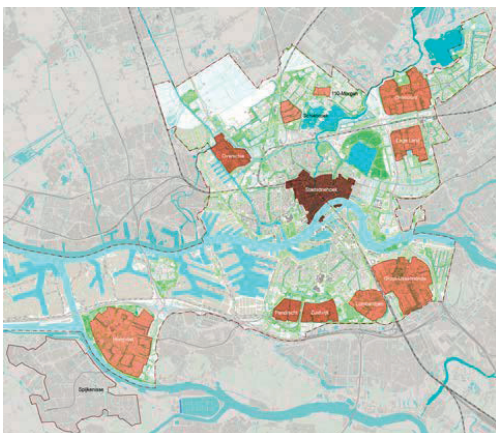


Figure 14. The Garden Cities of Rotterdam (Paul Broekhuizen in Provoost and Axinte, 2025)

In the Netherlands, at the end of the 1940s, ideas about the 'organic' and 'open' quarter were incorporated in an urban concept called the *wijkgedachte*, or 'residential district idea', as the key element between the individual and society, between private and public life, combining urban and rural qualities, fact the lead to critical approaches to *wijkgedachte* as 'anti-urban', 'sentimental' and 'regressive'.

Nevertheless, the concept became very popular and it was applied on a large scale in the two decades after the war (Van Hellemond, 2021). "The scientific approach offered landscape architects the possibility to design a variety of separate yet connected spaces, differing in rhythm, size and material. The downside was that it could lead to deterministic and fragmented urbanism." (Van Hellemond, 2021).



Figure 15. Urban plan for Pendrecht, 1952-1954 (City Archive Rotterdam in Provoost and Axinte, 2025)

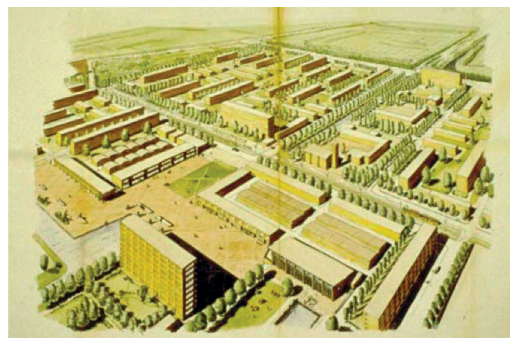


Figure 16. Lotte Stam-Beese - Bird eye view drawing of Pendrecht (Fabrizi, 2018)

The post-war reconstruction brought with it a new orientation in the garden cities planning towards collective housing, thus reinforcing the modernist vision of the future. Rotterdam's Garden Cities conceived during the post-war

reconstruction era were shaped by modernist urban planning ideals, by CIAM principles and Clarence Perry's neighbourhood unit concept, as exemplified by Lotte Stam-Beese's project for Pendrecht, one of the most representative projects of the urban theories developed in Europe in the post-war period (Figures 15, 16). The district is organised in four neighbourhoods laid around a traffic-free/ pedestrian square. Instead of the usual system of perimeter blocks, the fundamental unit of these neighbourhoods was the *wooneenheid* (or cluster): this form of spatial organization implements a physical and social connection between every single home and the neighbourhood considered as a whole (Fabrizi, 2018). The green spaces followed the same fractal structure, from public to community and individual.



Figure 17. Typical housing and collective green from the 1960s, Reyeroord - Stadsarchief Rotterdam, in Provoost and Axinte, (2025)

The new neighbourhoods of the Garden Cities “were designed as planned, socially oriented residential environments. Green spaces were interwoven in the built areas as sites of social learning and moral formation” (Figure 17). The planners employed the communal gardens and green buffers as normative spatial instruments. “Unlike the densely programmed city centre, the Garden Cities offer an abundance of green public space, a clear spatial structure, and a legacy of collective living”. They represent distinctive urban landscapes including green corridors, communal gardens, and wedge-shaped green spaces that serve as places for social life and values (Joris Vermeiren in Provoost and Axinte, 2025). But the green recreation was conceived in a very formal

manner, rather than informal hanging out, as already CIAM asked for green areas with well-defined purposes.

As an example of the second-generation Rotterdam's urban growth, Spijkenisse is no more a garden city, in fact, a neighbourhood directly related to the city centre, but a New Town in its own right, designated as a working-class neighbourhood and a ‘growth centre’ in 1976.



Figure 18. Spijkenisse has an extensive network of green spaces and waterways (Municipality Nissewaard, in Provoost and Axinte, 2025)

The Masterplan shows a clear structure of neighbourhood units, separated by green corridors and mainly located along the main roads, with important green spaces surrounding the New Town as a belt. The plan envisaged that the neighbourhood units would be structured around more or less central green spaces with public and social roles, although no major public facilities were built nearby in order to activate those spaces (Figure 18).



Figure 19. San Polo development plan by Leonardo Benevolo (Galli 2022)

In most cases, the green network of spaces wconsidered as one of the main infrastructures to sit at the foundation of the New Towns, in some others it was more of a utopian dream.

Such is the case of San Polo, Brescia. The great planner, city historian of the city and theorist Leonardo Benevolo realised the initial development plan, organised around a large park (Figure 19). He mastered the overall urban layout inspired by English new towns, the design research on building types, and the complex administrative system of the operation, able to permit a process of public urbanisation. Today, San Polo is the most densely populated district in the city of Brescia, with more than 19,000 inhabitants. The original design remains intact, but the large park that, in Benevolo's vision, should have delimited the entire district has never been completed (Galli, 2022).

A landscape plan for San Polo was realised by Sir Geoffrey Jellicoe, one of the most important figures in Landscape Architecture at the time (Figure 20), who completed the built-up area with a large English garden. Jellicoe's project visually connected San Polo to the historic centre and to the lakes south of the city through a system of micro-reliefs and water bodies. Even if it could have been an additional value for the initiative, it is just part of the list of missed opportunities (Galli, 2022). Instead of a landscape corridor, today San Polo is crossed and split in two by a railway.



Figure 20. San Polo's landscape proposal by Sir Geoffrey Jellicoe (Leonardo Benevolo archive, IUAV - in Provoost and Axinte, 2025)

Another practice between utopia, propaganda, and reality was the one present in Drumul Taberei, or, to some extent, in Nowa Huta, and not only. Nowa Huta (Figure 21) was built in the 1950s and remains until today as an example of proletarian monumental architecture from the Socialist-Realism period (with allegedly Renaissance influences), although it was equally

inspired by the neighbourhood unit concept devised by Clarence Perry (Kępa, 2017). But after 1954, under the influence of Hrusciov's discourse, the supremacy of proletarian monumental architecture of the Socialist-Realism style, dominated by classical traits, diminished or was replaced by modernism / international style, and the new architectural paradigm became the economical approach.

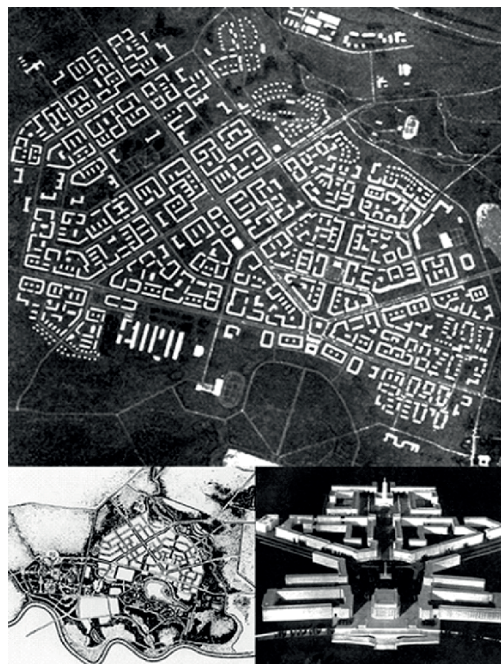


Figure 21. Development plan and model of Nowa Huta (Kantarek & Samuels, 2017)



Figure 22. Scale model of Drumul Taberei district (Arhitectura 1/1972, courtesy of Union of Architects in Romania – UAR - in Provoost and Axinte, 2025)

On its side, Drumul Taberei (Figure 22) is an emblematic urban assembly for a period of the "great opening" period of the '60s, built in a "free urbanism" style, although based on the *microrayon* concept, as the architects presented

it (the Soviet variant of neighbourhood unit, used also in Visaginas or Dimitrovgrad).

Usually, the generous planned green spaces were the first to be cut from the investments plan. So much so that, at least in Romania, many professionals, such as Gheorghe Petrașcu or Rică Marcus, in *Arhitectura* review no. 7/1957, are criticising the lack of attention for public spaces and landscape design, accusing the scarcity of paved alleys inside the *cvartals* and the absence of plants in the designated green spaces. In Eastern countries, the *cvartals*, naming buildings organised around inner courtyards (based on the Viennese *hof* model) were the specific urban development morphological unit in the 1950s. Later on, they were replaced, under Soviet influence, by the *microraiion*, a more modernistic approach inspired by the neighbourhood unit and the Charte d'Athènes principles (both models are illustrated in Figures 21, 22).

Arhitectura review no. 7/1957 is almost entirely dedicated to the green spaces and sports facilities projects and analyses, an interesting opportunity for some (discreet) critiques. Thus, Rică Marcus, well known for his book “Parks and Gardens in Romania”, criticised the use of typified architecture projects that generate monotonous and uninteresting urban landscapes – the reaction to this situation: people’s need to regain contact with nature.

Parks and gardens are presented as more than leisure spaces, but, in line with the new Soviet discourse, a means of education (such as libraries, theatres, exhibition halls, sports and playgrounds) is now integrated into the city’s green spaces. What is interesting about his standpoint is that he considers landscape design a means to regain the human scale in the new urban assemblies and to diminish the effects of standardisation.

Of course, the socialist city is the only one entitled to solve these contemporary problems, and that is going for almost the entire Eastern bloc. “As to the diffusion of the idea of setting up the plantations, it is worthy of notice that the masses’ interest in having parks and gardens cannot be fulfilled in the capitalist regime, in which the private property of the land leads to its being exploited to the limit. An eloquent example in this regard is that of American batching and even that of the so-called garden

cities, with lots measuring between 100-150 sq. m. and squeezed houses with only 4-6 m between them. None of these methods addresses the real needs of the population.” (Marcus, 1957).

Meanwhile, Gustav Gusti was deploring “the desolate situation on our construction sites, in which unfinished buildings go into use while still being surrounded by unsightly and unimproved terrains, in which the denizens struggle with dust and mud, organising as best as they can the playground, the garbage-depositing space, and the enclosures for poultry and domestic animals” (Gusti, 1954).

If the planting was set aside or minimised for economic reasons, and the New Towns were rather grey or brown than green (Figures 23, 24), the new dwellers took this into their own hands. Which leads us to the next chapter.



Figure 23. Nowa Huta in the 1960s. (photo: Henryk Grzęda/PAP in Kępa, 2017).



Figure 24. Drumul Taberei in the 1960s.
(<https://b365.ro/i-love-drumul-taberei-ce-a-fost-si-ce-a-ajuns-cartierul-bucurestean-comunist-cu-arhitectura-inspirata-de-le-corbusier-468250/>)

The Daily Life Landscapes

As discussed before, in many planned visions of New Towns, the project was never fully completed. While the housing stock and primary infrastructures were already in place, many of

the interstitial areas and spaces between blocks, around entrances, alongside paths, remained unregulated and undefined. These public spaces of proximity became open terrains for residents to act.” Immediately after moving in and over time, people began to plant trees in front of their windows or even along streets, organise gardens, place benches, install bird feeders, raise flowerbeds, and build informal fences (Provoost and Axinte, 2025).

Quite often, desert, sterile urban landscapes were transformed over time (Figure 25), through private/collective interventions or later administrations’ planting actions (in some eastern countries also done by ‘volunteer’ citizens’ work – in socialist states, these practices were formally supported by the state, through resources, organizational formats, and narratives), in rich ecosystems, flooded by greenery.



Figure 25. Drumul Taberei, 2022 (photo: Andrei Gherasim in INTI, 2024b)

Not only in the case of un-finished and unplanted green infrastructures had the open spaces become the playground of denizens. Even in cases of well-implemented green spaces, “collective spatial structures have been weakened by societal shifts, increasing individualization, and changing usage patterns. Many of the original spatial qualities, such as the abundance of green and open layouts, no longer function according to their intended social logics.” That we talk about under-designed spaces, left aside during the implementation of the plans, or about abandoned spaces, these underused spaces became a new resource for spontaneous and adaptive actions and reinterpretations (Vermeiren in Provoost and Axinte, 2025).

In many cases, underutilized green spaces, due to their initial under-planting/ design or lack of functional/ social clarity, or following under management and abandonment, now offer unique opportunities for spontaneous and adaptive reinterpretation, like fertile ground awaiting reuses and reinterpretations.

As an example, in Milton Keynes, volunteer groups have formed around specific parks or nature reserves, where they clear invasive species, install bird boxes, run guided walks and/or support environmental education. At Linford Lakes, volunteers have helped transform a neglected site into a wetland where birds are thriving, while “in Woughton a group cares for a community orchard, sharing knowledge about traditional fruit varieties and grafting techniques”. Other initiatives operate in more hybrid spaces, like the Forest Gardens programme, led by the City Council that aims to create ten small food-forests across the city (Provoost and Axinte, 2025).

Another example is The Wijktoin Ommoord (neighbourhood garden Ommoord) resident-led initiative. Ommoord is a typical mass-housing estate designed for working families, organised into spatial units with distinct boundaries, and subject to waves of disinvestment and regeneration, leading to social change. Wijktoin Ommoord started as a grassroots initiative in 1972 (Figure 26) and it has evolved into an ecological hub that integrates sustainability, ownership, and neighbourhood identity. Wijktoin defines itself as a ‘nature garden’, where everything native is allowed to thrive.



Figure 26. Neighbourhood garden in Ommoord, 1973 (Ary Groeneveld/Stadsarchief Rotterdam in Provoost and Axinte, 2025)

Therefore its savage aspect demands volunteer work to maintain accessibility. This means keeping the paths open by managing the herbaceous vegetation at knee.

height, prune overhanging branches and brambles at shoulder height, and remove obstacles. Wijktoin is a pioneering community project that operates as an alternative model of urban stewardship (Figure 27) and contributes to a more sustainable urban ecosystem, having profound ecological and social value, as it also “witnessing a gradual demographic shift, with increasing interest from younger residents and families, further solidifying its role as a cross-generational community asset” (Vermeiren in Provoost and Axinte, 2025).



Figure 27. Wijktoin Ommoord today (photo: Joris Vermeiren in Provoost and Axinte, 2025)

In other cases, the pre-existing landscapes play a role in collective memory. Like in the case of Ilijaš, where the natural/rural environment plays a dual role (Figure 28). On the one hand, the forests and rivers offer vital resources and a sense of continuity as residents speak of the hills, the picnics, and mushroom picking as childhood realms. But they are also the triggers of social unrest and activist movements seeking a different kind of spatial redesign, grounded in environmental justice and collective responsibility (Provoost and Axinte, 2025).



Figure 28. Ilijaš surrounding rural landscape (photo: Samna Durmo in Provoost and Axinte, 2025)

Designed with community input and managed by local groups, these bottom-up initiatives blur the line between formal and informal. Alongside the formal infrastructure, a quieter story has unfolded: a proliferation of informal green practices, often led by residents and volunteer groups. These initiatives - orchard care, wildlife monitoring, informal gardening, or tree planting are rarely planned in a conventional sense. Many of these practices emerge in what might be called the cracks of the city. “The motivations vary, being nostalgia, ecological concern, or a desire for quiet, but what links them is an orientation towards stewardship without ownership”. These are not community projects with funding and formal objectives; they are acts of maintenance that persist without fanfare, often unnoticed by passersby (Provoost and Axinte, 2025).

Informal green practices

‘Informality’ as a concept was borrowed from the economic sector, where it has been used to describe local ‘shadow economies’. Imported into urban studies, the concept evolved from informal housing or informal urbanism, as sub products of global neoliberalism, thus placing informality in subaltern positions. However, other studies on informality grew alongside the rediscovery of the ‘ordinary city’, as a reaction to the increasing transformation of cities into global market powerhouses. Numerous studies have documented the usefulness of informal practices in various contexts, such as the ad-hoc collective production of space, or the negotiated forms of governance. Calls for restoring social justice in cities were beginning to acknowledge the role of informal practices as valuable resources. Autonomous groups and their bottom-up projects and/or informal practices were recognized and valued as already responding to real social needs (Axinte in Provoost and Axinte, 2025).

In many cases, informality has no conscious political dimensions. It only starts with basic daily practices, quite often as a response to the lack of basic qualities (as planting what the administrations ‘forgot’ to plant). In most cases, the new dwellers simply started to plant around the blocks as a favourite pastime. Then and now, these practices responded to the shortcomings of institutional maintenance.



these cities (Figure 33). This inventory of informal practices showed a variety of interests and purposes, forms of organization, and relationships with the administration.

Thus, New Towns with a strong and long-term public planning culture, such as Grand Paris Sud, Milton Keynes, Rotterdam, Spijkenisse, or Zoetermeer, have developed various forms of partnerships, agreements, or contracts, which aim to facilitate the participation of less institutionalized actors in the management of public spaces, seeking both to regulate and to increase the inhabitants' participation (Axinte in Provoost and Axinte, 2025). This might create a persistent tension, as many administrative documents discuss facilitating citizen initiatives, but their overall attitude remains embedded in a formal, top-down strategy and, quite often, implies a structured, planned methodology that emphasizes how initiatives can be integrated into existing frameworks. Therefore, they are "supportive on the surface but regulatory in practice" and "can act as a constant brake on the fluid and experimental nature of informal green practices", thus risking an excessive formalization and transforming the administration into an organizer instead of a facilitator (Vermeiren in Provoost and Axinte, 2025). In these institutionalized landscapes, most of the informal actors are represented by citizen-volunteers, specific to associative frameworks such as groups, associations, initiatives, or projects, which participate and contribute as part of a community (Axinte in Provoost and Axinte, 2025). The overall image is a more institutionalized one, quite often based on a strong community infrastructure (Figure 34). "In the more loosely regulated contexts, actors of informality are usually identified as individuals, or at most as small groups, such as tenants, neighbours, elderly, retired, women, or residents with rural backgrounds, who are practicing informality out of need or habit. [...] Although at first sight these more discreet practices seem weaker, less organized, and with a lower community impact, they are part of a wider vernacular ecosystem of complementary spaces that are resourcing each other, thus making them more horizontally accepted and adopted as a way of life." (Axinte in Provoost and Axinte, 2025).



Figure 34. Collective garden De Zoete Aarde / Sweet Earth in Zoetermeer (photo: Municipality Zoetermeer in Provoost and Axinte, 2025)



Figure 35. Garden with a kiosk for meetings in Buclă / The Loop area, Drumul Taberei, Bucharest, 2022 (photo: Alex Axinte in Provoost and Axinte, 2025)

But, on the other hand, they are facing a more complicated relationship with the local administrations. Ranging from ignorance to occasional support (when political aims are demanding it) and passive or active (sometimes violent) interdictions and evictions, the administration's ambiguous and changing attitude is rather discouraging, as in the cases of Dimitrovgrad, Ilijaš or Dunaújváros. "It prevents the crystallization of extended and explicit communities of practitioners and fuels the perpetuation of conflicts over the use of space or appropriation through privatization or occupation" (Figure 35) (Axinte in Provoost and Axinte, 2025).

From the purpose and interest point of view, the informal practices range from basic needs (like

food production) to aesthetic desires (ornamental gardens around the blocs), social needs (meeting points and outside living spaces), ecological, educational, or mental health concerns. In this perspective, it is also visible differentiations between more publicly oriented actions, led by groups or larger organisations, and more private initiatives, fuelled by personal desires or needs and based on adaptation, domestication, and appropriation. The resulting picture of informality, seen from the perspective of these New Towns and the organisations that represent them, comes as a jigsaw composed of illegal and deregulated, to self-organized, grass roots or bottom-up, to everyday and convivial, up to artist, activist, or guerrilla practices. All of them are “compensating for either the excesses or the shortcomings in the organizational landscape, enacted by spatial practitioners who develop individual and collective agency, fuelled either by grand narratives, or everyday needs and habits; informality is like an imaginary glue necessary for articulating the multiple forms of living together in New Towns” (Axinte in Provoost and Axinte, 2025).

CONCLUSIONS

For more than 70 years now, the New Towns have evolved along diverse historical, economic, or socio-cultural trajectories, shaped by extremely different political and economic contexts. Might seems logical that everyday life in these districts has also evolved equally differently. “Despite the diverse ways in which residents adopted and adapted their districts through dwelling, there is still a certain degree of familiarity that transcends across different local contexts. Thus, as if coming full circle, the everyday life of New Towns is still a shared experience.” (Provoost and Axinte, 2025). While initially sharing a similar project of spatial organization and a similar attitude toward pre-existing landscapes (more often seen as *tabula rasa*), these districts spread all over the world and evolved in the past decades as more or less different habitats, responding to diverse contexts, historical trajectories, and specific local ingredients. If the original landscapes remain sometimes as punctual witnesses of a long-gone reality (like Nowa Huta’s meadow or the hills of Ilijaš), all development schemes

proposed elaborate green infrastructures and generous green-spaces networks.

From blueprints to reality, these green networks proved themselves as a cornerstone of the entire development. In some cases, the planted areas became the main feature, asset, and attraction of the new districts, presented as green paradises opposed to the grey, old city centres. Nevertheless, in most cases, housing was seen as too standardised and often monotonous, while green spaces were underused or poorly maintained, falling into abandonment and oblivion. In other cases, the initial green spaces were merely an absence, being brought into existence over time by the new dwellers and their informal planting. As with many planned visions, some projects were never fully completed: the housing stock and primary infrastructure were in place, but the interstitial areas and spaces between blocks remained unregulated and undefined. Thus, public spaces of proximity became openings for residents to act. They fenced private or collective gardens, placed benches in front of the entrances, organised the spaces around the carpet-beaters (a more or less eastern bloc typical feature), adopted stray animals and turned them into community pets. “What began as isolated gestures slowly grew into a layered landscape of informal green practices.” (Provoost and Axinte, 2025).

In both cases, the green spaces faced abandonment and social and/or administrative disengagement on the one hand, and a new bottom-up revival on the other. Thus, through these grassroots initiatives, the green infrastructures of the New Towns became the main resources at many levels. Greenery is the feature that characterizes the quality of these, otherwise not-so-attractive, areas. It represents equally the new social infrastructure that generates a sense of community (Provoost and Axinte, 2025). Beside “fixing” the green, informal green practices are capable of generating a new wave of investment and attachment, to build new communities, and addressing problems related to ecological pressures and resilience. Thus, informal practices are generating new landscapes that respond to specific problems and bring a new social, political, and economic balance.

Between anti-planning and un-planning, the new bottom-up landscapes emerging from informal

green practices are able to respond to both local and more general problems, to bring value to otherwise neglected spaces, and to generate new qualities of daily life (Figure 36).

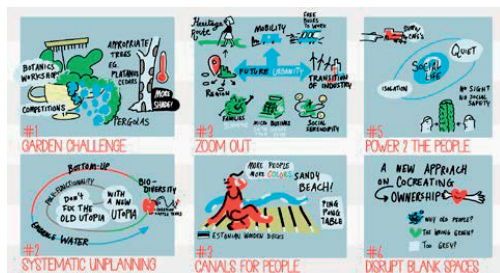


Figure 36. Graphic conclusions of Spijkenisse Lab, March 2024 (INTI 2024b)

In this context, “informal green practices do not constitute a counterforce to formal policy, but rather an essential complement to institutional planning in today’s complex and diverse urban landscape. Indeed, comprehensive examples of how municipalities effectively bridge the gap between their top-down planning frameworks and the organic, often spontaneous, nature of citizen-led collective greening remain relatively scarce.” The new social dynamics of the New Towns “demand a hybrid governance approach: one that bridges formal and informal logics, embraces diversity and failure, and understands urban quality as something co-produced through practice. Informal green practices not only green the city; they democratize it” (Vermeiren in Provoost and Axinte, 2025).

Passing from the original natural/ agricultural canvas, through top-down planned green networks and infrastructures to the new bottom-up generated daily-life green spaces, the landscape is the main value of the New Towns, playing a fundamental role in building the identities of these cities and districts. It should therefore stay at the centre of all regeneration, renovation, or sometimes patrimonial policies, as it is both the heritage of an already long past and a resource for a better future.

The New Towns epitomise the evolution of the landscape as viewed and described by John Brinkerhoff Jackson: “as an environment where permanence and change have struck a balance” instead of “a static utopia dedicated to ecological or social or religious principles” (Jackson, 1986). Thus, the New Towns’

landscapes passed from *Landscape One* – the vernacular/rural/long-time generated by the permanent management and use of the land, to *Landscape Two* - the political/planned/ imposed and image-based one, to *Landscape Three* - seen as a small-scale, balanced construction overlapping vernacular vitality over political schemes, and altering the flow of time (Jackson, 1986).

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SHARED STRATEGIES FOR SURVIVAL: A COMPARATIVE ANALYSIS OF LEAF DRY MASS SUGGESTS SIMILAR DROUGHT TOLERANCE TRAITS WITHIN TAXODIACEAE SPECIES UNDER URBAN CONDITIONS

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Abstract

Urban environments expose trees to intense drought stress due to elevated temperatures and reduced soil moisture. Identifying woody, drought-resistant species is essential for sustainable landscaping. This study assessed leaf dry-matter content (LDMC) as an indicator of drought tolerance in three species from the former Taxodiaceae (now Cupressaceae) family - *Metasequoia glyptostroboides*, *Taxodium distichum*, and *Sequoiadendron giganteum*, growing in urban settings in the city of Sofia, Bulgaria. Leaf samples were collected from mature trees at different sites. Results did not show significant interspecific differences, although slight numerical variation in LDMC was observed among the species. These findings suggest that under the studied urban conditions, the species share similar physiological investment in leaf dry matter, indicating comparable levels of drought resistance. This may be due to shared adaptive strategies to urban stress or insufficient divergence in structural leaf traits under prevailing environmental conditions. Overall, LDMC proved to be a practical and reliable proxy for assessing drought resistance and could be used for proper coniferous species selection in urban areas.

Key words: leaf dry-matter content, drought resistance, urban trees, Cupressaceae, Bulgaria.

INTRODUCTION

Urbanization imposes multiple abiotic stress factors on trees such as elevated temperatures, reduced soil moisture, sealed and compacted soils, irregular precipitation patterns associated with urban heat islands (UHI), and pollution (Percival, 2023). Altogether, this increases evaporative demand and depicts a challenge for plants' water balance, which affects their vitality, resilience, and life expectancy, respectively (Tams et al., 2024). Understanding and identifying reliable and easy to measure functional traits that reflect woody species' drought responses and adaptation strategies is essential for sustainable landscape design and urban forest planning (Haase and Hellwig, 2022).

Under urban conditions, where environmental pressures are strong and consistent, different species may end up with similar trait values because only certain trait combinations can withstand repeated drought, heat, and soil alteration (Su et al., 2021). Studies across urban environments showed that leaf characteristics such as leaf dry-matter content (LDMC) and specific leaf area (SLA) could change in consistent directions as urban stress increases

(Huang et al., 2020; Su et al, 2021). In this context, LDMC - the ratio between absolute dry leaf mass and fresh hydrated mass, is a widely used functional trait that integrates leaf structural investment and water content and often correlates with conservative water use strategies and drought tolerance in woody plants. LDMC is part of the leaf economics spectrum, as higher LDMC commonly indicates denser tissues, slower resource turnover, and greater tolerance of water deficit (Cornelissen et al., 2003; Pérez-Harguindeguy et al., 2013; Tsvetkova & Anev, 2017).

The former family Taxodiaceae, now treated within Cupressaceae (Kusumi et al., 2000; Farjon, 2005), comprises conifers such as deciduous species *Metasequoia glyptostroboides* Hu & W.C. Cheng (dawn redwood), *Taxodium distichum* (L.) Rich. (bald cypress) and the evergreen species *Sequoiadendron giganteum* (Lindl.) J. Buchh. (giant sequoia).

The three species differ in life history and local habitat preferences in their native ranges (Farjon, 2008). They also differ in their adaptive strategies. *Taxodium distichum* tends toward structural adaptation under stress, with increased

wood density and reduced hydraulic vulnerability under stress. Its growth and hydraulic conductivity decline sharply under drought, which may compromise long-term performance in water-limited urban environments (Stiller, 2009). Physiological ecology studies on *M. glyptostroboides* (Vann, 2005) show moderate drought tolerance, but sensitivity during phenological growth stages, such as bud flush and shoot elongation. Species relative water-use efficiency and leaf traits may limit survival under episodic urban water deficits. *Sequoiadendron giganteum*, while capable of surviving multiyear droughts in favorable microsites, displays pronounced canopy stress and foliage dieback under extreme water limitation (Ray, 2017; Baeza et al., 2021). In Bulgaria, these species have been widely introduced and cultivated as ornamental trees, particularly in parks, arboreta, and historic landscapes (Tsavkov and Anisimova, 2013; Sokolov et al., 2016; Anisimova, 2018; Stoyanov and Anisimova, 2025). *Taxodium distichum* and *Sequoiadendron giganteum* have been present in the country for more than a century now (Ganchev and Prokopiev, 1959; Bonev, 1961, 1966, 1972), reflecting their long-standing horticultural value and adaptability to local conditions. Their application across diverse urban landscapes provides an important context for examining their functional traits under contemporary urban stressors.

In this context, the aim of this research is to analyse leaf dry-mass content (LDMC) as a functional trait linked to drought tolerance in the three conifer species: *Metasequoia glyptostroboides*, *Taxodium distichum*, and *Sequoiadendron giganteum*.

MATERIALS AND METHODS

Mature, healthy individuals of each species were sampled from different sites within Sofia's green infrastructure: Doctors' Garden, Sofia University Botanical Garden, the Bulgarian Academy of Sciences (BAS) Botanical Garden, Sofia Zoo, Sofia Tech Park, Borissova Garden, Knyazhevska Garden, Business Park Sofia, Garden "Algeer", Park "Pancharevo Lake", the BAS Scientific Laboratory Complex, "Frederich Joliot Curie" str., "Bulgaria" boulevard, the University of Forestry Arboretum, and South

Park. Site distribution is shown on the map in Figure 1. These green areas represented a range of microclimatic and environmental conditions, differing in soil moisture availability, exposure to air pollution, different degrees of urban heat island influence, and different maintenance levels.

The field-based survey was carried out during the active growing season of 2025. To characterise the overall performance of the examined trees, the study included collecting data on the trees' height, diameter at breast height (DBH), and a phytosanitary status assessment. The latter was done using a five-grade scale adopted from Pencheva (2015), which classifies trees into the following categories: excellent condition (0), good condition (1), satisfactory condition (2), unsatisfactory condition (3), and poor condition or in a mortal state (4).

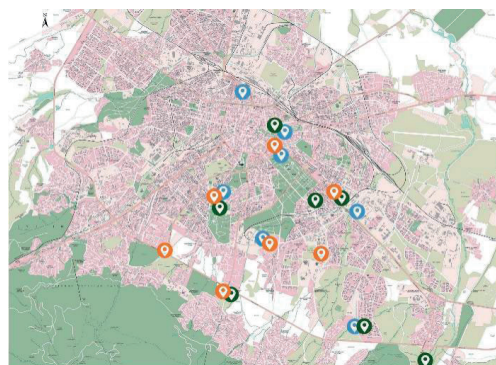


Figure 1. Map of the city of Sofia and site distribution Map underlay source: <http://www.isofmap.bg/> (green – *Metasequoia glyptostroboides*; orange – *Sequoiadendron giganteum*; blue – *Taxodium distichum*)

To evaluate leaf dry-matter content, leaf material was collected from sun-exposed branches at mid-crown height to ensure comparability. Fresh leaf mass was measured immediately after sampling using a precision electronic balance. Samples were then oven-dried at 80°C to constant weight and weighed again to obtain dry mass. LDMC was calculated as the ratio of oven-dry mass to fresh mass for each individual and used as a proxy for tissue density and physiological investment in leaf structure. The gathered data were statistically tested for interspecific differences and site-related variation.

RESULTS AND DISCUSSIONS

A total of 21 individuals were examined, with 12 leaf samples collected from each specimen. Analysis of the specimen-level dataset provides additional context for interpreting LDMC patterns and drought-related performance (see Table 1.). The examined individuals were cultivated in a broad range of urban conditions, including pedestrian squares, street plantings, green areas adjacent to business and scientific complexes, and large urban parks in the city of Sofia. According to Anisimova et al. (2025), Sofia's climate is characterized by pronounced temperature extremes, strong seasonal contrasts, and marked atmospheric drought during summer. Over the 10-year period from 2015 to 2024, daily maximum temperatures reached up to 48.1°C during late July and August, creating intense heat waves that contributed to early leaf discoloration and defoliation in sensitive tree species, particularly in non-irrigated urban areas. Relative humidity reached its lowest levels between June and September, frequently falling below 50%, and at times below 30%, indicating atmospheric drought conditions. Combined with limited irrigation and the urban heat island effect, these climatic stresses create challenging conditions for urban trees in Sofia. The selected specimens also varied substantially in size and maturity: heights ranged from 8.2 to

21.7 m for *Metasequoia glyptostroboides*, 8.2 to 18.3 m for *Sequoiadendron giganteum*, and 12.4 to 16.4 m for *Taxodium distichum*, and DBH values reflecting similar variability. The phytosanitary status was generally good across all species, with most individuals classified in categories 0 and 1, although occasional specimens showed signs of decline, typically in street plantings or in sites exposed to pedestrian pressure. Site-specific characteristics include differing levels of shading, soil sealing, and management intensity. This highlighted pronounced differences in environmental stress: most street-planted individuals received no irrigation, semi-intensive maintenance prevailed in pedestrian squares, and only a subset of park specimens benefited from regular watering. In particular, individuals planted in intensively maintained park settings tended to exhibit better phytosanitary conditions and more favourable growth parameters, whereas those in highly sealed, non-irrigated street environments showed increased stress indicators. Overall, the microhabitat conditions and management regimes vary widely across Sofia's urban landscape, highlighting the importance of considering functional traits such as LDMC in identifying species capable of maintaining stable performance under diverse and drought-prone urban conditions.

Table 1. Characteristics of sampled specimens and their specific site conditions in Sofia's green infrastructure

№	Species	Specimen characteristics			Site	GI' element	Other site specifics		
		Height (m)	DBH (cm)	Phytosanitary condition			Irrigation	Maintenance level	Planting type
1	<i>Metasequoia glyptostroboides</i>	17	45	0	BAS Botanical Garden	Urban garden / park	Yes	Intensive	Group
2	<i>Metasequoia glyptostroboides</i>	15.60	65	1	BAS Scientific Laboratory Complex	Pedestrian square	No	Semi-intensive	Solitaire
3	<i>Metasequoia glyptostroboides</i>	15	52	1	Business Park Sofia	Pedestrian square	No	Intensive	Solitaire
4	<i>Metasequoia glyptostroboides</i>	14	38	2	"Frederich Joliot Curie" str.	Street plantings	No	Extensive	Solitaire
5	<i>Metasequoia glyptostroboides</i>	18.20	71	0	Park "Pancharevo Lake"	Urban garden / park	No	Semi-intensive	Group
6	<i>Metasequoia glyptostroboides</i>	21	67	0	Sofia University Botanical Garden	Urban garden / park	Yes	Intensive	Group
7	<i>Metasequoia glyptostroboides</i>	9	33	0	South Park	Urban garden / park	No	Extensive	Group
8	<i>Sequoiadendron giganteum</i>	17.40	108	0	BAS Botanical Garden	Urban garden / park	No	Intensive	Solitaire
9	<i>Sequoiadendron giganteum</i>	16.20	93	1	BAS Scientific Laboratory Complex	Pedestrian square	No	Semi-intensive	Group
10	<i>Sequoiadendron giganteum</i>	17.10	81.50	1	"Bulgaria" boulevard	Street plantings	No	Extensive	Group
11	<i>Sequoiadendron giganteum</i>	8.20	64	2	Knyazhevska Garden	Pedestrian square	No	Intensive	Solitaire

12	<i>Sequoiadendron giganteum</i>	18.30	106	0	Sofia Zoo	Urban garden / park	No	Intensive	Group
13	<i>Sequoiadendron giganteum</i>	13.60	45.50	1	South Park	Urban garden / park	No	Extensive	Group
14	<i>Sequoiadendron giganteum</i>	16	57	1	The University of Forestry Arboretum	Urban garden / park	No	Semi-intensive	Group
15	<i>Taxodium distichum</i>	16	41	2	Borissova Garden	Urban garden / park	No	Semi-intensive	Solitaire
16	<i>Taxodium distichum</i>	12.40	38	1	Business Park Sofia	Street plantings	Yes	Intensive	Solitaire
17	<i>Taxodium distichum</i>	14.70	44	1	Doctors' Garden	Pedestrian square	No	Semi-intensive	Solitaire
18	<i>Taxodium distichum</i>	13.80	52	0	Garden "Algeer"	Pedestrian square	No	Semi-intensive	Solitaire
19	<i>Taxodium distichum</i>	7	16	0	Sofia Tech Park	Urban garden / park	Yes	Intensive	Solitaire
20	<i>Taxodium distichum</i>	12.60	36	1	Sofia Zoo	Urban garden / park	No	Intensive	Group
21	<i>Taxodium distichum</i>	13.60	39	1	South Park	Urban garden / park	No	Extensive	Group

¹GI – Green Infrastructure

The LDMC raw dataset (n = 21) showed moderate variation both among species and across sites. The LDMC raw dataset (n = 21) showed moderate variation both among species and across sites. Summary descriptive statistics (Figure 2.) indicated that *M. glyptostroboides* exhibited the lowest median LDMC (0.3153), *T. distichum* the highest (0.3587), and *S. giganteum* showed an intermediate value.

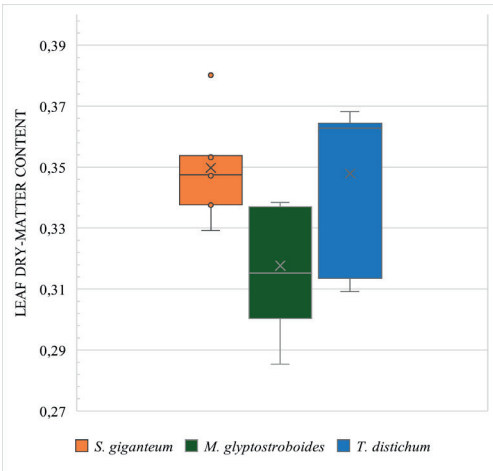


Figure 2. Distribution of leaf dry-matter content (LDMC) for *S. giganteum*, *M. glyptostroboides*, and *T. distichum*

These patterns were reflected across sampling locations. *S. giganteum* exhibited its highest LDMC at South Park (0.3802), *M. glyptostroboides* - its lowest at "Frederich Joliot Curie" Street (0.2853), while *T. distichum* showed comparatively consistent LDMC values across sites (0.3092-0.3682). Given the right-skewed distribution of the data and unequal sample sizes, a Kruskal-Wallis test was applied

to test for interspecific differences in LDMC. The results ($\chi^2 = 2.375$, $df = 2$, $p = 0.305 > 0.05$) indicated no statistically significant differences among the three species. Mean ranks followed the trend *T. distichum* (13.17) > *S. giganteum* (11.88) > *M. glyptostroboides* (8.14), but these differences were not statistically meaningful. High within-species variation, likely driven by microenvironmental heterogeneity, appears to overshadow differences attributable to species' identity.

Despite the lack of significant interspecific differences, the overall LDMC patterns support its use as a proxy for identifying drought-resistant trees. The convergence toward similar LDMC values suggests that all three taxa adopt comparable water-conserving leaf architectures when exposed to Sofia's harsh urban microclimate. Such functional convergence is consistent with environmental filtering, where only certain trait configurations particularly support survival under combined heat, drought, and pollution stress. This interpretation aligns with the "leaf economics spectrum" (LES) described by Wright et al. (2004), in which LDMC is inversely related to growth rate and nutrient turnover, and is associated with conservative resource-use strategies.

These findings are further supported by broader empirical evidence. Münchinger et al. (2023) demonstrated in a comparative study that drought-tolerant conifers typically possess thicker and more rigid leaves, lower specific leaf area (SLA), and greater tissue density, all traits closely aligned with higher LDMC and increased resistance to drought stress. Likewise, global analyses confirm that LDMC responds not only to phylogenetic constraints but also to

environmental pressures such as aridity and climatic stress (Dai et al., 2025).

In the present study, LDMC values did not consistently correlate with site-specific environmental variables (soil moisture, pollution proxies, or microclimate conditions), suggesting that within Sofia's urban context, both species identity and local site variation exert limited influence on leaf structural investment. Pooled values across species and sites fall within the lower-to-moderate LDMC range typical of evergreen conifers in temperate and urban settings, which range from approximately 0.25 to 0.40 g g⁻¹ (Tsvetkova & Anev, 2017), further reinforcing the idea that LDMC serves as an effective comparative indicator of drought resilience under urban stress conditions. The reduced spread of LDMC across the dataset also suggests that urban conditions constrain trait expression, narrowing the variation these species would otherwise display along the LES. However, LDMC represents only one dimension of drought tolerance. While it is a powerful and easily measured proxy for identifying species with more conservative water-use strategies, it does not directly capture hydraulic properties such as xylem vulnerability, stomatal behavior, rooting depth, phenology, or whole-plant water dynamics (Sterck et al., 2024). Studies of drought-induced conifer mortality further show that leaf traits alone cannot fully predict survival, as whole-plant hydraulic resilience and growth legacies often drive mortality risk under prolonged or repeated stress (Song et al., 2022). Similarly, weak coordination has been observed between LDMC and direct physiological indicators of drought tolerance, such as the turgor loss point or stomatal regulation thresholds (Vásquez, 2025).

CONCLUSIONS

Taken together, these findings highlight both the value and the limitations of LDMC as a functional trait proxy. The three studied species - *Metasequoia glyptostroboides*, *Taxodium distichum*, and *Sequoiadendron giganteum*, exhibited similar LDMC values. This indicates that they share a broadly convergent leaf structural investment, suited to drought-prone urban environments, and potentially similar drought-related strategies.

However, because LDMC reflects only one aspect of plant water-use strategy, these outcomes should be regarded as preliminary. A more comprehensive assessment of drought tolerance would require the investigation of additional functional traits and whole-plant hydraulic metrics.

As a pilot study, this research underscores the importance of trait-based approaches in guiding species selection for planning urban GI plantings. Importantly, the observed LDMC ranges align with established ecological strategies documented in the literature, demonstrating that even in the absence of statistical differences, functional traits offer meaningful insight into urban tree resilience and management needs. Even so, LDMC remains a practical indicator for identifying resilient woody species for urban landscaping under climate change, capable of withstanding prolonged heat, frequent drought events, and reduced moisture availability. The long-term survival and performance of the studied Taxodiaceae species in urban plantings in Sofia depend on careful micro-site selection and management.

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LANDSCAPING WITH UNDERUSED TREE SPECIES, A PLANT-BASED SOLUTION FOR GREENER CITIES

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Abstract

Present paper, “Landscaping with underused tree species, a plant-based solution for greener cities”, highlights the essential role of urban plant cultivation as a multifunctional strategy to address the major challenges of the 21st century. In the current context of rapid urban population growth, climate change, food crises, market inflation, and the degradation of the quality of urban life, the use of horticultural species is promoted as a Nature-Based Solution, offering multiple benefits in priority areas such as Food Security, Sustainability, and the Environment, with Socio-Economic Benefits. Because Urban Horticulture includes a wide range of activities, from small home gardens and community gardens to rooftop gardens, vertical farms, and park management, the use of a variety of horticultural species (fruits, vegetables, herbs, ornamentals, and medicinal plants) is presented not only as a food production activity, but as an integrated sustainable development strategy that improves the resilience of cities to climate change and increases the quality of life of citizens through the development of green spaces. The focus of this paper will be on using underused tree species for landscape design and as a main source of biodiversity and sustainability.

Key words: urban; horticulture; plant-based; sustainability; education.

INTRODUCTION

Tree species as part of an urban horticulture strategy represent a form of nature-based solution (NbS) that combines sustainability, participation, and self-sufficiency for improving communities and building greener and healthier cities. The practice of growing fruit and vegetable species within the city’s green spaces has been common in Italy for a long time (Petti B. et al., 2024; Dessì V. et al., 2025), but in other areas, it is an innovative practice. On roofs (Zlati C., et al., 2022; Orsini F. et al., 2014), balconies and facades (Cojocariu M. et al., 2022) abandoned areas, or public spaces (Negrea et al., 2010; 2011), urban horticulture reduces dependence on industrial supply chains and the carbon footprint (Armar-Klemesu M., 2020), while also promoting healthier habits, as one choice drove to another (diet, sports). Pollution is a common problem in urban spaces or post-industrial lands, but the situation can be improved by using compost and other common practices in gardens (Pascu R. et al., 2019; 2022). Shared lots or gardens strengthen social bonds (Ang T.W.B. et

al., 2025), promote environmental education (Girardet H., 2005), and encourage healthy lifestyles (Zlati C. et al., 2021; Song S. et al., 2022). Nevertheless, these practices are known for improving soil quality, reducing pollution (Pascu R. et al., 2021), and, very importantly, transforming neglected areas into productive and welcoming spaces. Thus, urban horticulture, through all known practices (Popa L.D. et al., 2024; Ciurlă L. et al., 2025; Sonea, 2025), proves to be a multifunctional solution, involved in regenerating the urban network, strengthening community relations and resilience.

Plant-based solutions (PbS) as part of NbS are capable of effectively addressing various challenges that may appear within the cities. Heat islands effect and high temperatures are mitigated by trees and green roofs (Zlati et al., 2022), air pollution is reduced due to vegetation that absorbs CO₂ and fine particles; the intensity of weather events is diminished and soil erosion is managed (Gupta K. et al., 2025).

At the same time, landscaping with tree species promotes the well-being of the population, firstly

because of the impact of green areas and community gardens.

These (PbS) approaches not only give us a nice aesthetic, beautiful landscapes, and delightful natural settings, but also promote the connection between people and the environment, contributing to community well-being and environmental sustainability (Kooijman E.D. et al., 2021).

Transforming a sterile place made of concrete into a living park with high vegetation, permeable soil, and small urban gardens makes heat more manageable, offering shade and shelter, promoting biodiversity, and creating an inclusive social space, place to different community events, all in one step (Bernardis R. et al., 2018).

It is known that PbS have the power to solve more problems simultaneously, being considered a valuable strategy for investing in healthier and more livable, greener cities (Gradinariu et al., 2007; Popek R., et al., 2024; Bernardis R. et al., 2024; Istrate M. et al., 2025).

MATERIALS AND METHODS

SWOT analysis was used as a planning tool and it focuses on the main Strengths, Weaknesses, Opportunities, and Threads of the opportunity of using tree species. It was used as a planning tool to ascertain the opportunity and feasibility of each project proposal.

Case studies (CSs)

Green School (CS1) - First case study is located in Valea Lupului commune, Privighetoarea Street, Valea Lupului Village, Iași County (Figure 1).

It is a school that embraces the Green School concept with specific elements that integrates 3 components: the courtyard, green facade, and green roof. For this study, we took into consideration the fruit tree species that are used as focal points or groups.

Urban Oasis (CS2) – The park located in Iași County, 70 Alexandru cel Bun Boulevard (Figure 2). The aim of this project is to restore a degraded neighbourhood park, transformig it into a sustainable urban playground. The central idea is the involvement of the local community in co-design and the use of edible plantings.



Figure 1. CS1 - Green School
Location: Privighetoarea Street, Valea Lupului village, Iași County (<https://earth.google.com/web>)



Figure 2. CS2 - Urban Oasis
Location: Iași County, 70 Alexandru cel Bun Blvd (<https://earth.google.com/web>)

Table 1. Case study comparison

Feature	CS1 Green School	CS2 Urban Oasis
Location Context	Rural/Suburban (Valea Lupului)	Dense Urban (Alexandru cel Bun Blvd)
Green Components	Courtyard Edible garden	Park, playground, edible garden
Primary User	Students Educators	Local community Residents
Method of Creation	Institutional design	Co-design (community involvement)
Ecological Focus	Micro-climate	Biodiversity Urban revitalization
Role of Edibles	Visual Educational Focal points	Participatory Social "Food Commons"
Methodological analysis	Expert-Led mode	Participatory/Co- design

The Green School demonstrates how to integrate nature into compact architecture for educational growth, while the Urban Oasis demonstrates how to integrate nature into dense urban fabric to foster social recovery.

Table 1 allows for a side-by-side evaluation of the two Iași-based projects.

Table 2. Species suited for this type of landscape design

Nr. crt.	Species name
1.	<i>Amelanchier x lamarckii</i>
2.	<i>Amelanchier alnifolia</i>
3.	<i>Aronia melanocarpa</i>
4.	<i>Asimina triloba</i>
5.	<i>Castanea sativa</i>
6.	<i>Cornus mas</i>
7.	<i>Cornus sanguinea</i>
8.	<i>Corylus avellana</i> 'Contorta'
9.	<i>Juglans nigra</i>
10.	<i>Lonicera kamtschatika</i> var. <i>aedulis</i>
11.	<i>Malus floribunda</i>
12.	<i>Mespilus germanica</i>
13.	<i>Pyrus salicifolia</i>
14.	<i>Pyrus calleryana</i>
15.	<i>Prunus cerasifera</i> 'Nigra'
16.	<i>Prunus serrulata</i>
17.	<i>Rosa centifolia</i>
18.	<i>Sambucus nigra</i>
19.	<i>Sorbus aucuparia</i> var. <i>aedulis</i>
20.	<i>Ziziphus jujuba</i>

Another objective of the present research is raising public awareness through strategic partnerships and training new generations of professionals prepared to build a greener future, which is considered of great value despite the economic, climatic, and all other risks.

Design tools

Images presented in the Results section are part of two projects that were designed using Lumion and SketchUp software. Both projects are in the implementation phase.

Planting material

The suitable variety of species (Table 2) offers décor throughout the seasons by flowering plants that bring colour and fragrance, bark, leaves, or fruits that persist on branches even in winter (Negrea R. et al., 2011a; Bernardis R. et al., 2022). This wide range contributes to a lively, fresh, and happy atmosphere.

Some of these species have already been used in the two case studies. Other species are recommended for PbS and could be replicated in other locations.

Main criteria for choosing species:

- high aesthetic value (persistent fruits on branches even in the cold season);
- a source of food for local fauna (birds);
- adaptability and resistance.

RESULTS AND DISCUSSIONS

The results we present involve two existing cases and a couple of PbS for various landscape design using underutilized tree species. Adopting PbS in urban landscapes is a necessary requirement in the context of climate change. To effectively adopt these solutions, important changes in urban planning are absolutely necessary.

In order to ensure that this PbS provides sustainable benefits to the community, we conducted a SWOT analysis.

Table 3 details the SWOT analysis that is proposed to be conducted before the preparation of each project.

Table 3. SWOT Analysis

Strengths	Weaknesses
- Nature-based solutions - New vision and educational alignment - Environmental impact and improved aesthetics - Community Involvement	- Locals resistance to change - Constant maintenance (watering, weeding, pruning) - Need for specialized information
Opportunities	Threats
- Raising public awareness - Training new generations of professionals - Strategic partnerships: (collaboration with different NGOs) - Promotion and image	- Competition for resources - Climate change (drought, flooding, or extreme temperatures) risks - Economic fluctuations - Urban regulations (possible legal conditions)

The main points of strength identified in the study were the new vision and educational alignment with the current social context, community involvement, strong environmental impact, and improved aesthetics.

Despite the high initial costs, constant maintenance, and resistance to change of the population, considered as potential weaknesses, the analysed opportunities pointed out the project feasibility, tipping the balance upwards.

The proposed theme can also be seen as a tool for enhancing urban resilience. Modern cities are presently perceived as "monocultures", dominated by a couple of species: *Tillia*, *Platanus*, or *Acer*, and this makes urban environments very vulnerable to pests and climate shifts. And, let's not forget that diversity is the best insurance policy against climate change.

Underused trees provide unique ecological niches that common ornamental trees might not. Introducing diversity in flowering periods could mean a wider range of birds and insects, providing pollination (Căpraru F. et al., 2009; Zlati C. et al., 2021).

PbS needs to be integrated into building regulations and urban planning, ensuring collaboration among public entities and the private sector, involving communities, and accessing adequate funding. Also, PbS requires an interdisciplinary approach; only with an integrated and long-term plan can cities truly benefit from all advantage, building a more sustainable, resilient, and safe tomorrow.

Green School. The interior courtyard within the school is an extension of the nature-oriented educational concept, providing a protected, quiet and stimulating space for student activities. Landscaping it has a vital importance. Green spaces have a important role of supporting the direct connection with the natural environment, providing a place for relaxation, reflection, and socialization, while also encouraging outdoor learning. Through its integration into the school circuit, the inner courtyard contributes to creating a pleasant microclimate, reducing stress, and promoting children's physical and emotional well-being. The presence of vegetation in learning environments has already a demonstrated impact on students' well-being and, mostly, on performance.

Numerous research highlights the fact that green spaces help reduce stress levels (Negrea R. et al., 2011; Armar-Klemesu M., 2020; Kooijman E.D. et al., 2021; Buțerchi I. et al., 2025), support concentration, and promote the development of emotional intelligence. It is known that children who carry out their activities in a natural setting are calmer, more

receptive, and show greater involvement in the educational process. Direct interaction with plants stimulates curiosity, supports exploratory learning, and contributes to the formation of a positive, lasting bond with the environment. In this context, the Green School in Valea Lupului is not only a sustainable building, but also a living educational space, where nature becomes an active partner in the training of children (Figure 3).



Figure 3. Green School project – fruit tree species (*Malus floribunda*) used as a focal point (Authors)

Urban Oasis. Vegetation in urban parks, playgrounds, and tree-lined avenues significantly lowers temperature through shading and evapotranspiration, effectively counteracting heat islands (Figure 4).



Figure 4. Urban Oasis - the playground layout plan (Authors)

Depending on the structure and composition of the vegetation, they can also play an important role in containing urban pollution. Green areas also provide crucial habitats for pollinating insects and other animals, contributing to urban

biodiversity. For citizens, parks and gardens offer the opportunity to engage in outdoor physical activities, socialize, and relax, thus contributing to mental and physical well-being. Scientific studies (Vladimir I. et al., 2024 and 2025) show that the presence of green areas is correlated with lower stress levels, a lower incidence of disease, and better overall health. Building urban oases in cities is therefore important for both the environment and the well-being of the population.

The idea of an urban oasis can also be replicated in other neighborhoods. In the example from Figure 5 we exemplify a PbS for another Urban Oasis.

This example includes *Prunus serrulata*, *Sorbus aucuparia* var. *aedulis*, *Rosa centifolia*, *Pyrus salicifolia*, *Juglans nigra*, and *Castanea sativa*. Figure 6 is a PbS example for smaller spaces: groups in private gardens or public institutions.

Based on the SWOT analyses, a comparative evaluation between the two case studies was made, as it can be seen in Table 4.

Table 4. Comparative evaluation between the case studies

Feature	CS1 Green School	CS2 Urban Oasis
Site context and typology	A semi-private, institutional space. The design is inward-facing, focusing on the well-being and development of students.	A public, urban commons. This is a "restorative" project located in a high-density neighborhood. Its logic is outward-facing, intended to pull the community into a shared physical space.
Vegetation strategy	Fruit trees as focal points, used for aesthetic structure and botanical observation.	Edible plantings, used for community foraging and "urban farming" concepts.
Architectural integration	Three-dimensional Integrates the built elements.	Horizontal/Landscape Focuses on ground-level park restoration and pathways
Social mechanism	Curricular Greenery is a tool for formal education and ecological awareness.	Participatory Uses co-design to ensure the neighborhood feels ownership.
Primary challenge	Maintenance within a school budget and student safety.	Reversing degradation and preventing vandalism in a public park.
Synthesis of Findings	Acts as a controlled ecological laboratory. It proves how school infrastructure can mitigate its own environmental footprint while providing a "living" focal point for children.	Acts as a social-ecological catalyst. It proves how "edible" urban design can reclaim neglected public spaces and turn them into functional playgrounds



Figure 5. A PbS for a Urban Oasis replica: includes *Prunus serrulata*, *Sorbus aucuparia* var. *aedulis*, *Rosa centifolia*, *Pyrus salicifolia*, *Juglans nigra*, and *Castanea sativa* (Photo Ina Asiminei)



Figure 6. PbS for groups and private gardens (Photo Ina Asiminei)

The use of fruit-growing species can be attractive, especially in rural and peri-urban areas, from the perspective of their availability and improving the natural resources quality (biodiversity, water, soil, landscape). Another advantage is that these species have melliferous potential, which is vital for biodiversity (Negrea R. et al., 2011b; Zlati C. et al., 2024).

In conclusion, if the Green School is about education through learning from nature, the Urban Oasis is about inclusion, living within nature. The use of fruit trees in both cases serves as the bridge between the two, transitioning Iași's landscape from purely decorative to actively productive.

CONCLUSIONS

Concluding our study on Landscaping with Underused Tree Species requires a synthesis of environmental resilience, urban aesthetics, and the urgent need for biodiversity. Integrating fruit species into landscape design is promoted as a closer connection with nature, creating a

friendlier and more harmonious space for the entire community and, at the same time, new sources of food, while ensuring biodiversity.

By approaching PbS design methods that integrate multiple factors, it is expected to create such spaces that combine aesthetics and utility, comfort, sustainability, and the durability of the whole community. Thus, the result will be improving the experience of residents and capitalizing on urban landscapes.

The reintroduction of underused tree species in urban landscape design is not only a botanical preference but a functional necessity. Building truly sustainable greener cities, we have to broaden the perspective on using a "safe" list of 5–10 species frequently selected and embrace a more diverse urban arboretum, including underutilized tree species. This approach reduces long-term maintenance costs, but more importantly, improves inhabitant well-being and also ensures that of our cities "green lungs" don't suffer when the climate changes.

Many of these species have unique canopy structures that could offer better shade density or wind protection compared to overused, thin-

canopied varieties. This could be considered a controlling microclimate strategy. Nevertheless, greener cities shouldn't just be green; they should also be visually engaging throughout the year. Species like *Amelanchier x lamarckii* of *Pyrus calleryana* offer spectacular autumn colors and bark textures that are often missing from the standard urban palette.

The transition to greener cities involves complex collaboration between urban planners, local nurseries, ecologists, and landscape architects to ensure these resilient species are grown and specified in public tenders (Gregory P.J. et al, 2019).

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MISCELLANEOUS



COMPARATIVE NUTRITIONAL ANALYSIS OF FRESH AND CANNED *AGARICUS BISPORUS*

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Abstract

Mushrooms, although they have a low energy value, contain antioxidants, vitamins, and minerals and are an important source of proteins and fibers. The aim of this study was to analyze the nutritional value of fresh and canned Agaricus bisporus using XRF analysis, completed with data from different scientific articles and databases. Fresh Agaricus bisporus generally have a slight nutritional advantage over the canned form, offering lower sodium content and higher levels of certain vitamins. Some protein content may decrease during canning. It is known that during thermal processing, some minerals (such as potassium, phosphorus, copper, selenium, and zinc) leach into the canning liquid (brine), while others may increase if added during processing (e.g., sodium, calcium). Our results show that, in canned mushrooms, compared with the fresh ones, the potassium content decreases and the calcium content increases. Canned Agaricus bisporus have a longer shelf life and provide a better source of fiber and certain minerals, but they also contain significantly higher amounts of sodium, which might be a concern for individuals with cardiovascular conditions.

Key words: XRF, minerals, thermal processing, health implications.

INTRODUCTION

Agaricus bisporus, being of high nutritional value, ranks among the most and consumed mushrooms in the world (El-Sebaaly, 2021).

In accordance with the USDA Food Database, 100 g of fresh *Agaricus bisporus* contain 2.89 g protein, 0.37 g fat, 0.83 g ash, 1.7 g fiber, 0.75 beta-glucan, 0.065 mg thiamin, 0.444 mg riboflavin, 0.077 mg vitamin B6, 9.07 µg biotin, 3.88 mg niacin, 35 µg folate, 0.9 IU vitamin D, 4.3 mg glutathione, 56 mg ergosterol, and the following amino acids: 0.477 g tryptophan, 1.88 g leucine, 1.15 g isoleucine, 1.41 g threonine, 1.7 g lysine, 0.37 g methionine, 1.12 g phenylalanine, 2.48 g valine, 0.655 g histidine, and 4 mg ergothioneine (USDA Food Databases). The main component in *Agaricus bisporus* mushrooms (champignons) that is indigestible in the raw state but becomes more digestible through thermal (heat) treatment, is chitin. The canning process is the treatment that provides long product shelf-life (Cağlarirmak et al., 2001).

During thermal processing, some minerals (such as potassium, phosphorus, copper, selenium,

and zinc) leach into the canning liquid (brine), while others may increase if added during processing (e.g., sodium, calcium).

Cağlarirmak et al. (2001) show that levels of vitamins for freshly *Agaricus bisporus* and canned and stored products (for six month) are: B1 (0.094 mg/100 g - 0.028 mg/100 g), B2 (0.396 mg/100 g - 0.176 mg/100 g), vitamin C (5.72 mg/100 g - 2.31 mg/100 g), folic acid (0.078 mg/100 g - 0.020 mg/100 g), pantothenic acid (2.29 mg/100 g - 1.22 mg/100 g) and niacin (5.35 mg/100 g - 4.29 mg/100 g). In the same study, the values registered for another parameters for fresh and canned *Agaricus* are: protein (3.43-2.24%), fat (0.35- 0.30%), total carbohydrate (3.78-3.84%) and ash (0.71-1.60%) (Cağlarirmak et al., 2001).

Falandysz et al. (2013), report the following values for sodium (mg/kg dry weight) for *A. bisporus*:

- 700 mg/kg dry weight (fresh caps);
- 860 mg/kg dry weight (fresh and whole);
- 16.000-25.000 mg/kg dry weight (conserved).

So, do canned *Agaricus bisporus* provide a better source of fiber and certain minerals? However, they also contain significantly higher

amounts of sodium, which might be a concern for individuals with cardiovascular conditions (Falandysz et al., 2013).

After storage of canned mushrooms, significant decreases in asparagine, alanine, methionine, and glutamine had been registered. Although the canning and storage process of mushrooms caused decreases in the levels of amino acids, the protein quality was not significantly lower than in fresh *Agaricus bisporus* (Jaworska et al., 2012).

In the preservation of mushrooms, mineral levels undergo changes caused mainly by the processes of scalding (blanching) and diffusion into the coating liquid.

According to the mushroom species, age, and the kind of substrate, different mineral contents were reported (Sinha et al., 2019).

The use of calcium-rich substrates (e.g., in casing soil or supplemented with calcium chloride/sulfate) can lead to higher levels in mushrooms (Tang et al., 2023).

Calcium chloride applied to irrigation of *Agaricus bisporus* crops increases the calcium content of the mushrooms and improves their post-harvest quality and shelf life (Miklus et al., 1996).

Shbeeb et al. found 3769 mgkg⁻¹ DW of calcium in whole canned *Agaricus bisporus* (Shbeeb et al., 2020).

Although several potentially toxic elements (Cu, Zn, Fe, Ni) are essential for the human body, they can cause allergic reactions, fatal neurological disorders, nephrotic effects, and hemolysis when ingested at high concentrations (Sinha et al., 2019).

Taking this into consideration, several countries and commissions, such as the USEPA, WHO, and FAO/WHO Codex, have specified maximum limits of potentially toxic elements in mushrooms (Siric et al., 2020; USEPA). Potentially toxic elements are non-degradable and can accumulate, posing high risks to human health when present in high levels in the food chain (Kumar et al., 2022).

Maximum limits for heavy metals such as cadmium, lead, and mercury in mushrooms are strictly regulated in the European Union (including Romania) to ensure food safety by Regulation (EU) 2023/915.

The content of micronutrients varies depending on whether the canned mushrooms are whole or

cut. Dawadi et al., 2022 in their research on *Agaricus bisporus* reached the conclusion that micronutrient contents are higher in whole canned than in cut canned and the fresh type. (Dawadi et al.; 2022).

In their study, Dobrinas et al. found higher values for chromium, copper, and iron in canned mushrooms than in fresh mushrooms (Dobrinas et al., 2013).

MATERIALS AND METHODS

Six commercial samples of fresh *Agaricus bisporus* and six samples of sliced and canned *Agaricus bisporus* mushrooms were collected from Romanian supermarket chains (Timișoara). The origin of the samples is Romania.

Immediately after collection, fresh and canned mushrooms were transported to the laboratory under refrigerated conditions.

We used a portable Hitachi XMET8000 device to determine the minerals by X-ray fluorescence, and we analyzed each sample in triplicate.

The moisture of the samples was determined using a Sartorius thermobalance.

The sterilized containers have the following ingredients: champignon mushrooms (*Agaricus bisporus*), water, salt, citric acid (acidity corrector) and ascorbic acid (antioxidant).

The samples are as follows:

- 1F - 6F = fresh mushroom samples,
- 7C - 10 C = canned in glass packaging,
- 11C - 12C = canned in metal packaging.

The nutritional parameters of canned mushrooms (100g), as indicated on the labels, are as follows: energetic value (15-20 kcal), fat (0.3-0.5 g), carbohydrates (0.2-0.5 g), fibre (2-2.8 g), protein (2.07-2.8 g) and salt (0.45-1 g).

For data analysis, we used PAST version 2.17c and MVSP version 3.22 softwares, in order to explore multivariate relationships among samples of the same mushroom variety in fresh and canned forms. Descriptive statistics (mean ± standard deviation) were first calculated, followed by multivariate techniques including principal component analysis (PCA) and hierarchical cluster analysis (HCA).

PCA was applied to diminish the dimensionality of the dataset and to categorize the main sources of variability in mineral composition among

fresh and processed (canned) mushrooms. PCA has the role of converting correlated variables into a smaller number of orthogonal components, permitting the visualization of patterns, separation between sample types, and identification of the variables contributing most to these differences. HCA was performed to complement PCA by grouping samples based on their general similarity. The combined use of PCA and HCA is widely recommended in food composition studies because it increases the robustness and interpretability of multivariate patterns (Matwijczuk et al., 2022).

RESULTS AND DISCUSSIONS

The moisture content in fresh samples ranged between 87% ÷ 90.2% and in canned samples between 90.6% ÷ 91.8%, respectively. Tables 1 and 2 show the intervals of the analyzed mineral contents for fresh and canned *Agaricus bisporus* detected in the laboratory using the XRF method or reported in the scientific literature.

Our results are similar to literature data, except calcium content in fresh *Agaricus bisporus*, which showed higher values (1946.4 mg/kg dry weight) compared with 1472.66 mg/kg dry weight reported by other researchers (Popescu et al., 2017).

We note that, for each element, there are large variations, a fact explained by the importance of the nature of the substrate and by the fact that *Agaricus bisporus* is a very good accumulator of heavy metals.

Table 1. Mineral’s content of fresh *Agaricus bisporus* (mg/kg dry weight)

Element	Literature Data Ranges	Fresh mushroom samples (1F to 6F)
K	31029÷45200 (Shbeeb et al., 2020) (Bernas et al., 2006)	35747÷ 40463
Ca	360÷1472.66 (Shbeeb et al., 2020) (Popescu et al., 2017)	983.2 ÷1946.4
Fe	49.9÷1596 (Vetter et al., 2003) (Gebrelibanos et al., 2016)	202.2 ÷ 417.6
Zn	46.8÷124.84 (Gebrelibanos et al., 2016) (Sinha et al., 2019)	67.8 ÷ 187.2
Cu	7.4÷125 (Gebrelibanos et al., 2016) (Muszyńska et al., 2017)	17.4 ÷51.6

Table 2. Mineral’s content of canned *Agaricus bisporus* (mg/kg dry weight)

Element	Literature Data Ranges	<i>Agaricus bisporus</i> canned in glass and metal packaging (7C to 12C)
K	1287÷35396 (Vetter et al., 2003)	1366.4÷24303
Ca	2563÷2937 (Vetter et al., 2003) (Shbeeb et al.,2020)	2172÷4132
Fe	116÷485 (Vetter et al., 2003) (Shbeeb et al.,2020)	186.6÷250.2
Zn	60.1 (Vetter et al., 2003)	40.2÷115.8
Cu	15.4 ÷165.2 (Vetter et al., 2003) (Dobrinás et al., 2013)	30.6÷58.2

In Figure 1 we observe that in preserved mushrooms potassium recorded lower values than in fresh mushrooms, while calcium recorded higher values, a fact confirmed by other researchers (Tables 1 and 2). The highest calcium values for calcium were registered in samples 8C and 11C and the lowest in samples 2F-6F.

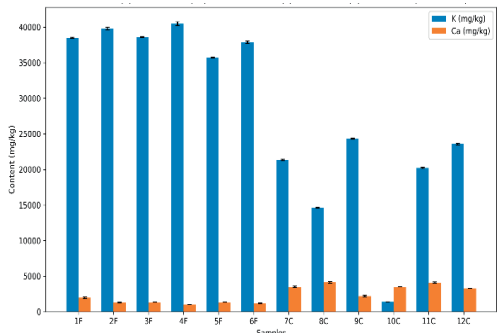


Figure 1. K and Ca contents in fresh (F) and canned (C) samples (Mean±SD) (mg/kg dry weight)

Legend: 1F-6F=fresh mushroom samples; 7C-10C= canned mushrooms in glass packaging; 11C-12 C= canned mushrooms in metal packaging

Analyzing Figure 2, we observe that the iron content in fresh mushrooms varies greatly from one sample to another. The highest iron values were recorded in fresh mushroom samples 1F, 3F and 5F and the lowest in following canned samples: 7C, 8C and 10C.

Regarding the zinc content, we observe that the highest values were found in fresh mushrooms samples 2F and 3F.

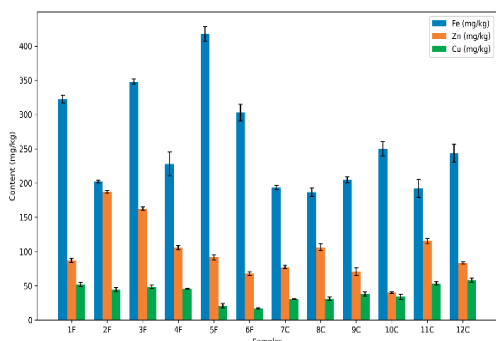


Figure 2. Iron, zinc and copper contents in fresh (F) and canned (C) (Mean $\pm$ SD) samples (mg/kg dry weight) Legend: 1F-6F=fresh mushroom samples; 7C-10C= canned mushrooms in glass packaging; 11C-12 C= canned mushrooms in metal packaging.

The copper content varies as follows: in fresh mushroom samples between 17.4 mg/kg DW (6F) and 51.6 mg/kg DW (1F), in samples preserved in glass packaging between 30.6 mg/kg DW and 38.4 mg/kg DW and in samples preserved in metal packaging recorded the highest values, of 53.44 mg/kg DW (11C) and 58.2 mg/kg DW (12C). The copper level in mushrooms preserved in metal packaging may increase due to the presence of citric acid and ascorbic acid in the coating liquid, since these acids, especially in humid environments and during sterilization processes, may favor the migration (or leaching) of copper from the metal packaging into the product.

The hierarchical cluster analysis was performed using the paired group algorithm (UPGMA – Unweighted Pair Group Method with Arithmetic Mean) in combination with the Euclidean distance as the similarity measure. Before analysis, the data were standardized (z-score normalization) to eliminate the influence of differing variable scales, due to the high sensitivity of Euclidean distance to magnitude differences. To assess the quality of the clustering, the Cophenetic Correlation Coefficient (CCC) was calculated, yielding a value of 0.90. A CCC value of 0.90 indicates an exceptional level of representation, signifying that the dendrogram offers a highly reliable approximation of the underlying data structure, with minimal distortion of the original similarities. Therefore, the clustering results can be considered robust and appropriate for interpretation in terms of grouping patterns and processing effects (fresh vs. canned samples)

(Figure 3). The Cluster Analysis reveals two clusters, separating the fresh samples, colored in green, from the canned samples, colored in red. The sample 10C appears separately from both groups due to the presence of low potassium and zinc contents.

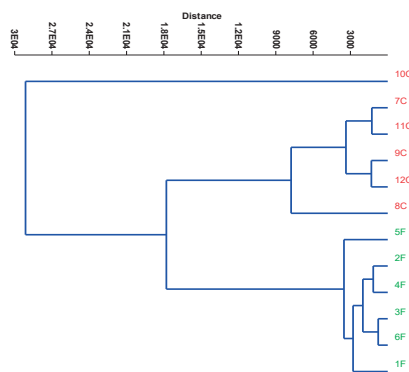


Figure 3. Cluster analysis legend: 1F-6F=fresh mushroom samples; 7C-10C= canned mushroomC= canned mushrooms in metal packaging

The fresh samples (1F–6F) are grouped more closely, indicating higher compositional similarity compared with canned samples. In contrast, canned samples showed more distinct sub-clustering, with pairs such as 7C-11C and 9C-12C exhibiting high similarity, while samples such as 10C and 8C presented greater divergence. These patterns suggest that processing conditions and preservation media contribute to increased variability among canned mushroom products.

Applying PCA using a correlation matrix, a Jolliffe cut-off of 0.7 as well as the broken stick method (Figure 4) shows that we can take in consideration two principal components, PC1 and PC2 (Figures 5 and 6).

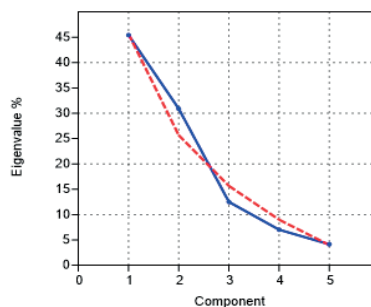


Figure 4. Broken stick

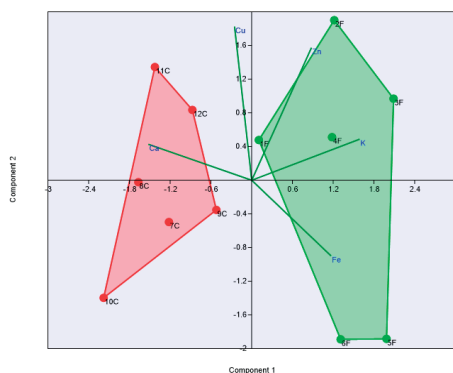


Figure 5. Graphical representation of data on principal components PC1 and PC2

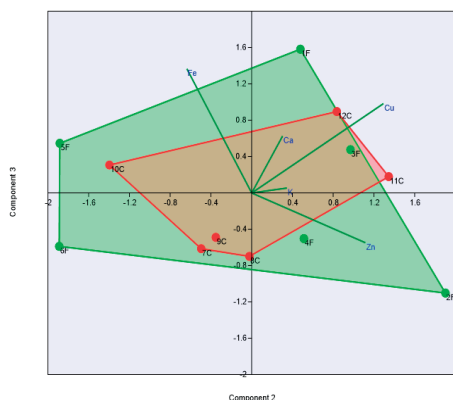


Figure 6. Graphical representation of data on principal components PC2 and PC3

The area of fresh samples is higher compared with that of canned samples, but at the same time it shows that both graphics present properties of the same mushroom assortment, with the difference that one is processed and the other is not processed.

Potassium, iron, and zinc are higher in the fresh samples, while calcium and copper show the highest values in the canned samples, as proven by PC1 and PC2. This indicates that Component 1 primarily reflects the effect of processing, separating fresh mushrooms from canned products. The results confirm the cluster analysis, where processing significantly alters the chemical profile of mushrooms. Fresh samples exhibited greater dispersion, reflecting natural variability, while canned mushrooms formed a more compact cluster, likely due to standardized processing conditions.

Table 3. The Partial Linear Correlation

0	K	Ca	Fe	Zn	Cu
K	0	0.10581	0.32241	0.24036	0.53427
Ca	-0.57431	0	0.77448	0.86133	0.23775
Fe	0.37328	-0.11186	0	0.49778	0.70394
Zn	0.43631	-0.068337	-0.26087	0	0.40252
Cu	0.23981	0.43849	-0.148	0.31915	0

The Partial Linear Correlation (Table 3) shows:

- strong positive correlations between calcium and zinc, and between calcium and iron;
- a high correlation between copper and iron;
- a moderate positive correlation between potassium and copper ;
- moderate positive correlations between zinc and copper, and between iron and zinc;
- a moderate negative correlation between calcium and potassium;

While many studies report high concentrations of both copper and iron together (as they are essential nutrients for mushrooms), the exact Pearson correlation coefficient varies significantly by species and location. The positive or negative linear correlations are highly dependent on the growth medium (substrate) composition. (Shbeeb et al., 2020; Vetter et al., 2003).

CONCLUSIONS

Agaricus bisporus mushrooms have a high nutritional value, regardless of whether they are fresh or canned.

Our results are similar to the literature data, except calcium content in fresh *Agaricus bisporus*, which registered higher values.

Our results show that, in canned mushrooms, compared with fresh ones, the potassium content decreases and the calcium content increases. Iron and zinc register decreases, and the copper content shows a slight increase. These conclusions are confirmed by other researchers (literature data from Tables 1 and 2).

The samples canned in metal packaging registered the highest values of copper content. Because minerals (especially zinc) can be found in large quantities in the canned liquid, discarding it reduces the final nutritional value of the meal.

Accordingly, canned *Agaricus bisporus* provide a better source of fiber and certain minerals, but they also contain significantly higher amounts of sodium, which might be a concern for individuals with cardiovascular conditions.

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PHENOLIC COMPOSITION OF WILD BLUEBERRIES AND WILD CRANBERRIES HARVESTED IN ROMANIA

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Abstract

Wild berries from spontaneous flora represent valuable plant resources due to their high content of phenolic compounds. The objective of this study was to determine the total phenolic content of wild blueberries (*Vaccinium myrtillus* L.) and cranberries (*Vaccinium vitis-idaea* L.) collected from different natural habitats in Romania, Cluj County. Total phenolic content was evaluated using spectrophotometric methods and expressed as mg gallic acid equivalents. The results revealed distinct differences between the two species. Blueberries showed the highest phenolic levels, with maximum concentrations of approximately 1154 mg GAE/100 g of plant material, whereas cranberries presented lower maximum values, reaching about 624 mg GAE/100 g. The findings underline the importance of Romanian wild blueberries and cranberries as natural sources of phenolic compounds and support their potential use in agriculture, food, and nutraceutical applications.

Key words: wild berries, *Vaccinium myrtillus*, *Vaccinium vitis-idaea*, total phenolic content, antioxidant activity.

INTRODUCTION

Wild berries from spontaneous flora have attracted increasing scientific interest due to their high content of bioactive compounds and their associated health benefits (Nowak et al., 2022; Nemzer et al., 2022; Giovanelli et al., 2009). Among these, species of the genus *Vaccinium*, including *Vaccinium myrtillus* L. (wild blueberry) and *Vaccinium vitis-idaea* L. (wild cranberry), are recognized as important sources of polyphenolic compounds with strong antioxidant properties (Ștefănescu et al., 2020). Polyphenols represent a diverse class of secondary metabolites involved in plant defense mechanisms against environmental stress factors such as ultraviolet radiation, pathogens, and oxidative stress. These compounds are widely studied due to their antioxidant capacity and their role in reducing oxidative stress and inflammation in biological systems (Bernal-Gallardo et al., 2022; Goławska et al., 2023). Both wild blueberries (*Vaccinium myrtillus* L.) and cranberries (*Vaccinium vitis-idaea* L.) are characterized by a complex phytochemical

composition, being rich in phenolic acids, flavonoids, and anthocyanins (Neuenfeldt et al., 2022; Nowak et al., 2022). Wild blueberries (*Vaccinium myrtillus* L.) are particularly known for their high anthocyanin content, which contributes to their strong antioxidant activity and potential health benefits, including cardiovascular and neuroprotective effects (Bunea et al., 2011; Hein et al., 2021). In contrast, cranberries (*Vaccinium vitis-idaea* L.) have a different phenolic profile, characterized by higher levels of proanthocyanidins and phenolic acids, which also contribute to their biological activity (Heiss et al., 2022; Gudžinskaitė et al., 2020; Šedbarė et al., 2022). The phenolic composition of these berries is influenced by multiple factors, including geographical origin, environmental conditions, altitude, and genetic variability. Consequently, significant differences may occur in both the qualitative and quantitative profiles of polyphenols, even within the same species (Ciulca et al., 2021; Dyukaryeva & Mallik, 2023; Aliman et al., 2020).

Although both blueberries and cranberries have been extensively studied individually, comparative analyses of their phenolic content, especially in relation to specific geographical regions, remain limited. This highlights the importance of investigating these species together in order to better understand their phytochemical variability and potential applications.

Romania represents a favorable environment for the growth of wild *Vaccinium* species due to its diverse climatic and geographical conditions, particularly in mountainous and subalpine regions. Previous studies have shown that wild berries collected from Romanian habitats exhibit significant variability in phenolic content and antioxidant activity depending on environmental conditions and location (Ciulca et al., 2021; Ștefănescu et al., 2020; Bunea et al., 2011; Brad et al., 2025).

However, despite the abundance of wild berry resources, data regarding the comparative phenolic composition of wild blueberries and cranberries from Romanian natural habitats remain limited. Therefore, the evaluation of total phenolic content in these species is of significant scientific and practical interest (Brad et al., 2025).

The aim of the present study is to determine and compare the total phenolic content of wild blueberries (*Vaccinium myrtillus* L.) and wild cranberries (*Vaccinium vitis-idaea* L.) collected from natural habitats in Romania and to assess their potential as sources of bioactive compounds.

MATERIALS AND METHODS

Sampling collection

Wild blueberries (*Vaccinium myrtillus* L.) and wild cranberries (*Vaccinium vitis-idaea* L.) were collected from five natural locations in Romania, Cluj County: Vlădeasa Mountain, Boaică (B), Pârâul Leucii (PL) and Poiana Horea (PH). In the case of Vlădeasa Mountain, two distinct sampling points were selected, corresponding to different altitudinal zones, namely Vlădeasa Răzoare (VR) and Vlădeasa

Sus (VS). The sampling locations map is described in Figure 1.

The sampling sites were located at altitudes ranging between 700 and 1400 m. Fully ripened fruits were manually harvested, as shown in Figure 2, during the harvesting seasons of 2024 and 2025.



Figure 1. Map of sampling locations



Figure 2. Manual collection of wild cranberry and wild blueberry samples

After collection, the samples were placed in clean containers and transported to the laboratory under refrigerated conditions to minimize biochemical degradation. Prior to analysis, the samples were processed immediately or stored at 4°C for a short period. The samples were coded as described in Table 1.

Table 1. Coding of the collected samples

Sample	Meaning
MVSA24	Cranberry samples collected from Vlădeasa Sus in August 2024
MVSS24	Cranberry samples collected from Vlădeasa Sus in September 2024
MVRA24	Cranberry samples collected from Vlădeasa Răzoare in August 2024
MVRS24	Cranberry samples collected from Vlădeasa Răzoare in September 2024
MB24	Cranberry samples collected from Boaică in 2024
MPL25	Cranberry samples collected from Pârâul Leucii in 2025
MVS25	Cranberry samples collected from Vlădeasa Sus in 2025
MVR25	Cranberry samples collected from Vlădeasa Răzoare in 2025
AB24	Blueberry samples collected from Boaică in 2024
AV24	Blueberry samples collected from Vlădeasa in 2024
AVR25	Blueberry samples collected from Vlădeasa Răzoare in 2025
AVS25	Blueberry samples collected from Vlădeasa Sus in 2025
APH25	Blueberry samples collected from Poiana Horea in 2025

Total Phenolic Content Determination

Total phenolic content (TPC) in wild blueberries (*Vaccinium myrtillus* L.) and wild cranberries (*Vaccinium vitis-idaea* L.) was determined using a modified Folin-Ciocalteu colorimetric method.

Fresh berry samples were collected from natural habitats and manually homogenized using a mortar and pestle prior to analysis.

For phenolic extraction, approximately 1 g of fresh homogenized sample was mixed with 10 mL of 60% ethanol. The mixture was subjected to maceration for 24 h, followed by incubation in a water bath for 3 h. The extracts were then cooled, filtered, and stored at 4°C until further analysis.

Subsequently, the extracts were centrifuged at 4000 rpm for 10 min, and the supernatants were collected for analysis.

An aliquot of 0.1 mL of extract was mixed with 1.4 mL of distilled water and 0.1 mL of Folin-Ciocalteu reagent. After 3 min of incubation, 0.4 mL of sodium carbonate (Na_2CO_3) solution was added. The reaction mixture was then incubated for 60 min at 25°C in the dark to allow color development.

The absorbance was measured at 765 nm using a UV-Vis spectrophotometer (Predescu et al., 2016).

Total phenolic content was calculated using a calibration curve based on gallic acid and expressed as mg gallic acid equivalents per gram of fresh weight (mg GAE g⁻¹ FW) (Figure 3).

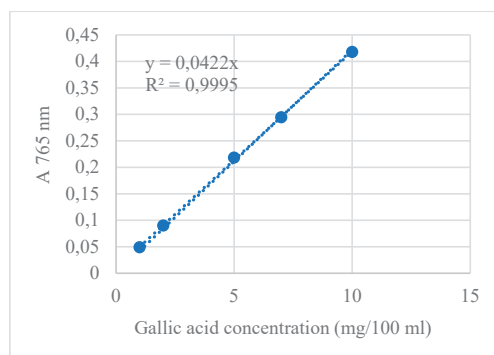


Figure 3. Gallic acid calibration curve used for the determination of the total phenolic content

Statistical Analysis

Statistical analysis was performed using MedCalc® statistical software version 23.4.9 (MedCalc Software Ltd., Ostend, Belgium). All measurements were carried out in triplicate, and the results are expressed as mean ± standard deviation. The normality of data distribution was assessed using the Shapiro-Wilk test, while the homogeneity of variances was evaluated using Levene's test. Since the assumptions for parametric analysis were not met ($P < 0.05$), non-parametric tests were applied. Differences between multiple samples were evaluated using the Kruskal–Wallis test followed by Conover post-hoc comparisons. Differences between wild blueberry and wild cranberry samples were assessed using the Mann-Whitney U test. Statistical significance was considered at $P < 0.05$.

RESULTS AND DISCUSSIONS

Total Phenolic Content of Wild Blueberry and Wild Cranberry

Assessing the phytochemical profile of wild berries is essential for understanding their biological activity and potential applications. Among the various bioactive constituents, phenolic compounds are of particular interest due to their strong antioxidant properties. In this context, the total phenolic content (TPC) of wild blueberries (*Vaccinium myrtillus* L.) and wild cranberries (*Vaccinium vitis-idaea* L.) was determined to provide a comparative evaluation of their antioxidant-related characteristics.

Using the Folin-Ciocalteu method, the total phenolic content (TPC) of wild blueberries (*Vaccinium myrtillus* L.) and wild cranberries

was determined and expressed as mg GAE/100 g fresh weight.

The mean phenolic concentrations of wild cranberry samples ranged from 382.99 to 546.02 mg GAE/100 g, while wild blueberry samples ranged from 491.67 to 1047.45 mg GAE/100 g. In wild cranberries, the lowest mean value was recorded for MVSS24 samples (382.99 ± 2.53 mg GAE/100 g), while the highest phenolic content was observed in MPL25 samples (546.02 ± 3.89 mg GAE/100 g). In wild blueberries, the lowest value was observed in AVS25 samples (491.67 ± 3.30 mg GAE/100 g), while the highest concentration was recorded for AB24 samples (1047.45 ± 7.16 mg GAE/100 g).

Table 2. Mean concentration and standard deviation for total phenolic content (mg GAE g⁻¹) of wild cranberries (*Vaccinium vitis-idaea* L.) samples

Cranberry Samples	Mean TPC (mg GAE /100 g fruit)
MVSA24	419.96bc+2.69
MVSS24	382.99c+2.53
MVRA24	412.16bc+2.67
MVRS24	457.71bc+3.21
MB24	439.18c+2.69
MPL25	546.02a+3.89
MVS25	475.32bc+3.26
MVR25	473.87b+3.22

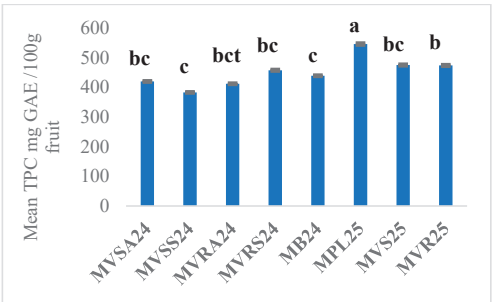


Figure 4. Total polyphenol concentrations in fresh cranberries (*Vaccinium vitis-idaea* L.)

Statistical analysis was performed using MedCalc software. The distribution of the data was assessed using the Shapiro-Wilk test, which indicated a deviation from normality ($P < 0.05$), while Levene's test showed heterogeneity of variances ($P < 0.001$). Therefore, non-parametric tests were applied.

For wild cranberries, significant differences in total phenolic content were observed depending

on location, as indicated by the Kruskal-Wallis test ($P = 0.000002$). Post-hoc analysis revealed that certain samples exhibited significantly higher phenolic concentrations, suggesting a strong influence of environmental conditions.

Table 3. Mean concentration and standard deviation for total phenolic content (mg GAE g⁻¹) of wild blueberries samples (*Vaccinium myrtillus* L.)

Blueberry Samples	Mean TPC (mg GAE /100 g fruit)
AB24	1047.45a+7.16
AV24	963.58a+7.23
AVR25	609.92b+4.06
AVS25	491.67b+3.3
APH25	581.01b+4.47

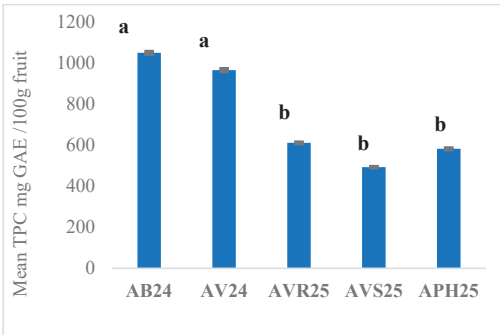


Figure 5. Means for total phenolic content in 100 g fresh blueberries (*Vaccinium myrtillus* L.)

Similarly, in wild blueberries, the Kruskal-Wallis test showed significant differences between samples collected from different locations ($P = 0.000856$), confirming variability in phenolic content depending on habitat.

When comparing the two species, the Mann-Whitney U test revealed a statistically significant difference in total phenolic content ($P < 0.0001$), with wild blueberry (*Vaccinium myrtillus* L.) samples exhibiting higher phenolic concentrations than wild cranberry samples.

These findings indicate that both species and environmental conditions significantly influence phenolic accumulation. The higher phenolic content observed in wild blueberries (*Vaccinium myrtillus* L.) may be associated with species-specific metabolic characteristics and their enhanced capacity for phenolic biosynthesis.

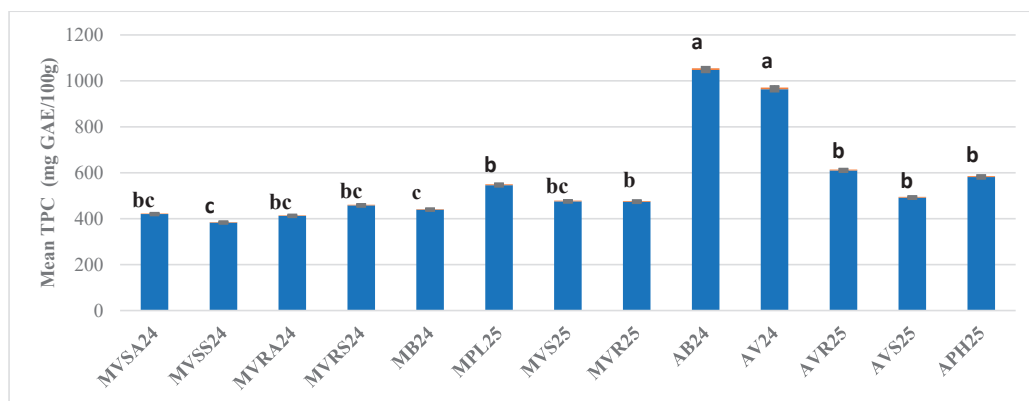


Figure 6. Means for total phenolic content in 100g fresh weight cranberries (*Vaccinium vitis-idaea* L.), respectively wild blueberries (*Vaccinium myrtillus* L.)

CONCLUSIONS

The present study demonstrated that both wild blueberries (*Vaccinium myrtillus* L.) and wild cranberries (*Vaccinium vitis-idaea* L.) represent valuable natural sources of phenolic compounds. However, clear differences in total phenolic content were observed between the two species as well as between sampling locations. Wild blueberry samples exhibited higher total phenolic concentrations, ranging from 491.67 to 1047.45 mg GAE/100 g, while wild cranberry samples showed lower values, ranging from 382.99 to 546.02 mg GAE/100 g. These results confirm that wild blueberries have a greater capacity for phenolic accumulation compared to wild cranberries.

Significant variability in phenolic content was observed among samples collected from different locations, indicating that environmental factors such as altitude and habitat conditions play an important role in influencing phenolic concentration.

Statistical analysis confirmed the presence of significant differences both between samples from different locations and between the two species ($P < 0.05$), highlighting the impact of both species-specific characteristics and environmental conditions on phenolic accumulation.

Overall, the findings emphasize the importance of wild *Vaccinium* species as natural sources of phenolic compounds and support their potential use in food, agricultural, and nutraceutical applications.

ACKNOWLEDGEMENTS

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RESEARCH ON THE DEVELOPMENT OF A LOW-CARB BREAD WITH ANTIOXIDANT POTENTIAL, FORTIFIED WITH *SPIRULINA*, INTENDED FOR THE PREVENTION AND DIETOTHERAPY OF DIABETES MELLITUS AND OBESITY

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Abstract

The adoption of an unhealthy lifestyle (a diet high in sugars and fats, physical inactivity), genetic predisposition, chronic stress, and environmental factors are major causes of diabetes mellitus and obesity. The adoption of a healthy diet, including products adapted to the metabolic disorders associated with these chronic conditions, is of considerable interest. This paper presents research on the development of a low-carbohydrate bread fortified with Spirulina powder (fortification levels of 1-4%), with high nutritional value and antioxidant potential, intended for individuals with diabetes and obesity. This bread exhibits superior sensory qualities and has a low content of total carbohydrates (35.60%) and available carbohydrates (25.65%). It is distinguished by its protein content (14.15%), total dietary fiber (9.95%), mineral content (K = 282.45 mg/100 g; Mg = 83.21 mg/100 g; Ca = 63.42 mg/100 g; Fe = 3.27 mg/100 g), total polyphenols (262.83 mg GAE/100 g) and antioxidant capacity (295.48 mg Trolox equivalents/100 g). Due to its complex biochemical composition and low content of total and available carbohydrates, this type of bread is suitable for the diet of individuals with diabetes and obesity.

Key words: bread, low carb, Spirulina, diabetics, obese individuals.

INTRODUCTION

Diabetes management, including the prevention and remission of type 2 diabetes, is based on effective evidence-based studies. Dietary recommendations and nutrition therapy are essential for improving both life expectancy and quality of life (Mette et al., 2023; Uusitupa et al., 2019). Among the key factors involved in the development of diabetes is the increase in postprandial glucose levels following the consumption of foods with a high glycemic index, such as wheat flour bread (Miller et al., 2024).

Bread is a staple food in countries around the world and is therefore important in global nutrition. The metabolic effect of bread depends on several qualitative aspects, such as the type of grains, carbohydrate and other nutrient content, and preparation methods, etc. Bread quality is determined by its content of dietary

fiber, whole grains, seeds, and, respectively, added sugar in its composition (Ludwig et al., 2018; Pagliai et al., 2021). High consumption of refined grains, which are low in dietary fiber and have a high glycemic index, is associated with an increased risk of type 2 diabetes and other chronic diseases. Therefore, improvements in bread composition could influence population health. Thus, international research has highlighted the beneficial effect of reformulated breads rich in dietary fiber, whole grains, and/or functional ingredients on fasting blood glucose concentrations in adults, mainly among those with type 2 diabetes (Schadow et al., 2023). The consumption of functional bakery products based on whole-grain flours, enriched with seeds and cereals, can improve health and reduce postprandial blood glucose levels as well as the risk of metabolic syndrome (Lange et al., 2024).

Burnete et al. (2020) developed a bread for individuals with diabetes using nutritionally valuable ingredients rich in bioactive compounds (polyphenols, carotenoids, etc.). The product is characterized by a low content of available carbohydrates (38.56-39.49%) and stands out due to its protein content (8.85-9.12%), total dietary fiber (3.15-3.70%), and total polyphenols (100.85-115.12 mg GAE/100 g), while also exhibiting antioxidant capacity (117.45-125.15 mg TE/100 g).

Chiranthika et al. (2024) conducted experiments to develop a functional bread intended for individuals with diabetes, fortified with flours derived from the rhizomes of *Lasia spinosa* and *Nelumbo nucifera* (fortification levels of 20, 40, 60, and 80%). Chemical analysis showed that the fortified breads had higher dietary fiber, resistant starch, and ash contents than the control breads, while protein, fat, and starch contents were lower. The authors also reported that bread fortified with 20% rhizome flour achieved the highest sensory score compared with breads produced with higher fortification levels. Moreover, it exhibited higher levels of dietary fiber, resistant starch, antidiabetic activity, and a lower starch digestibility rate than the control bread.

Jiménez et al. (2025) developed a low-glycemic-index white bread fortified with dietary fiber from *Oxalis tuberosa* (fortification levels of 3%, 6%, and 10%) as a functional ingredient providing nutraceutical benefits.

This paper presents research on the development of a low-carbohydrate bread fortified with *Spirulina* powder (fortification levels of 1-4%), with high nutritional value and antioxidant potential, intended for individuals with diabetes and obesity.

MATERIALS AND METHODS

Materials

“White bread” (Control sample) was obtained from white wheat flour, “Bucovina” still water, natural sourdough, and sea salt.

To obtain the product “Low Carb Bread Fortified with *Spirulina* Powder”, ingredients were selected to ensure a reduction in glycemic index and an increase in fiber content. For this purpose, in addition to *Spirulina* powder, the following were used: natural sourdough fortified

with *Spirulina*, whole wheat flour, *Aronia pomace* powder (20 g/kg whole wheat flour), a seed mix (85 g seeds/kg whole wheat flour; sunflower seeds 35%, flax seeds 20%, white sesame 15%, black sesame 15%, poppy seeds 5%, chia seeds 5%, millet 5%), chicory inulin. Natural sourdough (C) was obtained by fermentation at room temperature for 12 days of a mixture consisting of white wheat flour type 650, whole wheat flour, rye flour, and “Bucovina” still water. Then, 5%, of the amount of wheat flour used to feed the natural sourdough (C) was substituted with *Spirulina* powder. The fermentation of the culture obtained from the natural sourdough, white wheat flour, whole wheat flour, *Spirulina* powder and “Bucovina” still water was carried out under refrigeration conditions (3-8°C), for 30 days (Catană et al., 2024).

The use of wholegrain flour, sourdough, and oilseeds in the bread formulation leads to a reduction in carbohydrate content and glycemic index and, consequently, to a decrease in blood glucose levels approximately one hour after consumption (Lange et al., 2024).

Bread making

For the development of the “Low carb bread fortified with *Spirulina* powder”, four experimental variants were created (Figures 2-3), corresponding to the levels of fortification with *Spirulina* powder: 1%, 2%, 3%, and 4%. In parallel, a control variant was used (“White bread”- C) (Figure 1).



Figure 1. “White bread” (Control sample)



Figure 2. “Low carb bread fortified with *Spirulina* powder” (V1, V2)



Figure 3. "Low carb bread fortified with *Spirulina* powder" (V3, V4)

For the development of "White bread" and "Low carb bread fortified with *Spirulina* powder", a biphasic method was applied.

Methods

Statistical Analysis

"Low carb bread fortified with *Spirulina* powder" (V1, V2, V3, V4) and "White bread" (Control sample) were analyzed in triplicate, and the results are presented as the arithmetic mean and standard deviation.

Sensory analysis

The sensory analysis of "Low carb bread fortified with *Spirulina* powder" (V1, V2, V3, V4) and "White bread" (Control sample) was conducted by evaluating the sensory characteristics (appearance, color, taste, and smell) and by "Comparison method with unitary score scales" method (Burnete et al., 2020). Evaluation of the sensory attributes of the products by a panel of 10 tasters through comparison with scoring scales ranging from 0 to 5 points, followed by the calculation of the average score. Calculation of weighted average scores, summation of these to obtain the total average score, and determination of the product's sensory quality based on the total average score by comparison with a scale from 0 to 20 points.

Table 1. Correlation of total average score with product rating

Total average score	Product rating
18.1.....20	Very good
15.1....18	Good
11.1.....15	Satisfactory
7.1....11	Unsatisfactory
0...7	Failure

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 an Instron Texture Analyzer (model 5944, Illinois Tool Works Inc., USA) equipped with Bluehill 3.13 software.

Slices of bread with a thickness of 25 mm were compressed using a compression piston with a diameter of 12 mm. The analysis method included a compression cycle to a depth of 4 mm, with a compression speed of 1 mm/s. Three measurements were performed for each sample. The testing was carried out at room temperature, and the test parameters were:

- compression speed: 100 mm/min;
- load cell: 50 N.

Using the Bluehill 3.13 software, the following texture parameters were calculated: firmness, elasticity, and cohesiveness.

Physico-chemical analysis

Nutritional composition of the bread samples was determined by the AOAC method: 979.09 (protein content), 963.15 (fat content), 923.03 (ash content) and 985.29 (total dietary fiber). Moisture of the bread samples was determined by a Halogen Moisture Analyzer HE53 (Mettler Toledo). Total carbohydrates were calculated using the formula: 100 (%) -moisture (%) - protein (%) - fat (%) - total dietary fiber (%). The energy value was 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 and zinc was performed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS, model NexION300Q, PerkinElmer) after dry digestion of the samples. 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.

For the determination of minerals, two different analytical techniques were used in order to accurately quantify the mineral content of the studied elements (the zinc and iron content of the analyzed samples is significantly lower than that of potassium, calcium, and magnesium).

ICP-MS operating conditions are presented in Table 2, while HR-CS AAS operating conditions are presented in Table 3.

Table 2: ICP-MS operating conditions

Parameter	Value
Radio Frequency Generator	40 MHz
Radio Frequency Power	1000 W
Nebulizer Gas Flow	0.89 L/min
Auxiliary Gas Flow	1.20 L/min
Plasma Gas Flow	16 L/min
Sample Flow	1.0 mL/min
Replicate Read Time	15 s

Table 3. HR-CS AAS operating conditions

Element	Wavelength (nm)	Gas Flow (L/h)	Burner Height (mm)	Flame Type
Na	588.9953	90	6	Aer-acetilenă
K	766.4908	80	8	Aer-acetilenă
Ca	422.6728	80	6	Aer-acetilenă
Mg	285.2125	70	6	Aer-acetilenă

Bioactive compounds content

The determination of the total polyphenol content was carried out using the Folin-Ciocalteu spectrophotometric method, according to Horszwald and Andlauer (2011), with some modifications (extraction medium, extraction mode and time, and 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, and volume of extract used in analysis), using a UV-VIS Jasco V 550 spectrophotometer at a wavelength of $\lambda = 517$ 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 bread sample.

Microbiological analysis

The microbiological analysis of bread samples 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.

Determination of shelf life (Real-time shelf-life study)

To determine the shelf life of the product “Low carb bread fortified with *Spirulina* powder” (V1–V4), a real-time stability study was conducted. Test batches of the product (for each experimental variant) were produced and stored at room temperature in sealed polypropylene bags. The low-carb bread samples fortified with *Spirulina* powder were analyzed daily for 7 days, as follows:

- Sensory analysis (appearance, taste, smell, aroma);
- Instrumental texture analysis (firmness, elasticity, cohesiveness);
- Moisture analysis;
- Microbiological analysis (yeasts and molds, *Enterobacteriaceae*, water activity).

Failure thresholds (end of shelf life) were established for each analytical method (Table 4), and the shelf life was considered to have ended when any of the analyzed parameters (sensory, microbiological, texture, moisture) exceeded the established limits. A safety margin was also applied: if the product remained within the acceptable limits for all parameters during the 7-day period, its shelf life was defined as 4 days.

Table 4. Failure thresholds (end of shelf life)

Parameter	Failure thresholds (end of life)
Sensory analysis (appearance, taste, smell, aroma)	Sensory analysis (appearance, taste, smell, aroma) does not comply with the provisions of the technical specification of the product
Instrumental analysis of texture	
Firmness (N)	> 25 N
Elasticity	< 0.95
Cohesiveness	< 0.40
Moisture	> 45.4 %
Microbiological analysis	
Yeasts and molds	> 10 CFU/g
<i>Enterobacteriaceae</i>	> 10
Water activity	> 0.98

RESULTS AND DISCUSSIONS

Sensory analysis

The sensory analysis of the product “Low Carb Bread Fortified with *Spirulina* Powder” showed that the addition of *Spirulina* powder (fortification levels of 1-4%) does not negatively affect its taste or smell/aroma. Thus, breads

fortified with this microalga, at the specified concentrations, exhibit a pleasant taste and aroma characteristic of a well-baked bakery product with added *Spirulina* powder. The bread fortified with *Spirulina* powder has a pleasant, characteristic aroma resulting from the synergism between the bread's aroma and the specific aroma of this microalga.

At the same time, the product "Low carb bread fortified with *Spirulina* powder" has an elastic, dense crumb with uniform pores, brown in color with a slight purplish hue, free of lumps, in which seeds (sunflower, white and black sesame, flax, millet, and chia) are visibly distinguishable.

Additionally, the products "Control bread" and "Low-carb bread with antioxidant potential, fortified with *Spirulina*" were also evaluated from a sensory perspective by a panel of 10 assessors, using the "comparison method with unit scoring scales".

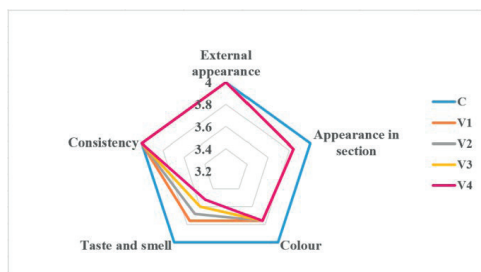


Figure 4. Sensory evaluation of the product "Low carb bread fortified with *Spirulina* powder" and of the "White bread" (C)

Following the sensory evaluation carried out using the "comparison method with unit scoring scales", the products received the rating "very good", achieving the following scores (Figure 4):

- "White bread" (control sample): 20 points;
- V1: 19.36 points;
- V2: 19.28 points;
- V3: 19.20 points;
- V4: 19.12 points.

The addition of ingredients required to reduce the carbohydrate content and glycemic index, as well as to increase antioxidant capacity, leads to a darker color of the product "Low carb bread fortified with *Spirulina* powder" compared to

the "White bread" (C). This is reflected in a 1.30-1.37-fold decrease in lightness (L^*) (Figure 5).

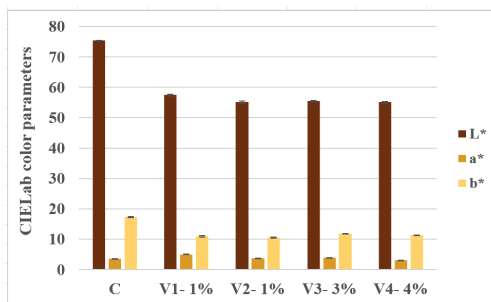


Figure 5. Color parameters of the product "Low carb bread fortified with *Spirulina* powder" (V1, V2, V3, V4) and of the "White bread" (C)

Instrumental texture analysis revealed an increase in firmness in the case of the product "Low carb bread fortified with *Spirulina* powder" compared to "White Bread" (control sample).

The increase in firmness of the low-carb bread is due to the ingredients used to reduce the glycemic index and enhance antioxidant capacity (whole wheat flour, seed mix, *Aronia* pomace powder, etc.).

Thus, 7 days after the date of manufacture, firmness recorded the following values:

- ✓ "White bread" (C)- Firmness = 7.09 N;
- ✓ "Low carb bread fortified with *Spirulina* powder" (V1)- Firmness = 12.89 N;
- ✓ Low carb bread fortified with *Spirulina* powder" (V2)- Firmness = 13.29 N;
- ✓ Low carb bread fortified with *Spirulina* powder" (V3)- Firmness = 23.39 N;
- ✓ Low carb bread fortified with *Spirulina* powder" (V4)- Firmness = 13.47 N.

Figures 6-10 present: the compression curves for the "White bread" (control sample) and the "Low carb bread fortified with *Spirulina* powder" (V1-V4).

The firmness of the product "Low Carb Bread Fortified with *Spirulina* Powder" (V1, V2, V3), measured 7 days after manufacture, is comparable to that reported by Burnete et al. (2020) for "Hypoglycemic Bread with Antioxidant Potential", also measured 7 days after manufacture (Firmness = 13.67 N).

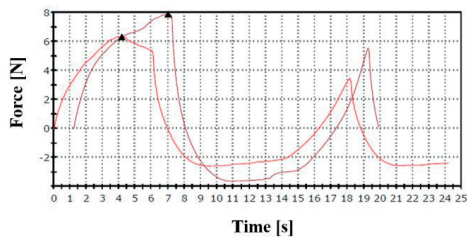


Figure 6. Compression curves for the product "White bread" (control sample)

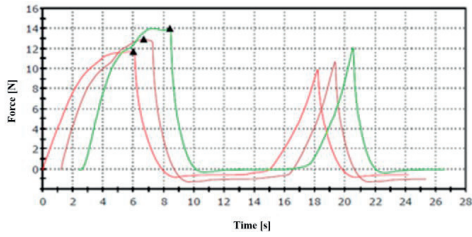


Figure 7. Compression curves for the product "Low carb bread fortified with *Spirulina* powder" (V1)

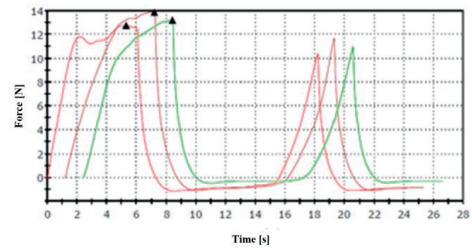


Figure 8. Compression curves for the product "Low carb bread fortified with *Spirulina* powder" (V2)

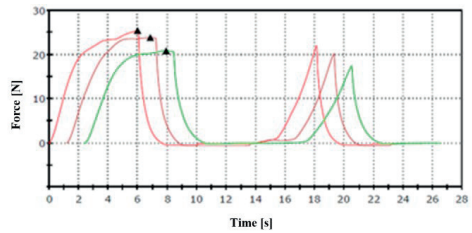


Figure 9. Compression curves for the product "Low carb bread fortified with *Spirulina* powder" (V3)

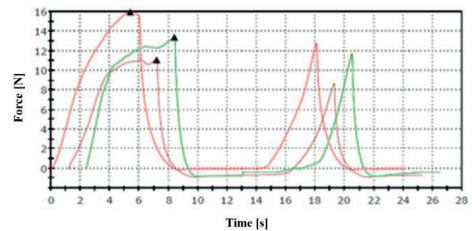


Figure 10. Compression curves for the product "Low carb bread fortified with *Spirulina* powder" (V4)

In Table 5 are presented texture parameters (firmness, elasticity and cohesiveness) of the products "Low carb bread fortified with *Spirulina* powder" and "White bread" (C).

Table 5. The textural properties of the products "Low carb bread fortified with *Spirulina* powder" and "White bread" (C)

Bread	Period (days)	Firmness (N)	Cohesiveness	Elasticity
"White bread"	7	7.09±0.42	0.45±0.04	1.18±0.03
V1	7	12.89±1.05	0.44±0.03	1.01±0.02
V2	7	13.29±0.28	0.43±0.03	1.03±0.03
V3	7	23.39±1.87	0.44±0.02	1.04±0.03
V4	7	13.47±1.86	0.45±0.02	1.02±0.02

Firmness is crucial for bread freshness and is often influenced by dietary fiber, which disrupt the gluten network, hinder gas retention, reduce bread volume, and increase crumb firmness (Djordjević et al., 2021; Ni et al., 2020). Despite high firmness, bread must also exhibit high cohesiveness to prevent crumbling and maintain freshness (Tóth et al., 2022).

Thanks to carefully selected ingredients that ensure proper moisture and high cohesiveness, the product "Low Carb Bread Fortified with *Spirulina* Powder" retains its freshness even 7 days after production. An important contribution to maintaining the freshness of this low-carb bread comes from the natural sourdough fortified with *Spirulina* powder used in its formulation, as well as the application of two-phase technology in its preparation.

Physico-chemical analysis

The physico-chemical composition of the products "Low Carb Bread Fortified with *Spirulina* Powder" and "White Bread" (C) is presented in Table 6.

Table 6. Physico-chemical composition of the products "Low carb bread fortified with *Spirulina* powder" and "White bread" (C)

Component	C	V1	V2	V3	V4
Moisture (%)	41.47±1.04	44.98±1.12	44.06±1.10	43.36±1.08	43.25±1.08
Acidity (degrees)	3.10±0.03	5.6±0.06	5.73±0.06	5.80±0.06	5.86±0.06
Ash (%)	0.47±0.007	1.60±0.02	1.73±0.03	1.86±0.03	1.95±0.03
Protein (%)	7.67±0.12	13.02±0.20	13.39±0.20	13.80±0.21	14.15±0.21
Fat (%)	0.76±0.01	4.77±0.06	4.89±0.06	4.98±0.06	5.05±0.07
Carbohydrates (%)	49.63±0.55	35.63±0.39	35.93±0.40	36.00±0.40	35.60±0.39
Available carbohydrates (%)	49.12±0.54	26.41±0.29	26.37±0.29	26.23±0.29	25.65±0.28
Total dietary fibers (%)	0.51±0.009	9.22±0.17	9.56±0.17	9.77±0.18	9.95±0.18
Salt (NaCl) (%)	0.72±0.005	0.70±0.005	0.69±0.005	0.68±0.005	0.67±0.005
Energy value (kcal/100g)	235	219	222	224	225
Energy value (kJ/100g)	998	921	933	943	943

The physico-chemical analysis revealed that the product "Low carb bread fortified with *Spirulina* powder" has a reduced carbohydrate content, specifically available carbohydrates, and stands

out for its protein, ash, and total dietary fiber contents compared to “White Bread” (C).

According to the results obtained, the moisture of the product “Low carb bread fortified with *Spirulina* powder” ranged between 43.25% and 44.98%, which is comparable to the values reported by Mahmoud et al. (2024) for breads fortified with the microalgae *Chlorella vulgaris*, *Phaeodactylum tricornutum*, and *Tetraselmis chuii* (4% fortification level), where moisture ranged from 41.47% to 42.93%.

Additionally, this bread has higher moisture and lower carbohydrate content compared to the low-glycemic white bread fortified with *Oxalis tuberosa* dietary fiber reported by Jiménez et al. (2025) (Moisture = 32.41%; Carbohydrates = 68.02% d.w.).

Furthermore, the carbohydrate content of “Low carb bread fortified with *Spirulina* powder” is comparable to that reported by Pereira et al. (2024) for multigrain (spelt and lupin) bread fortified with 1.9% (w/w of flour) *Chlorella vulgaris* (Carbohydrates = 36.23%).

The acidity of the product “Low carb bread fortified with *Spirulina* powder” is approximately 1.8 times higher than that of “White Bread” (C), due to the ingredients used in its formulation (whole wheat flour, *Spirulina* powder, *Aronia* pomace powder, etc.). It is comparable to that reported by Hoxha et al. (2023) for bread fortified with pumpkin flour at a 25% fortification level (Acidity = 5.5 degrees). The ash content of the product “Low carb bread fortified with *Spirulina* powder” (V3 and V4) is comparable to that reported by Sya'niy et al. (2026) for white bread fortified with *Moringa oleifera* green leaf stalk powder (Ash = 1.90%) and to that reported by Hoxha et al. (2023) for bread fortified with pumpkin flour at a 5% fortification level (Ash = 1.82%).

The protein content of “Low carb bread fortified with *Spirulina* powder” is 1.3-1.4 times higher than that reported by Zahorec et al. (2024) functional bread fortified with 15% carob pulp powder and 10% dry sourdough (Protein = 10.02%), while the available carbohydrate content is 1.8-1.9 times lower compared with the values reported by the same authors (Available carbohydrates = 48.76%).

Additionally, the protein content of the low-carb bread fortified with *Spirulina* powder is higher than that reported by Görünmek et al. (2025) for breads fortified with the microalgae *Chlorella*

vulgaris and *Micractinium simplicissimum* (fortification levels 1% and 3%; Protein = 9-12.3%).

It is noteworthy that “Low carb bread fortified with *Spirulina* powder” qualifies as a source of protein, as, according to Regulation (EC) No 1924/2006 of the European Parliament and of the Council, at least 12% of its energy value must come from proteins; in this case, proteins contribute 23.78-25.15% of the energy value.

The fat content of the product “Low carb bread fortified with *Spirulina* powder” is lower than that reported by Jiménez et al. (2025) for low-glycemic white bread fortified with *Oxalis tuberosa* dietary fiber at a 10% fortification level (Fat = 6.36% d.w.), but higher than that reported by Pereira et al. (2024) for multigrain (spelt and lupin) bread fortified with 1.9% (w/w of flour) *Chlorella vulgaris* (Fat = 2.55%).

The low-carb bread presented in this experimental study has a high total dietary fiber content (9.22-9.95%), comparable to that reported by Lange et al. (2024) for multiseed bread (Total dietary fibers = 9.3%), and 1.42-1.53 times higher than that reported by Zahorec et al. (2024) for functional bread fortified with 15% carob pulp powder and 10% dry sourdough (Total dietary fibers = 6.48%). Furthermore, the product “Low carb bread fortified with *Spirulina* powder” is rich in fiber, in accordance with Regulation (EC) No. 1924/2006 of the European Parliament and of the Council, having a fiber content greater than 6 g/100 g.

Dietary fiber, especially viscous fiber, can contribute to reducing the glycemic response after consuming carbohydrate-rich foods. Maintaining target blood glucose levels and controlling postprandial glycemia are essential for the prevention and management of diabetes (Giuntini et al., 2022). Additionally, Lu et al. (2023) reported that supplementation with viscous soluble dietary fibers offers potential benefits for glycemic and lipid control in patients with type 2 diabetes. The recommended supplementary dose is 8.3-10.2 g/day, and the recommended duration of supplementation is over 6 weeks.

The nutritional value of the product “Low Carb Bread Fortified with *Spirulina* Powder” is further enhanced by its mineral content (K, Ca, Mg, Zn, and Fe). The mineral element content of this product is presented in Figures 11 and 12.

According to the results obtained, the mineral content of the product increases proportionally with the level of fortification with *Spirulina* powder.

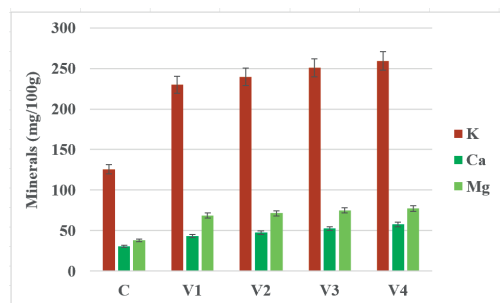


Figure 11. Mineral content (K, Ca, and Mg) of the product “Low Carb bread fortified with *Spirulina* powder” and of the “White bread” (C)

The potassium content of the product “Low Carb Bread Fortified with *Spirulina* Powder” ranged between 230.08 and 259.50 mg/100 g, with the highest value recorded in experimental variant V4 (fortification level with *Spirulina* powder: 4%). The potassium content of this functional bread is comparable to that reported by Zahorec et al. (2024) for functional bread fortified with 15% carob pulp powder and 10% dry sourdough (K = 247.44 mg/100 g), but 1.39-1.73 times higher than that reported by Mahmoud et al. (2024) for breads fortified with the microalgae *Chlorella vulgaris*, *Phaeodactylum tricornutum*, and *Tetraselmis chuii* at a 4% fortification level (K = 132.73-186.62 mg/100 g).

The calcium content of the product “Low Carb bread fortified with *Spirulina* powder” ranged from 43.23 to 57.50 mg/100 g, comparable to that reported by Hoxha et al. (2023) for bread fortified with pumpkin flour at 5% and 10% fortification levels (Ca = 41.61 mg/100 g and 50.51 mg/100 g, respectively), but lower than that reported by Mumtaz et al. (2025) for toasted and wholemeal breads enriched with buckwheat husk at fortification levels of 1.5-4.5% (Ca = 67.1-87.7 mg/100 g).

The magnesium content of the low-carb bread fortified with *Spirulina* powder is 1.8-2 times higher than that of “White Bread” (C) and 3-4.8 times higher than that reported by Mahmoud et al. (2024) for breads fortified with the microalgae *Chlorella vulgaris*, *Phaeodactylum*

tricornutum, and *Tetraselmis chuii* at a 4% fortification level (Mg = 14.05-25.10 mg/100 g). The iron (Fe) content of the product “Low carb bread fortified with *Spirulina* powder” ranged between 3.36 and 3.75 mg/100 g, which is higher than that reported by Mumtaz et al. (2025) for toasted and wholemeal breads enriched with buckwheat husk at 1.5-4.5% fortification levels (Fe = 0.97-1.95 mg/100 g).

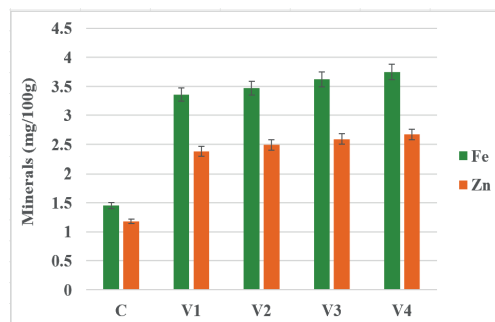


Figure 12. Mineral content (Fe and Zn) of the product “Low carb bread fortified with *Spirulina* powder” and of the “White bread” (C)

The zinc content of the product “Low carb bread fortified with *Spirulina* powder” is approximately 1.4 times lower than its iron content. However, the zinc content of this product is 1.7-1.9 times higher than that reported by Zahorec et al. (2024) for functional bread fortified with 15% carob pulp powder and 10% dry sourdough (Zn = 1.39 mg/100 g).

Bread, as a staple food consumed worldwide, offers significant potential for nutrient fortification due to its widespread popularity and simple production. Among staple foods, bread has been identified as a major contributor to macronutrients, minerals, and vitamins (Falsafi et al., 2025). In this context, fortifying bread with *Spirulina* is of particular interest for increasing its content of proteins, minerals, fiber, and other bioactive compounds.

Bioactive compounds content

Due to some of the ingredients used (whole wheat flour, *Spirulina* powder, *Aronia* pomace powder, sourdough, etc.), the product “Low carb bread fortified with *Spirulina* powder” has a high content of bioactive compounds (total polyphenols, total carotenoids) compared to “White bread” (C). Thus, the polyphenol content

of this bread variety ranged in the range of 244.76-262.83 mg GAE/100 g (Figure 13).

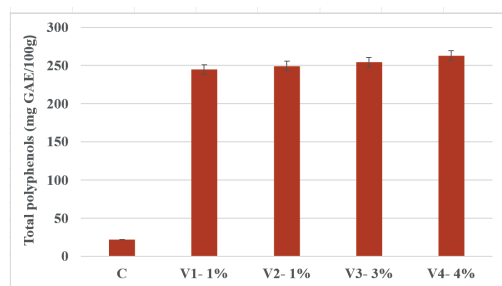


Figure 13. Total polyphenols of the product “Low carb bread fortified with *Spirulina* powder” and of the “White bread” (C)

The total polyphenol content of the product “Low carb bread fortified with *Spirulina* powder” is 1.3-1.4 times higher than that reported by Pandey et al. (2024) for bread enriched with black carrot (*Daucus carota* L.) powder at a 10% fortification level (total polyphenols = 185.78 mg GAE/100 g), and 2.4-2.6 times higher than that reported by Hernández-López et al. (2023) for bread fortified with 2.5% *Spirulina* (total polyphenols = 100 mg GAE/100 g).

Fortification with microalgae to increase polyphenol content is one of the main trends in functional foods aimed at preventing cardiovascular diseases and diabetes (Koli et al., 2022). Numerous studies support the antioxidant, anti-inflammatory, antithrombotic, anti-allergenic, antidiabetic, anticancer, and overall protective properties of polyphenols (Ahmed et al., 2025; Poti et al., 2019). In recent years, the complex symbiotic relationship between dietary polyphenols and the human gut microbiota has become a central focus of scientific investigation due to its profound implications for human health and disease (Jyoti and Dey, 2025). Considering these aspects, the consumption of low-carb bread with a high polyphenol content is of real interest.

The total carotenoid content of the product “Low carb bread fortified with *Spirulina* powder” ranged from 1.09 to 4.37 mg/100 g, with the highest value recorded at the 4% *Spirulina* fortification level (Figure 14).

The total carotenoid content of “Low carb bread fortified with *Spirulina* powder” is higher compared with that reported by Catană et al. (2025) for “Low carb Mini-Baguette Fortified

with *Spirulina* Powder” (total carotenoids = 1.03-3.92 mg/100 g).

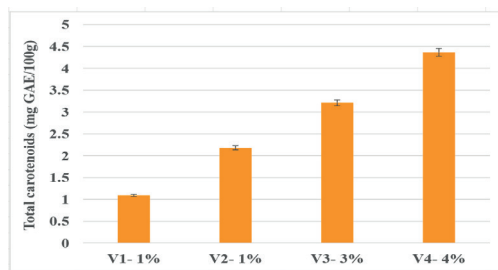


Figure 14. Total carotenoids of the product “Low carb bread fortified with *Spirulina* powder”

Carotenoids are tetraterpenoid pigments that are an essential part of the human diet and have a significant impact on human health. They possess antioxidant, cytoprotective, photoprotective, and other bioactive properties, enabling them to play a protective role against various diseases, including cancer, diabetes, cardiovascular diseases, obesity, and neurodegenerative disorders (Bufka et al., 2024).

Antioxidant capacity

Due to its content of antioxidants (phenolic compounds and carotenoids), the product “Low carb bread fortified with *Spirulina* powder” exhibits notable antioxidant capacity (Figure 15).

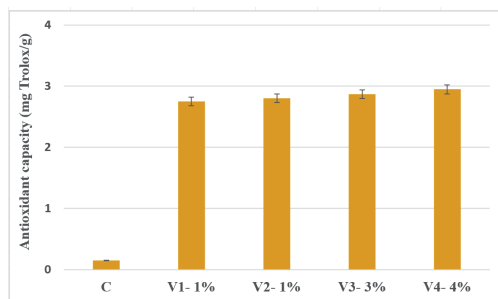


Figure 15. Antioxidant capacity of the product “Low carb bread fortified with *Spirulina* powder” and of the “White bread” (C)

The antioxidant capacity of this bread ranged between 2.75 and 2.95 mg Trolox/100 g, with the highest value recorded at the 4% *Spirulina* fortification level. This antioxidant capacity is 4.07-4.37 times higher than that reported by Calderón de la Barca et al. (2022) for bread made from a flour mix (68.7% wheat, 22.7%

amaranth, 8.6% orange sweet potato), where the antioxidant capacity was 0.675 mg Trolox/g.

Corroborating the results of sensory analyses, physicochemical properties, bioactive compound content, and antioxidant capacity, the experimental variant V4 of the product “Low carb bread fortified with *Spirulina* powder” was selected as the optimal formulation.

Microbiological analysis

The microbiological indicators of the “Low carb bread fortified with *Spirulina* powder” and of the “White Bread” (C) are presented in Table 7. The results of the microbiological analysis confirmed that both products comply with the requirements of the current legislation.

Table 7. Microbiological analysis of the product “Low carb bread fortified with *Spirulina* powder” and of the “White bread” (C)

Microbiological indicators	“White bread” (C)	“Low carb bread fortified with <i>Spirulina</i> powder”			
		V1	V2	V3	V4
Yeasts and molds (CFU/g)	< 10	< 10	< 10	< 10	< 10
<i>Enterobacteriaceae</i> (CFU/g)	< 10	< 10	< 10	< 10	< 10
Water activity	0.935	0.957	0.949	0.943	0.941

The water activity of the products ranged from 0.935 to 0.957. Based on sensory analysis (appearance, taste, smell, and aroma), instrumental texture analysis (firmness, elasticity, cohesiveness), moisture analysis, and microbiological analysis (yeasts and molds, *Enterobacteriaceae*, water activity), it was found that the product “Low carb bread fortified with *Spirulina* powder” (V1-V4) remained compliant even after 7 days from the date of manufacture.

According to the established protocol for shelf-life evaluation of the product, and based on the results of microbiological analyses, sensory evaluation, moisture content, and instrumental texture measurements, the shelf life of the product “Low carb bread fortified with *Spirulina* powder” (V1-V4) was set at 4 days.

CONCLUSIONS

The product “Low-carb bread fortified with *Spirulina* powder” has superior sensory qualities and a complex biochemical composition, making it suitable for the metabolic requirements of diabetics and obese individuals. Thus, this fortified bread has a low carbohydrate content (35.60-36.00%) and available carbohydrates (25.65-26.41%) and stands out

with its protein content (13.02-14.51%), total dietary fiber (9.22-9.95%), and minerals (K: 230.08-259.50 mg/100 g; Ca: 43.23-57.50 mg/100 g; Mg: 68.74-77.43 mg/100 g; Fe: 3.36-3.75 mg/100 g; Zn: 2.38-2.67 mg/100 g).

Additionally, “Low-carb bread fortified with *Spirulina* powder” is notable for its content of bioactive compounds: total polyphenols (244.76-262.83 mg/100 g) and total carotenoids (1.09-4.37 mg/100 g). Due to its antioxidant content, this product exhibits antioxidant capacity (2.75-2.95 mg Trolox/g).

The shelf life of “Low-carb bread fortified with *Spirulina* powder” was established at 4 days.

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ADVANCES IN GREEN AND CONVENTIONAL EXTRACTION METHODS FOR PHYTOCHEMICALS IN HALOPHYTIC PLANTS

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Abstract

*Halophytes are plants adapted to grow in high-salinity environments such as salt marshes and coastal areas. Halophytic plants exhibit biochemically distinct metabolite profiles that depend on various species (e.g., *Salicornia* sp., *Suaeda* sp.) and the extraction methodology; therefore, method selection must be tailored to the target compound class and the particular taxon studied. The modern green techniques, as ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), pressurized liquid extraction (PLE), supercritical fluid extraction (SFE) and natural deep eutectic solvent (NADES)/deep eutectic solvent (DES) based extractions, systematically increase mass transfer, reduce solvent consumption, and often improve recovery and the preservation of labile phenolics and lipophilic constituents relative to conventional maceration or Soxhlet extraction. In conclusion, rational extraction-process design enables the selection of efficient and sustainable extraction workflows that maximize the recovery of bioactive compounds while minimizing environmental degradation and solvent footprint.*

Key words: green extractions, halophytes, conventional extraction, phytochemicals, eco-friendly solvents.

INTRODUCTION

Halophytes represent a specialized group of salt-tolerant flora characterized by their ability to proliferate in substrates featuring high osmotic pressure and ionic toxicity (Lopes et al., 2021; Cioara (Andrei) & Mitelut, 2025). These organisms are distributed across diverse bioclimatic zones, where they have evolved complex adaptive mechanisms to navigate hypersaline conditions. This specialized physiological adaptation positions halophytes as viable candidates for alternative agriculture within saline environments. Furthermore, these taxa represent significant repositories of essential nutrients and secondary metabolites, offering substantial potential for integration into the food and pharmaceutical sectors, and not only (Oulahal & Degraeve, 2022; Palos-Hernández et al., 2022; 2025; Ribas-Taberner et al., 2025). While numerous halophytic species biosynthesize an extensive repertoire of bioactive compounds with pronounced economic and therapeutic utility, their utility is often complicated by the concomitant accumulation of supra-optimal concentrations

of sodium and chloride ions. Traditional extraction techniques, such as maceration or Soxhlet extraction, continue to be widely applied; however, modern green extraction methods, including supercritical fluid extraction or ultrasound-assisted extraction, provide improved efficiency and greater environmental sustainability (Balkrishna et al., 2024).

The extraction of bioactive constituents from halophytes has garnered considerable interest in recent years, driven by the escalating demand for natural and functionally active compounds across the pharmaceutical, food, and cosmetic sectors. Bioactive constituents present in these extracts - including polyphenols, flavonoids, alkaloids, terpenoids, and glycosides - exhibit a wide spectrum of pharmacological activities, notably antioxidant, anti-inflammatory, and antimicrobial effects (Giordano et al., 2021; Aware et al., 2022; Hulkko et al., 2022). Owing to their diverse biological properties, these metabolite compounds have attracted considerable interest for their incorporation into pharmaceutical products, nutraceuticals, functional foods, and cosmetic formulations (Balkrishna et al., 2024). Nevertheless, the

effective translation of their therapeutic potential into practical applications is highly dependent on the implementation of high-performance extraction technologies capable of maximizing extraction while preserving the chemical stability and biological functionality of these compounds.

Emerging extraction paradigms - encompassing green extraction methodologies and nanotechnology-enhanced approaches - are explored with particular focus on their potential to further augment extraction performance and sustainability (Chongo, 2025). The integration of environmentally benign solvents, process intensification techniques, and nanoscale extraction platforms represents a promising frontier in bioactive compound recovery.

Bibliometric data from research and experimental results from recent studies are incorporated to elucidate the application of different extraction techniques for target classes of bioactive compounds, including phenolics, flavonoids, alkaloids, and essential oils (Chaves et al., 2020; Chávez-González et al., 2020). These examples highlight comparative extraction results, retained bioactivity, and potential industrial utility of the resultant extracts (Usman I. et al., 2022; Usman M. et al., 2023).

Recent developments in extraction methodologies have arisen in response to expanding scientific and industrial interest in the bioactive compounds. These innovative methodologies are designed to isolate these compounds from diverse biomass and from natural matrices with greater specificity and reduced environmental burden compared to traditional extraction techniques (Sanoja-López et al., 2024). Phytochemicals, bioactive constituents of plant origin, have attracted considerable scholarly and commercial attention due to their wide-ranging therapeutic properties and utility across pharmaceutical, nutraceutical, and cosmetic industries (de Lima Cherubim et al., 2020). Efficient recovery of these compounds constitutes a crucial step in exploiting their functional potential.

Emerging extraction technologies, including supercritical fluid extraction (SFE), microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), instant controlled pressure drops, pressurized liquid extraction (PLE), and

negative pressure cavitation, have demonstrated enhanced yields, diminished solvent consumption, and improved environmental sustainability relative to conventional methods (Sanoja-López et al., 2024).

For example, SFE using supercritical carbon dioxide affords selective isolation of phenolics, essential oils, and antioxidants while mitigating the need for hazardous organic solvents and preserving thermally labile constituents (Wang et al., 2021; Wang et al., 2024). Similarly, ultrasonic and microwave-assisted techniques facilitate disruption of plant cell walls and improve mass transfer, thereby accelerating extraction kinetics and reducing energy input. Synergistic combinations of extraction approaches (e.g., ultrasonic-microwave-assisted extraction) further underscores the potential for optimizing recovery efficiency.

Recent evidence underscores the potential of integrating disparate extraction modalities to optimize both the quantitative recovery of metabolites and the preservation of their biofunctional attributes (Jha & Sit, 2022). The fundamental rationale for such "hybrid" or "coupled" extraction strategies is to capitalize on the discrete strengths of various techniques, thereby inducing a synergistic effect that transcends the inherent limitations and extractive capacities of any singular methodology (Capaldi et al., 2024).

By strategically sequencing or concurrently applying diverse energy sources - such as acoustic cavitation, electromagnetic radiation, or enzymatic catalysis - researchers can achieve higher process intensification, leading to superior mass transfer rates and the stabilization of sensitive molecular structures that might otherwise be compromised in traditional monolithic systems.

Integration of these advanced extraction strategies within biorefinery frameworks underscores their alignment with circular economy principles and valorisation of plant biomass toward zero-waste paradigms (Alara et al., 2021; Alvi et al., 2022).

As scientific literature has mentioned, several factors such as the types of plant materials, extraction techniques/time, solvents, pH, temperature, pressure, and material-to-solvent ratio can influence the extraction efficiency of bioactive compounds from plant matrices.

Therefore, further investigations to optimize these factors could be advantageous for recovering bioactive compounds from halophyte extracts.

Sophisticated analytical methodologies are pivotal in the characterization and identification of secondary metabolites derived from halophytic matrices. Phytochemical profiling serves as a fundamental cornerstone in elucidating the bioactive constituents within these salt-tolerant taxa, providing the empirical basis for their pharmacological evaluation (Pai et al., 2022). For initial separation and chromatographic fingerprinting, thin-layer chromatography (TLC) and its high-performance evolution (HPTLC) function as economically viable diagnostic tools for high-throughput preliminary screening.

Conversely, high-performance liquid chromatography (HPLC) and its advanced iterations - ultra-high-performance (UHPLC) and ultra-performance liquid chromatography (UPLC) - remain the established benchmark for the sensitive quantification of polyphenolic compounds, flavonoids, and glycosides (Chuo et al., 2022). For the analysis of lipophilic and volatile fractions, gas chromatography-mass spectrometry (GC-MS) is uniquely optimized for profiling volatile oils and terpenoid assemblages (Hubschmann, 2025).

The synergy of these integrated analytical approaches fortifies quality control protocols, ensures rigorous standardization, and accelerates the trajectory of natural product-based drug discovery. Consequently, systematic phytochemical analysis continues to serve as a critical nexus, bridging ethnobotanical traditional knowledge with the rigorous requirements of contemporary pharmaceutical applications (Kumar et al., 2023).

A paramount challenge in the field of halophyte extraction involves the rigorous standardization of extracts, as the phytochemical profile of an extract is subject to significant fluctuations dictated by taxonomic specificity, geoclimatic provenance, environmental stressors, and the phenological stage at the time of harvest.

This inherent biochemical heterogeneity complicates the maintenance of inter-batch consistency, posing a substantial impediment to the development of pharmaceutical and nutraceutical formulations, where the

preservation of specific bioactivity levels and toxicological safety profiles is mandatory. To mitigate these challenges, sophisticated analytical platforms, including high-performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC-MS) (Hubschmann, 2025), and nuclear magnetic resonance (NMR) spectroscopy, have become indispensable for the comprehensive metabolomic profiling and qualitative standardization of halophyte extracts.

The integration of these advanced instrumental techniques not only facilitates enhanced reproducibility across production cycles but also provides a quantitative framework for identifying optimized extraction methodologies that ensure the maximum retention of bioactive constituents.

The present review synthesizes recent progress in phytochemical extraction, critically examining the influence of novel techniques on human health outcomes, ecological sustainability, and industrial applications (Custodio et al., 2022). It also delineates current challenges and prospects for improving extraction efficiency and eco-sustainability, including the adoption of innovative solvents, process intensification strategies, and hybrid technologies.

Extraction methods of phytochemical constituents

The extraction of bioactive compounds represents the fundamental and most critical prerequisite for the subsequent separation and characterization of phytochemical constituents (Mandal et al., 2015; Shikov et al., 2022; Bommakanti et al., 2023; Bouizgma et al., 2025).

Phytochemical extraction separates bioactive constituents from plant matrices using methods chosen based on compound polarity, stability, and desired purity, applying conventional techniques like maceration, decoction, or Soxhlet extraction (solvent-based, often high-temperature), or modern, efficient methods such as Microwave-Assisted Extraction (MAE), Ultrasound-Assisted Extraction (UAE), High Voltage Electrical Discharge (HVED-assisted extraction) (Abbaspour et al., 2024) or Supercritical Fluid Extraction (SFE) (Verma &

Himshweta, 2023; Veršić Bratinčević et al., 2023).

This process necessitates a series of preparatory stages - including pre-washing, dehydration (via solar desiccation or lyophilization) and pulverization in order to ensure sample homogeneity and to maximize the surface-area-to-volume ratio, thereby enhancing solvent penetration and mass transfer. Effective extraction requires careful solvent selection: polar (water/ethanol) for phenolics or non-polar (hexane) for terpenoids, to ensure maximum yield and stability of compounds (Mgoma et al., 2025).

1. Conventional Extraction Methods

These are widely used, simple, and cost-effective methods, though they often require longer times and higher solvent volumes (Manousi et al., 2019; Martins et al., 2023).

✓ **Maceration:** Plant material is soaked in a solvent (e.g., ethanol, water) at room temperature for several days, requiring occasional stirring.

✓ **Percolation:** Solvent is passed slowly through a packed column of plant powder, ensuring continuous exposure to fresh solvent, making it more efficient than maceration.

✓ **Soxhlet Extraction:** Used for continuous extraction, where the sample is placed in a thimble and continuously washed with hot, vaporized solvent. It is efficient for lipids and less-soluble compounds but is not suitable for heat-sensitive substances.

✓ **Hydrodistillation & Steam Distillation:** Used primarily to extract volatile essential oils. Plant material is boiled in water, with compounds separating based on volatility and immiscibility.

✓ **Decoction & Infusion:** Specifically for water-soluble, heat-stable compounds (decoction) or delicate herbs (infusion) in water. Best for water-soluble and thermostable compounds.

2. Advanced/Modern Extraction Methods

These methods are faster, more efficient, and use less solvent (Green Extraction) (Zhang et al., 2020; Yeasmen & Orsat, 2023; Pogorzelska-Nowicka et al., 2024; Zaky et al., 2024).

➤ **Ultrasound-Assisted Extraction (UAE):** Uses high-frequency sound waves to induce cavitation, breaking cell walls to release compounds and improving mass transfer (Yusoff et al., 2022; Shen et al., 2023; Tymczewska et al., (2023)

➤ **Microwave-Assisted Extraction (MAE):** Uses electromagnetic energy to heat the sample directly, accelerating extraction and breaking down cell walls via internal pressure. Highly efficient for polar compounds.

➤ **Supercritical Fluid Extraction (SFE):** Employs supercritical fluids (usually) to extract lipophilic compounds under specific temperature (40–80°C) and pressure (35–70 MPa) making it ideal for thermolabile compounds and non-polar lipophilic substances (terpenoids, carotenoids).

➤ **Pressurized Fluid Extraction (PFE)/Accelerated Solvent Extraction (ASE):** Uses elevated pressure (50-300 psi) and temperature (50-200°C) to keep solvents in a liquid state, above their boiling point, which significantly increases extraction speed and efficiency.

➤ **Enzyme-Assisted Extraction (EAE):** Uses enzymes (e.g., cellulase, pectinase) to break down plant cell walls, releasing compounds (Costa et al., 2020).

➤ **Pulsed Electric Field (PEF) Extraction:** A non-thermal technique that increases cell membrane permeability (electroporation).

➤ **Natural Deep Eutectic Solvent (NADES) Extraction:** A green method using biocompatible, biodegradable solvents (Costa et al., 2022; Aktaş & Kurek, 2024; Suthar, 2025).

Table 1 Extraction of biochemical compounds from halophytes

No.	Biochemical compounds		Best Methods	Solvents	Conditions	Resources
1	Polyphenols (Flavonoids, Tannins, Phenolic Acids)		MAE, UAE and SFE are favored for high efficiency. EAE is used to break down cell walls to release bound polyphenols.	Polar solvents (methanol, ethanol, water, acetone) or their mixtures (70% ethanol-water).	Moderate temperatures (40°C–60°C) are preferred to avoid thermal degradation.	Chávez-González et al., 2020 Alara et al., 2021 Alvi et al., 2022 Bener et al., 2022 Anaya-Esparza et al., 2023 Chocieł et al., 2024 Bouizgma et al., 2021
2	Terpenoids (Essential Oils, Monoterpenes, Sesquiterpenes)		HD is the traditional standard, while DS is used for volatile components. For higher quality and yield is used particularly for non-polar terpenoids.	Non-polar solvents like hexane, petroleum ether, or chloroform.	Room temperature for maceration (to protect delicate oils) or high-temperature distillation for stable terpenoids.	Usman I. et al., 2022 Usman Mm et al., 2023
3	Alkaloids		Percolation or maceration Modern techniques like MAE or UAE are used to enhance yield.	Ethanol, ether, or chloroform.	Acidified alcohol is often used to form salts, increasing solubility.	Mandal et al., 2015 Soares et al., 2023 Bagade & Patil, 2021 Pogorzelska-Nowicka, 2024
4	Pigments	Carotenoids/Chlorophylls (Lipophilic)	Soxhlet, cold press methods or green extractions (NADES, SFE)	Non-polar solvents (hexane, petroleum ether, chloroform, acetone)	Low temperature and protection from light are critical due to instability.	Bashir et al., 2023 Hulkko et al., 2022
		Anthocyanins (Hydrophilic)		Acidified polar solvents (methanol or ethanol with HCl).		Wu et al., 2020
5	Polysaccharides and Dietary Fiber		Hot water extraction or decoction.	Water.	Water extraction followed by ethanol precipitation (water extraction and ethanol precipitation - WEEP).	Espro et al., 2021 Martins et al., 2023 Soares et al., 2023
6	Proteins and Amino acids		Enzyme-Assisted Extraction (EAE)	Aqueous buffers (acetic acid/sodium acetate)	Low temperatures	Mandal et al., 2015 Pogorzelska-Nowicka, 2024
7	Glycosides (e.g., Saponins) and Alkaloids		Maceration, percolation or reflux extraction.	Aqueous ethanol or methanol.	Can break down the cell wall matrix to improve yield	Mandal et al., 2015
			Enzyme-assisted extraction or chromatography-based methods	Cellulases/pectinases (enzymes derived from <i>Aspergillus sp.</i> , <i>Bacillus sp.</i>)		Martins et al., 2023 Mgoma et al., 2025

MATERIALS AND METHODS

A comprehensive and systematic literature search was performed across the Scopus, PubMed, and Web of Science databases. The comprehensive search encompassed included studies, articles, review papers, and books published from 2020 onward, as publications prior to this period did not address the central focus of the present study (green extraction methodologies) (Yeasmen et al., 2021; Kraemer et al., 2025). The search was limited to English-language articles published within selected dates in the databases.

The search strategy combined terms related to halophytes, types of extraction, bioactive compounds, and kinds of solvents using the following Boolean expression: (“extract” OR “extraction” OR “extracting”) AND (“solvent” OR “soluble” OR “solution”) AND (“bioactive compounds” OR “phytochemicals” OR “phytonutrients”) AND halophyte.

To further refine the results, a secondary search component will be incorporated using the

Boolean operator “AND NOT” with the following terms: “chemical composition” OR “nutraceutical” OR “bioresources”. This approach is implemented to exclude studies not pertaining to halophytes and those extracts.

Additionally, the reference lists of all included articles were manually screened to identify further relevant studies and ensure comprehensive coverage of the literature. The reference lists predominantly include studies from recent years, as halophytes have only relatively recently become the subject of extensive scientific investigation and analytical research.

VOSviewer is a software tool for constructing and visualizing bibliometric networks. These networks may include journals, researchers, or individual publications, and they can be constructed based on citation, bibliographic coupling, co-citation, or co-authorship relations (<https://guides.lib.purdue.edu/c.php?g=706710&p=10841603>).

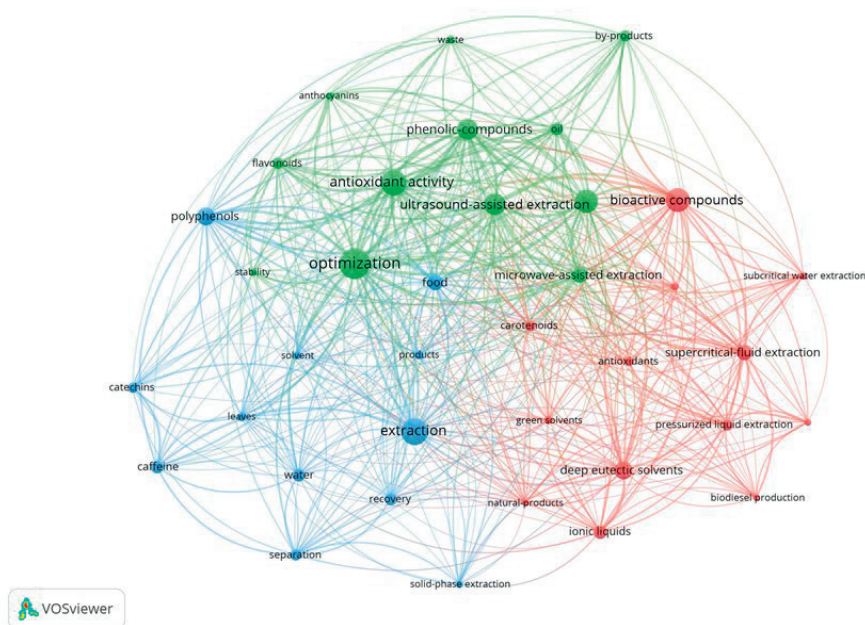


Fig. 1 Map based on bibliographic data

Cluster 1, highlighted in red, includes 13 keywords closely linked to the green extraction of natural products. Central to this cluster is "bioactive compounds", which represents bioactive compounds that combat free radicals, enhancing health benefits. The term "natural products" further emphasizes their effectiveness in applications like health, nutraceuticals, etc. Together, these keywords underscore the interconnectedness of sustainable practices and the extraction of beneficial compounds in green extraction research.

Cluster 2, highlighted in green, encompasses 12 keywords that are vital for understanding green extraction. The term "optimisation" serves as a core concept, referring to the process of isolating valuable compounds from natural sources. The keyword "antioxidant activity" indicates the focus on bioactive compounds that provide health benefits by mitigating oxidative stress. Together, these keywords illustrate the essential components and innovative techniques that characterize green extraction, emphasizing the importance of sustainability and the extraction of beneficial compounds.

Cluster 3, highlighted in blue, comprises five key terms that are essential for understanding the green extraction of natural products. The

term "extraction" refers to natural substances that have significant effects on living organisms, playing a crucial role in health and nutrition. "Polyphenols" encompass the various effects these bioactive compounds have, including antioxidant, anti-inflammatory, and antimicrobial properties, highlighting their potential applications.

RESULTS AND DISCUSSIONS

Conventional extraction methods and their limitations

Decoction, infusion, and maceration represent three fundamental solid-liquid extraction methodologies extensively utilized for the isolation of bioactive secondary metabolites from diverse botanical matrices (Hidayat & Wulandari, 2021). Each technique is governed by distinct thermodynamic and kinetic parameters, which dictate its specific procedural application and resultant extractive efficiency. A critical appraisal of the operational nuances inherent to these processes is imperative for the selection of an optimized extraction protocol, as the efficacy of the methodology is strictly contingent upon the morphological characteristics of the plant material, such as

lignification and tissue density, and the physicochemical stability of the target phytoconstituents.

Decoction represents a rigorous thermal extraction methodology involving the sustained boiling of vegetal material within an aqueous medium to facilitate the liberation of bioactive secondary metabolites. This technique is specifically efficacious for the processing of recalcitrant and highly lignified tissues, such as rhizomes, barks, and seeds, where elevated temperatures are requisite to disrupt the robust cellular architecture and enhance the mass transfer of intracellular solutes.

Notwithstanding its utility, decoction is subject to several inherent limitations, including prolonged processing durations and the potential for the thermal degradation of heat-sensitive nutritional constituents. Furthermore, continuous exposure to high temperatures frequently induces the volatilization of essential oils, leading to a substantial loss of volatile phytochemicals and a potential alteration in the qualitative pharmacological profile of the resulting extract.

Infusion represents a moderate thermal extraction methodology characterized by the submersion of botanical matrices in either ambient or sub-boiling aqueous solvents, deliberately avoiding the rigorous kinetic conditions of decoction. This technique is primarily indicated for the processing of non-lignified tissues (foliar, floral, and herbaceous structures) where the preservation of labile volatile constituents is prioritized over exhaustive leaching.

While advantageous for maintaining the chemical integrity of sensitive compounds, infusion typically demonstrates a reduced total extractive yield relative to more aggressive thermal methods, as it lacks the thermal energy required to penetrate recalcitrant cellular barriers effectively. Furthermore, the methodology often proves inadequate for the comprehensive recovery of bioactive metabolites from specialized taxa, such as halophytes, where complex physiological adaptations and specialized integuments may necessitate more robust extraction parameters to facilitate complete solute liberation.

Maceration represents a classical solid-liquid extraction methodology traditionally employed

in the preparation of phytopharmaceuticals, remaining a cornerstone of natural product isolation due to its inherent cost-effectiveness and technical simplicity. During this process, crushed botanical matrices are submerged in an appropriate solvent inside a hermetically sealed vessel. The system is maintained in a static state for a prolonged incubation period, ranging from several hours to several days, augmented by intermittent agitation to minimize boundary layer resistance and facilitate solute transport. This extended contact time allows for passive intra-particle diffusion of the solvent across the semi-permeable plant cell walls. Consequently, the solvent facilitates the solvation and subsequent leaching of intracellular bioactive secondary metabolites into the bulk liquid phase until a thermodynamic equilibrium is approached. Unfortunately, the process has several major disadvantages: very slow and time-consuming, consuming, and the solvent required is considerable.

The percolation technique utilizes a conical, narrow-necked apparatus known as a percolator, characterized by apertures at both the proximal and distal ends. In this technique, the solid matrix is pre-moistened with a solvent prior to its introduction into the vessel to facilitate the efficient extraction of bioactive compounds (Luksta & Spalvins, 2023). Percolation offers several distinct advantages over traditional maceration, including enhanced temporal efficiency, the ability to isolate thermolabile constituents without thermal degradation, and high suitability for the extraction of potent or high-value pharmacological agents. Conversely, the technique is subject to certain limitations, most notably substantial solvent consumption and extended processing durations when compared to Soxhlet extraction. Furthermore, the process requires work experience, as the particle size of the solid material is a critical parameter to ensure optimal extraction kinetics and prevent the formation of preferential flow paths.

The **Soxhlet extraction** protocol constitutes a preeminent methodology for the continuous, iterative solid-liquid extraction of botanical or synthetic matrices via a heated organic solvent within a dedicated glass refluxing assembly (López-Bascón & De Castro, 2020). As a highly standardized and well-established technique, it

frequently exhibits superior performance and higher recovery yields relative to conventional batch extraction methods. The defining feature of the Soxhlet mechanism is the cyclic exposure of the comminuted matrix to fresh, distilled solvent, which maintains a maximal concentration gradient and ensures the exhaustive leaching of solutes into the bulk liquid phase. While the method is widely utilized in the isolation of nutraceuticals and offers a cost-effective operational framework, it is subject to several inherent limitations. Notably, although the user mentions its use for thermolabile compounds, the continuous boiling of the extract in the collection flask frequently poses a significant risk of thermal degradation for heat-sensitive constituents. Furthermore, the process is characterized by prolonged extraction durations, high solvent-to-feed ratios, and the absence of mechanical agitation within the extraction thimble, which may limit the kinetics of mass transfer for certain recalcitrant analytes.

Hydrodistillation (HD) and Steam Distillation (SD) are conventional methods used to extract essential oils by vaporizing volatile compounds from plant material using heat and steam, which are then condensed and separated. HD involves immersing plant material in boiling water, while SD passes steam through the halophytic material. SD is often better for reducing distillation time, whereas HD can cause decay due to prolonged contact with boiling water. Although HD can often result in higher yields for some halophytes, SD can produce higher-quality oils in specific applications.

Advanced extraction techniques for enhanced bioactivity

Microwave-assisted extraction (MAE) operates through the dual mechanisms of dipole rotation and ionic conduction, driven by the displacement of charged ions within both the solvent and the botanical matrix (Camel, 2000; Mketo & Nomngongo, 2023). While MAE is highly effective for the isolation of specific polyphenolic subclasses, such as flavonoids, its application is often constrained by the intense thermal kinetics involved (Aguiar et al., 2022). Consequently, more sensitive phytochemicals, including tannins and anthocyanins, may undergo significant thermal degradation or structural transformation, necessitating precise

calibration of irradiation power and duration to preserve the biochemical integrity of the extract (Bhadange et al., 2024).

Ultrasound-assisted extraction (UAE) utilizes acoustic energy at frequencies exceeding 20 kHz to facilitate the mechanical disruption of botanical cell walls, thereby augmenting the effective surface area available for solvent penetration and the subsequent liberation of intracellular secondary metabolites (Dzah et al., 2020; Demesa et al., 2024). UAE accelerates extraction kinetics and significantly reduces both processing durations and solvent consumption while maximizing the total extractive yield. However, the intense energy levels associated with UAE may initiate sonochemical reactions, necessitating careful optimization of sonication power and pulse cycles to preserve the biochemical integrity of the extract (Anaya-Esparza et al., 2023, Bagade & Patil, 2021).

Supercritical fluid extraction (SFE) represents a sophisticated analytical and industrial-scale separation methodology that employs fluids maintained beyond their thermodynamic critical temperature and pressure as the primary solvent. Supercritical carbon dioxide remains the preeminent medium for SFE due to its chemically inert nature, inherent non-toxicity, and favourable critical parameters ($T_c=31.1\text{ }^{\circ}\text{C}$, $P_c=73.8\text{ bar}$), which facilitate the extraction of labile bioactive compounds under relatively mild thermal conditions (Dias et al., 2021; Herzyk et al., 2024). While is the established benchmark for most phytopharmaceutical and nutraceutical applications, alternative fluids such as ethane, propane, or even supercritical water may be strategically employed depending on the specific polarity, molecular weight, and solubility requirements of the target analytes.

Accelerated Solvent Extraction (ASE), also widely recognized as **Pressurized Fluid Extraction (PFE)** is characterized by minimized solvent consumption, high analytical throughput, and significantly expedited extraction kinetics compared to conventional methodologies.

To achieve optimal recovery yields of specific secondary metabolites, several critical process parameters must be meticulously calibrated; these include, but are not limited to, the

extraction temperature (T), hydrostatic pressure (P), and the duration of static and dynamic extraction cycles. The precise control of these variables allows for the exhaustive leaching of phytochemicals while maintaining a high degree of reproducibility across complex biological samples.

Enzyme-assisted extraction (EAE) is predicated on the biocatalytic cleavage of covalent linkages within the plant cell wall matrix, typically requiring an aqueous environment to facilitate enzymatic activity. By targeting specific structural polysaccharides such as cellulose, hemicellulose, and pectin, these enzymes induce the systematic disintegration of the cellular architecture, thereby significantly augmenting the permeability of the botanical substrate (Vo et al., 2023)

Notably, enzymatic reactions proceed with high efficiency under relatively mild conditions - typically characterized by moderate pH levels and low thermal input - over a condensed temporal framework ranging from several minutes to a few hours. In the majority of experimental cases, the integration of an enzymatic pretreatment phase demonstrates a substantial positive correlation with overall extraction efficacy and the qualitative enrichment of the final biochemical profile.

Deep eutectic solvents (DES) are defined as eutectic systems comprising stoichiometric mixtures of hydrogen bond donors (HBDs) and hydrogen bond acceptors (HBAs), characterized by a significant depression in melting point relative to their individual components and highly correlated properties. (Serna-Vázquez et al., 2021; Aduloju et al., 2023). A specialized subclass, **natural deep eutectic solvents (NADES)**, is uniquely formulated from primary metabolites - including amino acids, saccharides, and organic acids - that are endogenously present within biological systems (Alam et al., 2021; Ferreira & Sarraçuca, 2024). This intrinsic composition ensures a high degree of biocompatibility and environmental sustainability (Stanisz et al., 2024).).

Their favourable ecological profile is further underscored by their inherent biodegradability and negligible toxicity, particularly when composed of plant-derived constituents (Socas-Rodríguez et al., 2021; Bashir et al., 2023).

Furthermore, NADES-mediated extractions are effectively conducted under mild operational conditions, which are critical for preserving the molecular integrity of thermolabile constituents while simultaneously reducing total energy consumption (Pinho et al., 2024). Collectively, these attributes establish NADES as a transformative medium in green chemistry, offering a synergistic balance of operational efficiency and environmental stewardship for modernized extraction processes (Ivanovic et al., 2020; Lu & Liu, 2020).

Pulsed Electric Field (PEF) technology represents a non-thermal, ecologically sustainable processing modality based on the application of high-intensity, short-duration electrical pulses. The fundamental mechanism of PEF involves the induction of electroporation, a phenomenon whereby the application of an external electric field exceeding the membrane's critical dielectric strength results in the formation of transient or permanent aqueous pores. This methodology is particularly effective for the selective isolation of proteins, bioactive secondary metabolites, and natural pigments from diverse vegetal substrates.

Factors influencing extraction efficiency and bioactivity

Extraction efficiency and the bioactivity of compounds are primarily influenced by solvent polarity, temperature, extraction time, pH, etc. Optimal conditions (e.g., proper solvent, moderate heat) maximize yield, while extreme conditions (high heat/long time) can degrade compounds, reducing bioactivity (Khan & Dashti, 2023). Advanced methods like UAE/MAE offer higher efficiency.

The cumulative yield of phytochemical extraction is intrinsically contingent upon the unique physicochemical attributes and structural stabilities of the target analytes. Empirical evidence underscores that a specific set of operational parameters - such as temperature, solvent polarity, or pH - may exert divergent or even antagonistic effects on the recovery of distinct phytochemical classes (Wu et al., 2020). For instance, while elevated temperatures may enhance the solubility and mass transfer of certain thermostable polyphenols, the same thermal energy can simultaneously induce the

oxidative degradation of more sensitive constituents, such as anthocyanins, thereby resulting in an inverse correlation between extraction efficiency and yield across disparate molecular species within the same botanical matrix (Veršić Bratinčević et al., 2023). This phenomenon necessitates a multi-objective optimization approach when attempting to isolate a broad spectrum of secondary metabolites, as the "optimal" conditions for one chemical species may be detrimental to the recovery of another (Qasim et al., 2016).

A one-way analysis of variance (ANOVA) test can be applied to detect differences among the different extraction techniques (used at a given time) in terms of the extraction yield amount affected by solvent type and extraction time. Each test is conducted at least three times, and the data are presented as the mean $\pm$ standard deviation (SD). The data results are calculated using Microsoft Excel and analyzed with Prism software until the differences are significant at $p < 0.05$.

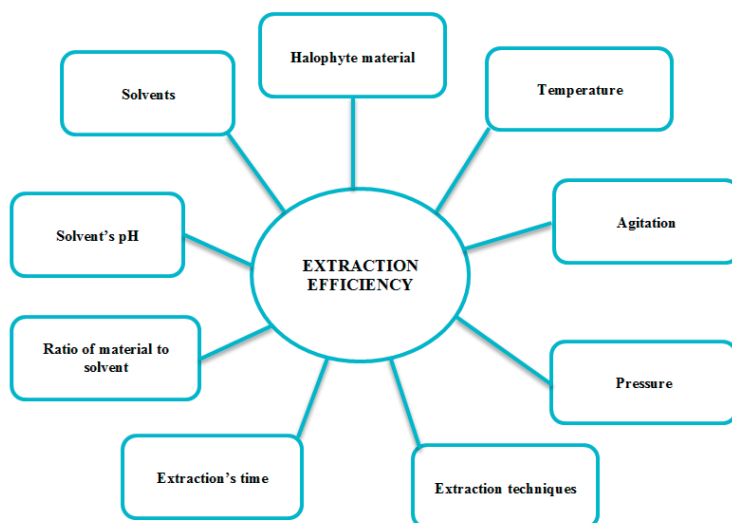


Fig. 2 Factors that can influence the extraction efficiency of halophyte's bioactive compounds

• **Solvent Selection and Polarity:** The most critical factor is solvent polarity, must match the target compounds (e.g., water/ethanol for polar phenolics and hexane for non-polar). Natural Deep Eutectic Solvents (NADES) are emerging as a sustainable, non-toxic, and highly efficient alternative to conventional organic solvents, particularly for extracting bioactive compounds from plants and food waste (Prabhune & Dey, 2023; Foroutani et al., 2024). Composed of natural metabolites such as choline chloride, sugars, or organic acids, they are characterized by strong hydrogen bonding, low volatility, and high customizability. Conventional solvents, traditionally used for extraction and industrial processes, are primarily characterized by their volatility, high solvency power, and petroleum-derived origins, but they are increasingly

scrutinized due to environmental and health hazards (Claux et al., 2021).

• **Temperature:** higher temperatures generally increase solubility and mass transfer, but excessive heat can degrade bioactive compounds (Antony & Farid, 2022)

• **Extraction time and kinetics:** yield increases with time until reaching equilibrium, often within 30–60 minutes, after which prolonged extraction can lead to compound degradation. And heat, can also cause degradation if prolonged. Advanced techniques can significantly decrease processing time from hours to minutes.

• **Particle size and pretreatment:** reducing particle size (grinding/milling) increases the surface area, enhancing solvent penetration and yield.

- **pH levels:** pH affects the solubility and stability of specific compounds; for instance, acidic conditions (pH < 5) favor the extraction of anthocyanins.

- **Extraction method:** green technologies are more efficient than conventional methods, providing higher yields in less time while preserving bioactivity.

- **Solid-to-solvent ratio:** a higher proportion of solvent to solid can increase yield, but an excessive amount may complicate downstream processing.

The choice of parameters directly affects the structural integrity of the compounds and their final antioxidant, antimicrobial, or therapeutic potential (Rodríguez et al., 2020).

Analytical techniques for phytochemical profiling

Several methods can be used in the identification of compounds from halophyte plant extracts. Phytochemical studies play a crucial role in the identification of bioactive compounds present in halophytes (Machado et al., 2025; Szydłowska-Czerniak et al., 2025). These compounds mainly include alkaloids, flavonoids, tannins, terpenoids, and phenolic substances, which are widely recognized for their potential (Tzanova et al., 2020; Soares et al., 2023; Valisakkagari et al., 2024; Wehbe et al., 2024; Sun et al., 2025).

Physicochemical properties include total polyphenol content, total flavonoid content, antioxidant activity, and polyphenol identification (Câmara et al., 2020; Cañadas et al., 2021; Fredsgaard et al., 2023; Buscaglia et al., 2024)

Biological properties include antimicrobial properties, biodegradation, and long-term stability (et al., 2024).

For the detection of functional group, presence of multiple bonds and rings, hydrogen and carbon arrangement, as well as full structural elucidation the techniques used include Mass Spectroscopy (MS), Ultraviolet Spectroscopy (UV), Infrared Spectroscopy (IR), Thin Layer Chromatography (TLC), High Performance Liquid Chromatography (HPLC), Gas Chromatography-Mass Spectrometry (GC-MS) remain reliable and widely used. More recent advancements, including Nuclear Magnetic Resonance (NMR), Liquid Chromatography–Tandem Mass Spectrometry (LC-MS/MS),

Fourier Transform Infrared Spectroscopy (FTIR), metabolomics approaches, and machine learning–assisted spectral analysis, have further improved the accuracy and sensitivity of detecting and profiling complex plant metabolites.

The integration of these analytical and extraction methods contributes significantly to quality control, supports the standardization of plant-derived products, and facilitates the discovery and development of natural product-based pharmaceuticals, nutraceuticals, and more (Bener et al., 2022; Choudhary et al., 2021).

Challenges and limitations

Green extraction has increasingly gained prominence as an advanced and rapidly developing area within analytical chemistry, reflecting the broader global movement toward more sustainable and environmentally responsible industrial and scientific practices (Lefebvre et al., 2021; Majid et al., 2023). This approach seeks to reconcile the growing demand for the efficient isolation of high-purity secondary metabolites with the necessity of reducing the environmental impact associated with conventional extraction processes. Consequently, ongoing developments in this field are directed toward improving both the efficiency and selectivity of extracting biologically active compounds, while simultaneously minimizing the consumption of hazardous solvents, energy, and other resources throughout the analytical procedure. The adoption of such strategies contributes to the development of more sustainable analytical methodologies and supports the broader objective of environmentally conscious research and production.

Solvents employed in the extraction of halophyte constituents are typically selected according to their polarity, which strongly influences the efficiency and selectivity of phytochemical recovery. These solvents can be broadly classified into polar solvents, such as water and various alcohols; solvents with intermediate polarity, including acetone and dichloromethane; and nonpolar solvents, for example *n*-hexane, ether, and chloroform. The choice of solvent is therefore a critical factor in determining the range of bioactive compounds that can be isolated from plant matrices. A

variety of extraction techniques are used to obtain these compounds, ranging from conventional methods to more advanced approaches. Commonly applied procedures include maceration, decoction, infusion, percolation, and Soxhlet extraction, as well as superficial extraction. In addition, modern extraction strategies such as ultrasound-assisted extraction and microwave-assisted extraction have been increasingly adopted due to their potential to improve extraction efficiency and reduce processing time (Geow et al., 2021).

RESULTS AND DISCUSSIONS

The selection of an appropriate extraction technique represents a crucial factor in effectively harnessing the therapeutic potential of natural products. This choice influences not only the extraction yield and the resulting phytochemical profile but also, and perhaps more importantly, the preservation of the intrinsic bioactivity of the compounds present in the extract (Ruiz-Medina et al., 2025).

Conventional extraction methods are characterized by several significant limitations, most notably the protracted temporal requirements and the necessity for substantial solvent volumes, often involving labor-intensive, multi-stage iterative procedures. A critical constraint within these frameworks is the inherent susceptibility of thermolabile phytochemicals to thermal degradation or oxidative transformation during heating phases, which can significantly compromise the biochemical integrity of the resulting extract. Despite these technical impediments, these conventional techniques maintain widespread utility in the isolation of volatile essential oils and aromatic fractions. This persistent application is primarily attributed to their operational simplicity and the minimal instrumentation overhead required, making them accessible and cost-effective alternatives to more contemporary, capital-intensive extraction technologies.

Modern extraction techniques, specifically ultrasound-assisted (UAE), microwave-assisted (MAE), enzyme-assisted (EAE), and supercritical fluid extraction (SFE) (Kumar et al., 2021; Paucar et al., 2023), represent a paradigm shift toward process intensification in

phytopharmaceutical research (Islam et al., 2023). Novel methods have been demonstrated to improve selectivity, reduce solvent consumption, and enhance extraction kinetics relative to conventional approaches (Sánchez-Camargo et al., 2020).

These methodologies align with the principles of Green Chemistry, ensuring that higher recovery yields are achieved with enhanced selectivity and reduced environmental impact, positioning these "green" technologies as superior alternatives to traditional, labor-intensive benchmarks.

The review also critically evaluates key process parameters that influence extraction efficiency, such as solvent type and polarity, extraction duration, temperature regimes, and ultrasonication amplitude, and discusses strategies for their optimization in various extraction contexts (Espro et al., 2021).

CONCLUSIONS

Further research is needed into the optimization of alternative combinations of green solvents; may provide valuable insights into improving the extraction performance of halophytes (Hessel et al., 2022). In particular, the investigation of modified extraction parameters, including variations in extraction time, solvent-to-sample ratio, and other operational conditions, may further clarify the efficiency and selectivity of the process. The encouraging outcomes reported for these environmentally friendly extraction approaches also highlight their potential for broader practical utilization. Additionally, phytochemical profiling what utilizes advanced analytical techniques to separate, identify, and quantify bioactive compounds like flavonoids, terpenoids, and alkaloids is a necessity (Mondal et al., 2025). Extracts obtained through such sustainable methodologies are often enriched in high-value bioactive compounds, thereby presenting promising opportunities for different and practical applications (Lemoine et al., 2024; Lemoine et al., 2025). The integration of these extracts into healthcare, pharmacy, and agriculture applications could support the exploitation of currently underutilized halophytes and contribute to the advancement of

circular economy principles (Socas-Rodríguez et al., 2021).

This review emphasizes that the future valorisation of halophytes and their extracts will likely depend on the strategic integration of hybrid extraction approaches designed to enhance compound recovery while simultaneously addressing sustainability and economic feasibility. The implementation of a carefully selected and rigorously optimized extraction methodology therefore represents a fundamental initial stage in the effective exploitation of the extensive chemical diversity inherent to halophytes. Such an approach facilitates the transformation of these into safe and efficacious bioactive ingredients with potential applications in both pharmaceutical and nutraceutical fields.

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ADDING VALUE TO THE AGRI-FOOD SIDE-STREAMS – A REVIEW ON THE ROMANIAN APPROACH TO APPLE POMACE

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Abstract

Due to the industrialization of both fruits and vegetables, significant quantities of plant waste are generated annually, accounting for over 40% of the total processed plant products. An important by-product of this industrial process is apple pomace, which results from the processing of apples into products such as juice and fruit preserves, which are obtained through pressing, boiling, and fermentation processes. Although often perceived as a seemingly valueless by-product, apple pomace holds significant potential as a source of valuable compounds. Recent research on its use as a substrate in microbial processes has influenced the production of low-alcohol beverages that are richer in organic acids or single-cell proteins. Recent trends indicate a growing interest in using residues from apple processing as by-products in the food industry to extract value-added products, including dietary fibers, proteins, natural antioxidants, biopolymers, pigments, and compounds with unique properties. In this study, we focused on methods of valorization of apple pomace from different varieties grown in Romania.

Key words: bioeconomy side streams, apple pomace, organic acids, valorization, Romania.

INTRODUCTION

Romania has a rich history of apple growing due to favorable climatic conditions. However, apple production has steadily declined, ranking fourth out of five European apple-producing countries, according to a 2022 study (I. M. Vlad et al., 2022). The country's orchards are dominated by apple varieties of the genus *Malus* sp., family *Rosaceae*, including *Starkrimson*, *Jonathan*, *Red Delicious*, *Idared*, *Golden Delicious*, *Florina*, *Jonagold*, *Romus 3 and 4*, *Generos de Voinești*, *Topaz*, *Frumos de Voinești*, *Granny Smith*, *Domnesc*, *Crețesc*, *Poinic*, *Pățul*, *Ardelean*, *Ciprian*, *Poinic*, *Sovari Nobil* and *Gala* (Popescu, 2012; I. M. Vlad et al., 2022).

One of the prominent agro-industrial wastes in Romania includes apple peel/pomace, along with red and white grape pomace, as well as red beet pomace/skin. Industrial processing of apples generates significant volumes of vegetable waste each year, from juice extraction or fermentation processes to produce apple

brandy/pălincă (a Romanian-specific alcoholic beverage), vinegar, and cider. If unmanaged, the high moisture of apple waste can pose risks to human health, air quality, soil, and ecosystems (Ghinea et al., 2019; Vodnar et al., 2017). Despite the potential value of apple waste, there remains a significant disposal problem in Romania. In particular, apple peel, a major residue from fruit processing, possesses antioxidant properties and may serve as a cardioprotective and anticarcinogenic agent, containing flavonoids such as quercetin glycosides and anthocyanins that are usually absent in fruit pulp (Vodnar et al., 2017).

The main purpose of this review is to analyze and describe the valorization of apple waste in Romania, to perform this analysis, articles published in 2009 were studied, consulting international databases such as PubMed, Google Scholar, Web of Science, Science-Clarivate, using keywords such as "apple waste in Romania", "industrial waste in Romania", and "Romanian apple varieties".

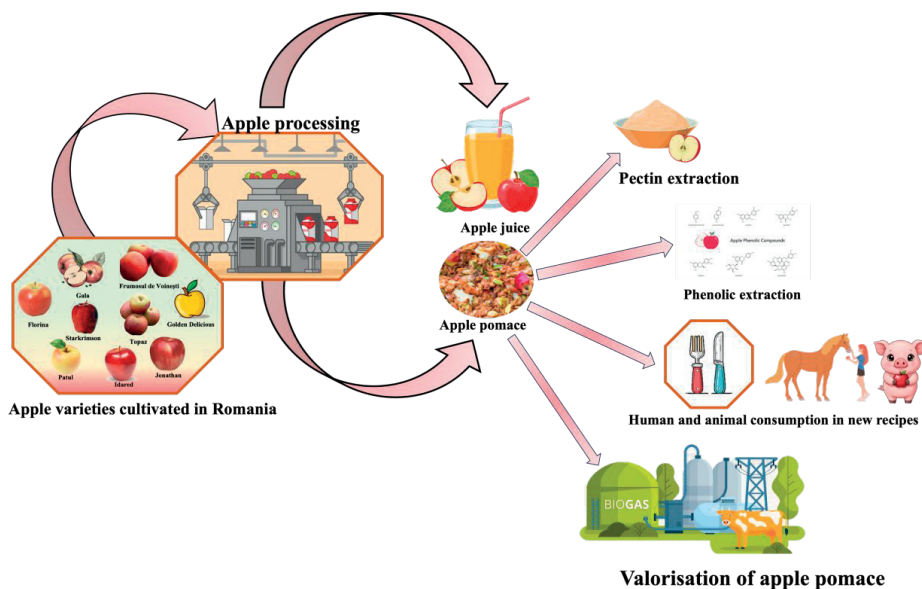


Figure 1. Bioeconomic model for the use of agro-industrial waste from apples

BIOCHEMICAL COMPOSITION OF APPLE POMACE

Apples are highly valued for their rich content of bioactive compounds, such as flavonoids, hydroxycinnamic acids, vitamins, fiber, and minerals, making them a popular choice for consumption as both whole fruit and juice. The nutritional profile of apples, which is high in water (85%), carbohydrates (14%), pectin (0.2%), fat (0.6%), and essential vitamins and minerals, varies according to the climatic zone of cultivation (Campeanu et al., 2009; Popescu, 2012; Velcirov et al., 2022).

Apple waste, composed mainly of pulp (54%), seeds (7%) and peel (34%), is fiber-rich and suitable for food/feed enrichment. An emerging trend in the food industry involves the re-use of by-products from fruit processing to enrich food for both humans and animals, providing additional bioactive compounds (Vasiliu et al., 2018).

Polyphenols, abundant in apples, are bioactive secondary metabolites with strong antioxidant, antimicrobial, antidiabetic, and anticarcinogenic properties, making them valuable for pharmaceutical, cosmetic, and food applications (Lang et al., 2024; Szabo et al., 2022; Vasiliu et al., 2018).

Pectin, a complex polysaccharide extracted from apple residues, finds applications in various

industries as a thickening agent in foods and as a capsule substitute in the pharmaceutical and medical sectors (Marcon et al., 2005; Vasiliu et al., 2018).

In addition, the high carbohydrate and fiber content of apple residues allows them to be used as a substrate in fermentative processes, which could lead to biogas and contribute to ongoing bioenergy research (Călinoiu et al., 2018; Ciuta et al., 2016; Vasiliu et al., 2018).

Apple waste (AW) is therefore proving to be a versatile resource, with applications ranging from the food industry to pharmaceutical.

PECTIN FROM APPLES

Pectin, an intricate polysaccharide originating from plants, contains linear polymers such as homogalacturonan and rhamnogalacturonan, interspersed with neutral sugars such as L-rhamnose, L-arabinose, and D-galactose (Canteri-Schemin et al., 2005; Gurev et al., 2023; Müller-Maatsch et al., 2016). This adaptable compound finds widespread application in numerous sectors, including food, pharmaceuticals, textiles, and more, owing to its outstanding gelling properties, stability, and ability to thicken substances (Wang et al., 2014; Wikiera et al., 2015).

In addition to its industrial uses, pectin serves significant biological roles. It functions as a

stabilizer and protective agent in various food and beverage products, demonstrates immunomodulatory properties, reduces serum cholesterol levels, and has been observed to induce apoptosis in colon cancer cells. Its diverse array of advantages positions it as a prized ingredient for product enhancement and human health promotion (Girón-Hernández et al., 2023; Marcon et al., 2005; Wang et al., 2014).

The efficiency of pectin extraction, according to studies conducted so far, is influenced by the extraction method (Dranca et al., 2020; Gurev et al., 2023; Camelia C. Vlad et al., 2022). Among the most commonly used methods for extracting pectin AW obtained from Romanian-grown apple fruits (generally *Malus domestica*) are physicochemical methods (ultrasound-assisted extraction, microwave-assisted extraction), enzymatic methods (endo-xylanase and endo-cellulase), alkaline extraction, and classical methods such as citric acid extraction (Dranca et al., 2020).

Ultrasound-assisted extraction (UAE) enhances pectin extraction from AW by using ultrasonic waves and solvents to increase mass transfer efficiency. This method is energy-efficient and requires minimal solvent use (Canteri-Schemin et al., 2005). The improvement in extraction is attributed to ultrasonic cavitation and mechanical mixing, which disrupt cell walls, promote solvent penetration, and facilitate compound diffusion through mild heating (Egüés et al., 2021; Gurev et al., 2023; Wang et al., 2014).

Microwave-assisted extraction (MW) uses microwave energy to rapidly heat the solvent, enhancing its penetration into apple waste matrices and enabling efficient extraction of pectin, cellulose, and hemicellulose (Wang et al., 2007). Studies have demonstrated that MW significantly increases pectin yield while requiring less solvent and shorter extraction time, making it a fast and high-efficiency method (Bagherian et al., 2011; Spinei et al., 2022; Zarei et al., 2017).

Pectin is generally extracted using acids such as H_2SO_4 , HNO_3 , and HCl , or citric acid, and the extraction process can lead to the corrosion of production line components as well as the generation of large amounts of acidic industrial waste (Wikiera et al., 2021). As a sustainable

alternative, enzymatic extraction is preferred due to its selectivity and minimal impact on polymer structure (Wikiera et al., 2015; Wikiera et al., 2015a). In addition, pectin extracted by acid extraction has a high molecular weight and low polygalacturonic acid content. Compared to acid extraction, enzymatic methods using enzymes such as endo-xylanase and endo-cellulase produce different pectin in terms of molecular weight and sugar content, as in terms of the high extraction yield of galacturonic acid (Wikiera et al., 2016). In general, a combination of physicochemical-enzymatic or classical methods is preferred (Wikiera et al., 2015).

Pectin characterization commonly involves titrimetric methods (Gurev et al., 2023), and physicochemical techniques such as FT-IR (Fourier Transform Infrared Spectroscopy) in the wavelength range of $4000-400\text{ cm}^{-1}$. FT-IR is employed for determining the degree of esterification, which is defined as the number of carboxyl groups esterified, typically assessed at the wavelength of 1730 cm^{-1} . The degree of esterification (DE) can be calculated based on the sum of areas of bands between 1730 and 1600 cm^{-1} , corresponding to the total carboxylic groups. This proportionality aids in understanding the esterification level of the pectin (Dranca et al., 2020).

The characterization of pectin extracted from apple waste (*Malus domestica* Fălticeni) involves two essential parameters - the degree of esterification (DE) and the galacturonic acid content - both of which critically influence its structure and functional behavior. The degree of esterification, determined by infrared spectrophotometry, can be calculated using specific analytical formulas (Dranca et al., 2020; Naqash et al., 2021). This parameter represents the proportion of carboxyl groups ($-COOH$) in galacturonic acid units that are esterified with methyl radicals ($-OCH_3$), thus determining the physicochemical characteristics and gelation mechanism of pectin. Complementarily, the galacturonic acid content is quantified using methods such as the sulfamate/m-hydroxydiphenyl test (Dranca et al., 2020; Müller-Maatsch et al., 2016), which measures the proportion of D-galacturonic acid in the polysaccharide structure. A high galacturonic acid content, usually above 65%, indicates higher structural purity and is associated with

superior gel-forming capacity, increased viscosity, and improved biological functionality, as free carboxyl groups actively participate in ionic interactions and the gelation process (Cui et al., 2023; Naqash et al., 2021).

Furthermore, the DE value determines the type of gelation and the application potential of pectin. Pectins with high methoxyl content (DE > 50%) form gels in the presence of sugar and under acidic conditions, making them suitable for conventional food products such as jams and jellies. In contrast, pectins with low methoxyl content (DE < 50%) require calcium ions for gelation and can form gels even in low-sugar systems; thus, they widely used in food and pharmaceutical formulations (Löfgren & Hermansson, 2007; Rascón-Chu et al., 2019). DE also affects other functional properties, including solubility, water-binding capacity, and molecular interactions: pectins with high DE values tend to be more hydrophobic and gel more quickly, while those with low DE values have a higher number of free carboxyl groups, conferring greater chemical reactivity and biological activity (Morris et al., 2010).

The interaction between galacturonic acid content and esterification degree defines both the quality and versatility of pectin application. An optimal balance between these two parameters ensures desirable gelling properties and extends its use in several sectors - from the food industry, where it serves as a gelling, thickening, and stabilizing agent, to the pharmaceutical and biomedical fields, where it functions in controlled release systems and as a component of biocompatible materials (Dranca et al., 2018; Huang et al., 2022; Naqash et al., 2023). Further characterization includes monosaccharides by HPLC (High-Performance Liquid Chromatography) (Wikiera et al., 2015a, 2015b), thermal analysis through calorimetry, microstructure examination through scanning electron microscopy (SEM), and rheological analysis all of which provide valuable insights into pectin structure and the effects of extraction

methods on its functional properties (Dranca et al., 2020).

These characterization methods provide essential information about the composition, structure, and quality of pectin, aiding in its utilization in various industries, including food, pharmaceuticals, and cosmetics (Franchi et al., 2014).

To determine the most efficient method for pectin extraction from AW, various techniques have been evaluated, including citric acid extraction, classical extraction combined with UAE or MW, as well as enzymatic extraction.

Studies (Dranca et al., 2021) conducted using treatment with citric acid, at a ratio of 1:10 (w/v) with *Malus domestica* apple residue from Fălticeni, highlighted that the optimal extraction parameters were a temperature of 85.71°C and a time of 147 minutes, resulting in an extraction yield of 23.26%, a galacturonic acid concentration of 99.25 g/100 g, and an esterification degree of 81.18%

In another study (Dranca et al., 2019) from 2019, combining classical extraction with UAE, followed by thermal treatment, resulted in an extraction yield of 6.8%, a GalA content of 69.694 g/100 g, and an esterification degree of 80.09% at a temperature of 90°C, pH of 1.92, and time of 147 minutes and 43 seconds. Through MW at 560 W, pH of 2.2, and a time of 120 seconds, an extraction yield of 24%, a GalA content of 91.82 g/100 g, and an esterification degree of 88.52% were obtained. Compared to classical extraction with citric acid, this method showed better results in terms of extraction yield, galacturonic acid content, and esterification degree. Regarding enzymatic extraction, a study indicated that the optimum was achieved after 18 hours, at a temperature of 48°C, using 43 µl/g of enzyme, resulting in an extraction yield of 6.8%, a galacturonic acid content of 97.46 g/100 g of pectin, and an esterification degree of 96.02% (Dranca et al., 2019).

Table.1 Synthesis of data regarding the extraction of pectin from Romanian apple waste

Extraction	Yield, %	Gala, g/100 g	DE, %	Reference
CE	23.26	86.5	81.18-84.40 37.68	(Dranca et al., 2021; Dranca et al., 2020; Camelia Cristina Vlad et al., 2022)
MW	23.32	90.6 47.31- 73.02	73.80-88.5 38.69-71.37	(Dranca et al., 2021; Dranca et al., 2020; Gurev et al., 2023)
UAE	9.18	92.83 49.16- 78.71	81.18-77 36.47-73.78	(Dranca et al., 2021; Dranca et al., 2020; Gurev et al., 2023)
EEC	7.17	78.8	53.5	(Dranca et al., 2020)
EECL	6.76	85.2	44.6	(Dranca et al., 2019; Dranca et al., 2020)
UAE-H	6.86	69.06	80.7	(Dranca et al., 2019; Dranca et al., 2020)
EE-UT	7.95	75	47.6%	(Dranca et al., 2020)

CE – citric acid extraction, MW – microwave extraction,

UAE – ultrasound-assisted extraction, EEC – enzyme-assisted extraction with cellulase, EECL – enzyme-assisted extraction with Celluclast, UAE-H – ultrasound-assisted extraction – heating treatment, EE-UT – enzyme-assisted extraction – ultrasound treatment

In Table 1, it can be observed that the highest extraction yield of pectin from AW derived from *Malus domestica* apples cultivated in Romania is achieved through MW, with a yield of 23.32% (Dranca et al., 2021; Dranca et al., 2020; Gurev et al., 2023). Additionally, the classical extraction method using citric acid also yields high extraction efficiency. On the other hand, the lowest extraction yield is obtained from enzymatic treatments with Celluclast 1.5 L (Dranca et al., 2019). In terms of galacturonic acid content, the highest value, depending on pH, extraction time, and method, is obtained through UAE at 92.83 g/100 g of sample. Moreover, the highest degree of esterification is observed in pectin samples obtained through citric acid treatment followed by UAE (Dranca et al., 2019; Dranca et al., 2020; Gurev et al., 2023).

The pectin extracted through classical and microwave methods was subsequently analyzed using HPLC at a wavelength of 250 nm. (Dranca et al., 2021). In the study conducted by Dranca et al., 2021, it was observed that pectin obtained from the secondary product of apples through citric acid extraction has a galacturonic acid content of 78.2% mol², arabinose 7.51% mol, rhamnose 4.81% mol, xylose 1.16% mol, glucose 6.37% mol, and mannose 1.84% mol, with a molecular weight of 2.63×10^{-5} . Meanwhile, through MW, the galacturonic acid content was found to be 86.21 mol², arabinose 4.66% mol, rhamnose 3.10% mol, xylose 0.97% mol, glucose 3.71% mol, and mannose 1.48% mol, with a molecular weight of 2.64×10^{-5} . Because of their similar molecular weights, it

was reported in previous research that neither method affects the structure of pectin throughout the extraction process (Mao et al., 2019).

Through SEM analysis of pectin, it has been demonstrated that, after testing numerous extraction protocols on apple pomace from Romania that pectin obtained through classical extraction exhibits a homogeneous appearance with a porous surface. Conversely, pectin obtained through microwave- and ultrasound-assisted extraction and heating appears very rough and slightly ruptured. On the other hand, enzymatic extraction imparts a homogeneous and smooth appearance to the pectin, indicating that the extraction method significantly influences the structure of the obtained pectin (Dranca et al., 2020).

POLYPHENOLS FROM APPLE WASTE

Polyphenols are important plant secondary metabolites with various biochemical and biological properties and applications (Bertelli et al., 2021; Sridhar et al., 2021). Polyphenols from AW include phenolic acids, such as hydroxycinnamic acids (caffeic acid, chlorogenic acid, coumaric acid), hydroxybenzoic acids (gallic acid, protocatechuic acid, ellagic acid), anthocyanins (cyanidin-glucoside), dihydrochalcones such as phloridzin, flavonols (rutin, quercitrin, myricetin, epicatechin), flavan-3-ols (theaflavin, catechin, cafestol, procyanidin A1, procyanidin B1), flavanones (kaempferol, hesperidin, apigenin) (Gurev et al., 2023a; Sereti et al., 2024; Camelia C. Vlad et al., 2022; Vodnar et al.; 2017).

Romanian researchers have produced few articles regarding the extraction, analysis, and valorization of polyphenols from AW. Table 2
provides data on the extraction methods, along with details regarding the profile and quantity of the extracted polyphenols from AW.

Table 2. Synthesis of data regarding the extraction of polyphenols from apple pomace

Method and conditions of extraction	Polyphenolic compounds	References
Conventional solvent extraction with 80% methanol solution (v/v) at 25°C and 150 rpm, for 2 h	Gallic acid – 86.42 ± 2.26 mg/100 g DM; Caffeic acid – 3.62 ± 0.80 mg/100 g DM; Theaflavin – 71.73 ± 0.72 mg/100 g DM; Hesperidin – 42.83 ± 0.27 mg/100 g DM; Myricetin – 4.84 ± 0.01 mg/100 g DM	(Camelia C. Vlad et al., 2022)
Acid hydrolysis with 2M HCl at 100°C for 1 h	Caffeic acid – 4978.00 ± 900.00 mg/100 g DM; Protocatechuic acid – 2144.20 ± 37.60 mg/100 g DM; Quercetin 3-β-D-glucoside – 236.60 ± 3.12 mg/100 g; Kaempferol 27.80 ± 1.36 mg/100 g	
Conventional solvent extraction with continuous stirring for 30 min at 45°C, 1:4 ratio between apple pomace and extraction solution composed of 50% MeOH: 0.3 % HCl (v/v)	TPC – 2163.6 ± 78.0 mg GAE/100g; 3,4-Dihydroxybenzoic acid – 308.47 ± 16.99 µg/g; Gallic acid – 99.76 ± 4.52 µg/g; 4-Hydroxybenzoic acid – 169.51 ± 12.56 µg/g; Chlorogenic acid – 59.60 ± 4.26 µg/g; Catechin – 37.63 ± 2.84 µg/g	(Sereti et al., 2024)
UAE, 1:100 (w/v) apple pomace and 50% ethanol solution (v/v) at a frequency of 37 kHz and a temperature of 40 ± 1°C, for 30 min	TPC – 6.03 ± 0.21 mg GAE/g DW TFC – 2.13 ± 0.13 mg QE/g DW TT – 0.61 ± 0.03 mg TAE/g DW	(Gurev et al., 2023a)
UAE, 1:5 (w/v) apple pomace and methanol at room temperature for 15 min	Chlorogenic acid – 1173.13 ± 0.19 µg/g; Gallic acid-glucoside – 106.62 ± 0.14 µg/g; Epicatechin – 736.21 ± 0.44 µg/g; Isoquercitrin – 358.58 ± 0.07 µg/g; Quercitrin – 164.07 ± 0.09; Phloridzin – 720.39 ± 0.09 µg/g; Total phenolics – 4952.40 ± 0.08 µg/g	(Teleky et al., 2022)
UAE, extraction solution composed of HCl:MeOH:H ₂ O (1:80:19) at 40°C for 30 min	TPC – 564 ± 39.5 mgGAE/100gDW; Caffeic acid-4-O-glucoside – 2.492 mg/100 g DW; Quercetin 3-O-rutinoside – 4.318 mg/100 g DW; Phloridzin – 5.714 mg/100 g DW; Phloretin 2'-O-glucoside – 1.125 mg/100 g DW	(Vodnar et al., 2017)

¹DW - dried weight, DM - dried mass

In these articles, polyphenols quantification from AW was performed by colorimetric methods with the Folin-Ciocalteu reagent (TPC) (Gurev et al., 2023a; Sereti et al., 2024) and chromatographic analysis by HPLC coupled with different detectors: a multi-wavelength detector (MWD) (Gurev et al., 2023a; Camelia C. Vlad et al., 2022). photodiode array detector (PDA) (Sereti et al., 2024), diode-array detector and electrospray ionization mass spectrometer (HPLC-DAD-ESI-MS) (Vodnar et al., 2017), variable wavelength detector (VWD) (Teleky et al., 2022). The polyphenolic composition and extraction yield are significantly influenced by apple cultivar, extraction technique, solvent polarity, the physical state of the biomass (fresh, frozen, or dried), and pretreatments applied prior to extraction (Vodnar et al., 2017). UAE has been widely reported for its efficiency, yielding TP) values between 5.64 ± 0.395 and

6.03 ± 0.21 mg GAE/g DW, depending on the extraction solvent system, such as 50% ethanol (Gurev et al., 2023a) vs. HCl:MeOH:H₂O (1:80:19) (Sereti et al., 2024). These little differences are also attributed to the raw material characteristics. (Gurev et al., 2023a) used apple waste derived from the Golden Delicious variety, wich underwent citric acid pretreatment to prevent oxidation prior to drying at 70°C, whereas (Vodnar et al., 2017) extracted polyphenols directly from fresh apple peel obtained of the Ionatan de Voineşti variety. In another study on Romanian apple cultivars by (Catană et al., 2018), reported TPC values ranging between 17.83 and 38.83 mg GAE/g DW, with Golden Delicious exhibiting the lowest content and Jonathan thehighest. The same study demonstrated that extraction efficiency was significantly influenced by solvent, with 70% acetone showing a superior

extraction yield compared to 50% methanol and 50% ethanol. The effectiveness of the extraction solvent is closely associated with solvent polarity, phenolic solubility, degree of polymerization, and interactions with other matrix components, which can form insoluble complexes and limit extractability (Perussello et al., 2017; Venkatesan et al., 2019).

Polyphenols in apple cell walls are presented in both free and bound forms, with the latter typically esterified with polysaccharides like cellulose and hemicellulose. Extracting bound forms is more challenging due to their association with structural components (Fernandes et al., 2019; Vidot et al., 2020). The common extraction method involves first extracting free polyphenols using an aqueous organic solvent, followed by extracting bound polyphenols using base and/or acid hydrolysis (Li et al., 2020). Camelia C. Vlad et al. (2022) studied this sequential extraction of polyphenols from freeze-dried Idared AW. They found that using a 2M HCl acid solution and a high temperature (100°C) improved the polyphenol profile and content compared with extraction using 80% methanol at 25°C, reducing the extraction time from 2 hours to 1 hour. This method enhanced the content of certain phenolic acids (e.g., caffeic acid content increased from 3.62 ± 0.80 mg/100 g DM to 4978.00 ± 900.00 mg/100 g DM), altered flavonol and flavanone profiles, but reduced flavan-3-ol content in the bound fraction compared with the free fraction extracted with methanol at 25°C for 2 hours, and some compounds were poorly extracted or undetected.

In their study (Vodnar et al., 2017) investigated the impact of thermal pre-treatment (10 minutes at 80°C) on polyphenol extraction from apple peel in comparison to fresh peel. They observed a significant effect of the thermal pre-treatment on TPC and certain polyphenols, such as caffeic acid-4-O-glucoside and 3-caffeoylquinic acid, as determined by HPLC-DAD-ESI-MS. Interestingly, some polyphenol levels, like phloridzin and phloretin 2'-O-glucoside, decreased after the thermal pre-treatment. Their findings indicated both positive and negative impacts of thermal pre-treatment on polyphenol content, depending on the type of polyphenols (hydroxycinnamic acids or dihydrochalcones). These effects are attributed to the characteristics

of low-temperature thermal pre-treatments (below 90°C), which promote macromolecule de-flocculation (Wang et al., 2022) while minimally affecting the solubilization of lignocellulosic matter ultimately leading to increased phenolic compound content through polysaccharide hydrolysis (Jain et al., 2015; Rodríguez-Gutiérrez et al., 2019),

Apple Pomace: From Agro-Industrial Waste to a Valuable Component in Food and Bioprocesses

Apple waste is a valuable source of bioactive compounds, such as polysaccharides, fatty acids, and polyphenols, with promising applications in biotechnology and the food industry (Pop et al., 2021). Due to their nutritional composition, these by-products have been used as a fermentation substrate with lactic acid bacteria (*Loigolactobacillus bifermentans*, *Lactobacillus plantarum*, *Lactobacillus casei*), fructolactobacilli (*Fructilactobacillus florum*), and yeasts (*Saccharomyces cerevisiae*, *Saccharomyces boulardii*), to obtain functional foods, such as sourdough bread or soy milk (Camelia Cristina Vlad et al., 2022).

In recent years, research in Romania has focused on exploiting this potential by developing innovative foods enriched with valuable bioactive compounds (Martau et al., 2021; Camelia Cristina Vlad et al., 2022).

A relevant example is provided by studies conducted on Romanian AW from apple varieties, including the Idared (Galați, Romania) and Jonathan (Bistrița-Năsăud, Romania) varieties, which demonstrated prebiotic effects due to their high pectin and polyphenol content. The addition of a small amount of dried pomace to the culture medium significantly increased prebiotic activity compared with the control. Optimization of this process enables the development of functional products rich in nutrients and antioxidants, contributing to improved health outcomes. (Martau et al., 2021; Camelia Cristina Vlad et al., 2022).

Further research Plămadă (2025) confirmed that pomace from Red Delicious and Granny Smith varieties also promotes the growth of beneficial microorganisms such as *L. plantarum*, *L. casei*, *S. boulardii* at low and moderate concentrations, thus supporting its prebiotic potential, although

the prebiotic index remained lower than that of the control (Plamada et al., 2025).

During the fermentation process, the pH of the samples decreases due to the production of lactate and lactic acid, other organic acids, which also leads to changes in secondary metabolites. This effect has been observed in sourdough bread enriched with Jonathan apple pomace, where fermentation enhanced both acidity and bioactive compound content (Magyar et al., 2016; Martău et al., 2021).

Apple pomace has also been used as a fermentation substrate to obtain a nutraceutical product enriched with lipophilic carotenoid pigments through fermentation with *Zygomycetes* (Dulf et al., 2023), such as strains of *Actinomucor elegans* and *Umbelopsis isabellina* - fungal strains having the role of producing significant amounts of enzymes, lipids or pigments (Klempová et al., 2020). The bioactive compounds and antioxidant content of the resulting product were screened during this process (Dulf et al., 2023).

Another application in Romania that has been studied is the incorporation of apple pomace as a functional ingredient in foods such as biscuits, breads, muffins, jelly candies, and fruit protein bars (Ciurlă et al., 2024; Mandache et al., 2025; Mureșan et al., 2024; Pop et al., 2021). Due to its high fiber content, comprising approximately 35-60% dietary fiber (approximately 25% lignin, 10% pectin, 40% cellulose, and 25% hemicellulose), these figures are influenced by the variety of apples from which they originate (Pop et al., 2021; Rațu et al., 2023). The fibers from apple pomace play the role of retaining water and forming gels, increasing viscosity, as demonstrated by their incorporation into biscuits or jelly (Ciurlă et al., 2024; Fărcaș et al., 2022). In addition to the high fiber content, AW inclusion significantly enhanced the phenolic profile and antioxidant capacity of the final food products (Mandache et al., 2025).

Apples, which are rich in bioactive compounds, polysaccharides, carbohydrates, and fibers, as well as a valuable source of pectin, have been used as a stabilizing agent for oil-in-water emulsions, replacing the margarine used in conventional biscuit recipes, resulting in a new product that offers high nutritional value and antioxidant activity (Sereti et al., 2024; Velciov et al., 2022).

Apple pomace, including the Golden Delicious variety, is also used in fruit bars, where pectin extracted from the pomace acts as a gelling agent, forming a protective layer that prevents the physicochemical changes that may occur during storage. These changes include reducing antioxidant activity, pH, and acidity alterations, as well as variations in water activity, which could promote the development of pathogens such as yeasts and molds, thereby compromising the safety of the product for consumption (Gurev et al., 2023).

In the framework of current challenges regarding animal nutrition and restrictions imposed on the use of antibiotics, it has become necessary to identify sustainable and safe alternatives. One such direction is the utilization of agri-food by-products, which, instead of being discarded, can represent valuable sources of antimicrobial compounds, fibers, and essential nutrients. In this regard, Anghel et al. (2024) investigated the antimicrobial activity of apple extract before and after fermentation with *Saccharomyces cerevisiae*, highlighting its potential for use as a natural substitute for antibiotics and zinc oxide in the feed of piglets (Anghel et al., 2024). Complementarily, Filip (2024) (Filip et al., 2024) analyzed the biochemical composition of red and yellow apples before and after the drying process, with the aim of evaluating their applicability as a functional ingredient in animal feed.

In 2022, Telekey and his collaborators adopted an innovative approach to the recovery and reuse of biological materials using apple pomace from the Red Delicious variety to produce biodegradable biopolymers, replacing chemically produced polymers. This initiative addressed two important and topical issues, namely the valorization of raw materials and their integration into the food industry.

This study focused on the formation of films from apple pectin, which was mixed with poly(vinyl alcohol) (PVA) and itaconic acid, obtained from filamentous fungi of the *Aspergillus terreus* species, and with the addition of extracts from apple pomace. The formulation of these films resulted in materials with improved rheological properties because of the combination of pectin, PVA, and itaconic acid with polyphenols extracted from apple residues. In addition, this formulation

significantly improved the physicochemical properties of the films, particularly in terms of antioxidant activity, mechanical resistance, and their ability to resist vapor and dissolve less in water (Dranca et al., 2021; Teleky et al., 2022). Another study from 2016 focused on the valorization of Romanian agro-industrial residues, primarily focusing on biogas production. This energy source can be naturally generated through organic degradation under anaerobic conditions (Ciuta et al., 2016). Apple pomace, with its high moisture content, provides the perfect environment for this microbial degradation, both in aerobic and anaerobic processes (Dalpaz et al., 2020; Maragkaki et al., 2018). It contains approximately 90% volatile solids and a moisture content of approximately 75-80%.

An essential aspect of the efficiency of anaerobic degradation is the optimal carbon-to-nitrogen ratio, which ranges between 15 and 30 according to previous studies (Ciuta et al., 2016). By analyzing the elements composing the degradation consortium, it can be classified according to the UNI CEN/TS 15359 (Rada et al., 2012) standard into one of the five essential classes, providing valuable information about the compatibility of the co-digestion process. The rapid degradation of apple pomace, occurring in just two days, and its potential to produce biogas in significant quantities, highlighted by a respirometric index of $4888 \pm 804 \text{ mg O}_2 \cdot \text{kgVS}^{-1} \cdot \text{h}^{-1}$ and anaerobic biogas potential of $60.84 \text{ (Nm}^3 \cdot \text{tq}^{-1})$, represent a promising perspective for the valorization of these residues in energy production (Ciuta et al., 2016).

As well as using apple pomace in making food and functional supplements for people and animals, and in making biopolymers and biogas, there has been a big focus on using it in farming over the last few years. In 2025, Pădureanu et al. all evaluated the potential of apple pomace extract as a biostimulant. Although studies conducted to date have not shown significant effects on stimulating wheat plant growth, researchers have nevertheless suggested its use as a bioherbicide, given the persistent challenges associated with weed control in Romanian agriculture (Padureanu et al., 2025).

Water pollution with heavy metals is a major environmental problem. Recent research has

shown that apple pomace can be used as a natural biosorbent, without chemical treatments, to remove lead ions from water. Research has shown high adsorption efficiency and high metal retention capacity, demonstrating its potential as an environmentally friendly and sustainable solution for treating contaminated water, while also making use of an abundant agri-food by-product (Ungureanu et al., 2024).

CONCLUSIONS

Based on studies conducted to date on the utilization of apple pomace, we have concluded that apple waste is a complex and sustainable source of bioactive compounds with multidisciplinary applications. Optimizing extraction processes, detailed characterization of chemical components, and the development of environmentally friendly recovery technologies contribute to transforming this agro-industrial waste into a strategic resource for sustainable development and industrial innovation.

The most important compound extracted from apple pomace is pectin, which has valuable functional properties, mainly determined by the degree of esterification (DE) and galacturonic acid content. These parameters are essential for determining both the quality and the areas of application of pectin. Pectins with a high degree of esterification ($\text{DE} > 50\%$) are suitable for classic food products such as jellies and jams, while pectins with a lower degree of esterification ($\text{DE} < 50\%$) are frequently used in pharmaceutical and biomedical applications. In addition, modern extraction methods - such as ultrasound-assisted extraction (UAE) or microwave extraction (MW) - have proven to be more efficient than conventional methods, offering higher yields, greater purity, and improved structural properties of pectin.

On the other hand, apple pomace is an important source of phenolic compounds, which have a wide range of biological benefits, such as antioxidant, antimicrobial, and anticarcinogenic activity. Due to these properties, phenolic compounds are recommended as valuable ingredients in the formulation of functional foods, food supplements, and cosmetics. The composition and concentration of these compounds vary depending on the apple variety,

the extraction method and solvent used, and the preliminary treatments applied to the waste.

The application of apple by-products in biotechnology and industry has undergone significant development. They can be used as a substrate in fermentation processes to obtain biogas or fermented products with high functional value, but also in the production of biodegradable materials based on pectin and natural polymers. Furthermore, the use of apple waste as a food additive or natural feed offers nutritional and antimicrobial benefits, and recent research confirms its potential as an environmentally friendly biosorbent for removing heavy metals from contaminated water.

Overall, the recovery of apple waste contributes to reducing pollution, promoting a circular economy, and developing sustainable, innovative, and high-value-added products, demonstrating its strategic importance for the future of the bioeconomy.

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STUDY ON THE EFFECTIVENESS OF LAVENDER ESSENTIAL OIL FOR ANTIFUNGAL PROTECTION OF STRAWBERRIES IN POSTHARVEST

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Abstract

Post-harvest fungal decay, particularly caused by *Botrytis cinerea* (grey rot), is the primary source of economic losses in the strawberry supply chain for sales markets. The main objective of the study was to evaluate the use of lavender essential oil (LEO) Munstead cultivar to extend the shelf life of strawberries while monitoring antifungal activity and preserving nutritional quality over 12 days of storage. The chemical composition of LEO, as determined by GC-MS, highlights a predominant profile of esters and monoterpenols. The *in vitro* antifungal activity of *Botrytis cinerea* using the „poisoned medium” method was determined for: 0.25; 0.5; 1; 2; 4; 8; 10; 12; 14(%). The results demonstrated a concentration-dependent inhibitory effect of LEO on mycelial growth. The minimum concentration with a fungistatic effect was 1%, and 8% had a fungicidal effect, providing the best efficacy and practical applicability. For the *in vivo* test, coating mixtures based on sodium alginate, enriched with LEO at 1%, 2%, and 4%, were used. Treatments with LEO-enriched alginate reduced strawberry weight loss by 6.8%, decreased fungal incidence, and maintained firmness and ascorbic acid content.

Key words: alginate nanoparticles, *Botrytis cinerea*, edible coatings, lavender essential oil, postharvest.

INTRODUCTION

Strawberry fruits (*Fragaria* × *ananassa*) are some of the most appreciated fruits worldwide due to their special sensory qualities and rich content in bioactive compounds, such as vitamin C, anthocyanins, and other natural antioxidants. With a high water content, a high respiration rate, and a fragile tissue structure, strawberries are highly perishable, making them extremely susceptible to post-harvest spoilage. In the storage, transport, and marketing stages, quantitative and qualitative losses are substantial, significantly affecting the supply chain and producers' profitability (Romanazzi et al., 2016; Terry et al., 2013). Microbial degradation is one of the main causes of post-harvest losses in horticultural products, with a major impact on food quality and food safety (Șumălan R.M., 2022).

Among the factors responsible for post-harvest degradation, fungal diseases are the main cause of strawberry losses. *Botrytis cinerea*, the etiological agent of grey rot, is the most common and destructive pathogen of strawberries in the post-harvest period (Petrasc

et al., 2019). Recent studies have demonstrated the effectiveness of alternative antifungal control strategies in maintaining post-harvest strawberry quality, reducing spoilage, and preserving organoleptic properties (Șumălan et al., 2021). *Botrytis cinerea* can infect fruits in the field, at early stages of development, and during storage, developing rapidly through the appearance of mycelium and grey fruiting, softening of tissues, and depreciation of commercial appearance. The effective control of this pathogen is a major challenge in the post-harvest technology of small fruits (Ribeiro et al., 2021). Traditionally, the fight against post-harvest fungal diseases has relied on chemical fungicides. However, the intensive use of these substances has led to pathogen resistance, the accumulation of chemical residues on fruits, and growing concerns about food safety and environmental impact. In this context, there is increased demand from consumers and industry to identify and use alternative, natural, and sustainable solutions that protect fruits without compromising their quality and safety (Tripathi & Dubey, 2004).

In recent years, essential oils have gained particular interest as natural antifungal agents, due to their complex biological activity and broad-spectrum of action. They contain volatile compounds, such as monoterpenes, sesquiterpenes, and esters, which can interfere with the structure of microorganisms' cell membranes, inhibiting the growth and development of pathogenic fungi. Numerous studies have highlighted the effectiveness of essential oils in controlling phytopathogens and reducing post-harvest spoilage in fruits (Burt, 2004; Hyldgaard et al., 2012; Prakash et al., 2015).

The application of essential oils, including as vapours, has demonstrated significant potential for controlling fungi responsible for post-harvest spoilage and maintaining fruit quality, as shown for lavender, mint, and basil (Şumālan et al., 2020).

Lavender essential oil (*Lavandula angustifolia* Mill., LEO) is recognised for its antimicrobial, antioxidant, and antifungal properties and is used in both the food and pharmaceutical industries. Recent studies have highlighted a significant variability in the yield and chemical composition of lavender essential oil depending on genotype and pedoclimatic conditions (Costea et al., 2023; Boelens & Jiménez, 1991; Lis-Balchin, 2002).

The chemical composition of lavender essential oil is dominated by monoterpenols and esters, especially linalool and linalyl acetate, compounds responsible for both its characteristic aroma and its high biological activity (Cavanagh & Wilkinson, 2002; Lis-Balchin, 2002; Bakkali et al., 2008; Coriţar, 2024). These constituents give the essential oil a high potential to prevent microbial alteration of horticultural products by inhibiting the growth of phytopathogenic bacteria and fungi.

However, the direct application of essential oils is limited by their high volatility and potential effects on the sensory properties of fruits and vegetables, which require the development of alternative application systems and methods to control the release of active substances (Ribeiro et al., 2024; Baschetti et al., 2020).

In this regard, the use of biopolymer-based edible films is a promising strategy for protecting fruit in the post-harvest period (Hassan et al., 2018; Valdés et al., 2017).

To protect fruits during the post-harvest period, they delay respiration and ripening and limit the growth and multiplication of spoilage microorganisms (Valizadeh et al., 2024). Sodium alginate is valued for its biocompatibility, biodegradability, and non-toxicity, being approved for use in food applications. In addition, incorporating bioactive compounds, such as essential oils, into the polymer matrix enables controlled release of antimicrobial substances (Pandey et al., 2022; Baschetti et al., 2020; Donsi & Ferrari, 2016).

In the polymer matrix, controlled release of antimicrobial substances is enabled, enhancing the effectiveness of post-harvest protection without significantly affecting fruit properties (Baschetti et al., 2020).

Edible films act as semi-permeable barriers, reducing water loss and they gas exchange, and can simultaneously function as controlled-release systems for bioactive compounds, including essential oils. Integrating lavender essential oil into the alginate matrix can improve its stability and antifungal effectiveness (Valdés et al., 2017).

In the polymer matrix, the controlled release of antimicrobial substances is allowed, increasing the effectiveness of post-harvest protection without significantly affecting the sensory properties of the fruits (Pandey et al., 2022; Baschetti et al., 2020).

MATERIALS AND METHODS

The biological material used

The study was carried out using LEO from the Munstead cultivar, harvested in 2024, from the experimental field located in Mănăstur, Arad County, western Romania. The plant material was harvested at the optimal flowering stage to ensure a high content of bioactive volatile compounds. The essential oil was obtained by hydro-distillation and stored in dark-coloured glass containers at a low temperature (5±1°C) until practical use.

The phytopathogen, *Botrytis cinerea*, from the microbial collection of the discipline of Microbiology, Faculty of Engineering and Applied Technologies, code BC-15-2016, stored on PDA medium, was used to evaluate the antifungal efficacy of LEO. The fungal inoculum was obtained from 72-hour-old

cultures in the active mycelial growth phase. All experimental steps were carried out under sterile conditions to prevent external contamination and to ensure the reproducibility of the results.

Essential Oil Chemical Composition Analysis (GC-MS)

The chemical composition of lavender essential oil was determined by gas chromatography-mass spectrometry (GC-MS) using an Agilent 7890A GC-MS system equipped with an HP-5MS capillary column (30 m × 0.25 mm × 0.25 µm). Helium was used as a carrier gas at a constant flow rate of 1 mL/min. The injector temperature was maintained at 250°C, and the compounds were ionised at 70 eV. The mass spectra were recorded in electron impact ionisation (EI).

The identification of the compounds was achieved by comparing mass spectra with reference libraries and using retention indices. The results were expressed as relative percentages of the total essential oil composition.

In vitro evaluation of antifungal activity

The *in vitro* antifungal activity of lavender essential oil was evaluated using the poisoned medium technique (Pitt, 2008). The malt extract agar (MEA) medium was used to support optimal fungal development. LEO was incorporated into the MEA medium (30 mL) at different concentrations to achieve the following percentages: 0.25, 0.5, 1, 2, 4, 8, 10, 12, and 14%. After solidification, using a sterile cork borer, the medium was cut at two distal places, and mycelial discs of *Botrytis cinerea* (Ø 8 mm) were subsequently distributed from mycelium in the active growth phase. The inoculated plates were incubated at 25 ± 1°C in the dark for 5 days. Each concentration was tested in four replicates (n = 4), and the experiment was repeated independently to ensure reproducibility. If the fungal growth was 0, the mycelial disc was transferred to an LEO-free medium to determine whether the effect was fungistatic or fungicidal. Mycelial growth was evaluated by measuring two diameters perpendicular to the mycelial colonies, and the percentage of growth inhibition was calculated by reference to the control using the following calculation formula:

$$\text{MGI \%} = (\text{C-TLEO})/\text{C} \times 100$$

Where:

MGI - mycelial growth inhibition (%);

C - average diameter of fungal mycelia in control (mm);

T-LEO- average diameter of fungal mycelia for essential oil treatments (mm).

Obtaining the coating mixture based on LEO-enriched alginate nanoparticles

The alginate nanoparticle solution was formulated based on sodium alginate and used as a delivery system for LEO using the AquaWave 9381 ultrasonic bath (Barnstea Lab-line) according to the Valizadeh, 2024 protocol. Briefly, the base solution was prepared by dissolving 0.25% sodium alginate (250 mg/100 g distilled water) with constant stirring until a homogeneous dispersion was obtained. To stabilise the nanoparticles' structure, a 0.06% calcium chloride solution (60 mg/100 g water) was used, applied according to the ion cross-linking method.

For emulsification of the essential oil, Tween 80 (0.2%) was added. LEO has been incorporated at concentrations of 1%, 2%, and 4%. This method was also used for the LEO-0 as a control. The coating solutions were transferred to spray ampoules and stored in the refrigerator until use.

In vivo testing of coating mixtures based on LEO-enriched sodium alginate and evaluation of fruit quality indicators

For the evaluation of the *in vivo* effect, strawberry fruits (*Fragaria* × *ananassa*) were used, selected for uniformity in terms of size, maturity, and absence of visible defects, with 30 fruits/cover treatment variant. Before applying the LEO-enriched coating solutions, the fruits were washed with distilled water and allowed to dry at room temperature.

The coating solutions were applied by uniform spraying. After application, the fruits were allowed to dry at room temperature to form the protective film. A third of the fruits thus prepared were used to test the antifungal effect, for which they were artificially inoculated with a spore suspension of *Botrytis cinerea* at a density of 5 × 10⁵/mL, applied by spraying. After drying, the strawberries were placed in the containers and stored under conditions similar to those of the non-inoculated treatment at 5 ± 1°C for 12 days. At the end of storage, quality

indicators (weight loss, firmness, total soluble sugar content, pH), and the degree of fungal spoilage (FSD %).

To establish the FSD % (fungal spoilage degree), the Botrytis Spoilage Severity (BSS) was estimated for each treatment variant using the formula:

$$BSS = \Sigma (r \times n)$$

Where:

r - grade assigned to the degree of severity: 0 - for unaltered; 1 - altered 1-25%; 2 altered 26-50%; 3- altered 51-75%; 4 - altered 76-100%.

n - number of fruits with the same grade.

Formula for estimating the degree of fungal spoilage:

$$FSD \% = (BSS \times 100) / BF$$

Where: BF-initial number of inoculated strawberries multiplied by grade 4.

Statistical analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) to evaluate the effect of treatments on the measured parameters. When significant differences were detected, Tukey's multiple-comparison test was used to separate the means at the $p < 0.05$ significance level. All experimental data were expressed as mean $\pm$ standard deviation (SD), based on three independent replicates.

RESULTS AND DISCUSSIONS

Chemical composition of essential oil (GC-MS)

The chemical composition of lavender essential oil used in the study is presented in Table 1.

Table 1. Chemical composition (%) of lavender essential oil (Munstead cultivar) determined by GC-MS

No.	Identified compound		tr (min.)	LRI _C	LRI _r	C (%)
1	α -Thujene	MH	7.05	920	918	0.09
2	α -Pinene	MH	7.25	938	937	0.17
3	Camphene	MH	7.77	954	956	0.11
4	3-Octanone	O	9.15	986	984	0.78
5	β -Myrcene	MH	9.28	990	991	0.67
6	Butanoic acid, butyl ester	O	9.56	994	995	0.13
7	Acetic acid, hexyl ester	O	10.15	1016	1011	0.49
8	p-Cymene	MH	10.46	1025	1024	0.20
9	D-Limonene	MH	10.63	1029	1030	0.24
10	Eucalyptol	MO	10.69	1033	1034	0.54
11	β -trans-Ocimene	MO	10.98	1036	1037	1.17
12	cis- β -Ocimene	MO	11.35	1040	1039	0.67
13	Linalool oxide (cis)	MO	12.17	1074	1075	0.24
14	Linalool oxide (trans)	MO	12.76	1085	1084	0.11
15	Linalool	MO	13.32	1100	1099	29.50
16	Octen-1-ol acetate	O	13.64	1104	1106	0.89
17	3-Octanol acetate	O	14.07	1110	1111	0.16
18	Camphor	MO	14.82	1145	1144	0.18
19	Propanoic acid, 2-methyl-, hexyl ester	O	15.06	1168	1170	0.12
20	1-Terpineol	MO	16.10	1188	1189	4.05
21	2-Cyclohexen-1-one, 4-(1-methylethyl)-	MO	16.29	1198	1200	0.52
22	Butanoic acid, hexyl ester	O	16.65	1214	1216	0.67
23	Linalyl acetate	MO	18.67	1255	1256	43.24
24	Bornyl acetate	MO	19.73	1285	1283	0.15
25	Geranyl isobutyrate	MO	19.84	1292	1290	3.72
26	Epoxy- β -terpenyl acetate	MO	21.82	1340	1341	0.20
27	Epoxy- α -terpenyl acetate	MO	21.93	1346	1345	0.12
28	Nerol acetate	MO	22.31	1364	1362	0.17
29	Geraniol acetate	MO	22.96	1388	1382	0.38
30	Hexanoic acid, hexyl ester	O	23.21	1394	1393	0.17
31	3,7-Nonadien-2-ol, 4,8-dimethyl-	MO	23.30	1396	1395	0.06
32	β-Caryophyllene	SH	24.09	1406	1405	4.11
33	α -Bergamotene	SH	24.61	1415	1414	0.16
34	α -Caryophyllene	SH	25.21	1422	1420	0.09
35	β-Farnesene	SH	25.30	1444	1446	3.51

No.	Identified compound		t _R (min.)	LRI _c	LRI _r	C (%)
36	Germacrene D	SH	26.02	1482	1480	0.56
37	β-Sesquiphellandrene	SH	26.16	1490	1488	0.07
38	γ-Cadinene	SH	27.02	1514	1513	0.13
39	Caryophyllene oxide	SO	28.98	1580	1581	1.42
40	Hexahydrofarnesyl acetone	SO	36.25	1846	1845	0.09
Total constituents			-			≈100.00
from which						
MH (monoterpene hydrogenase)						1.48
MO monoterpene oxygenase						85.02
SH sesquiterpene hydrogenate						8.63
SO sesquiterpene oxidizate						1.51
Other oxygenated compounds						3.36

The relative content of volatile compounds was calculated as a percentage of the total area of chromatographic peaks obtained in ICT mode, which was set to 100%. Of the compounds identified, linalyl acetate and linalool were the most abundant, confirming that the essential oil analyzed belonged to a chemotype dominated by esters and monoterpene alcohols. These compounds are frequently associated with antifungal activity, which may explain the effectiveness later observed in biological tests. The results obtained are in line with the literature on *Lavandula angustifolia* essential oils (Cavanagh & Wilkinson, 2002; Lis-Balchin, 2002; Bakkali et al., 2008; Costea et al., 2023).

Antifungal activity of LEO established in vitro

The application of lavender essential oil (LEO) resulted in a progressive reduction in the mycelial growth of *Botrytis cinerea* with increasing concentration, compared to the control sample characterized by vigorous mycelium development. At low concentrations (0.25% and 0.5%), LEO showed a weak to moderate fungistatic effect, causing partial inhibition of fungal growth. At a concentration of 1%, a pronounced fungistatic effect was observed, and increasing the concentration to 2% intensified the inhibitory effect. At concentrations of 4% and above, mycelial growth was completely inhibited, indicating a fungicidal effect of lavender essential oil on *Botrytis cinerea*. The results presented in Table 2 show a concentration-dependent inhibition, with the fungistatic effect being evident starting at a concentration of 1% LEO, and the fungicidal effect being observed at concentrations of 4% and above.

The appearance of mycelial growth in the presence of different concentrations of LEO is illustrated in Figure 1, where progressive inhibition of fungal growth is observed with increasing LEO concentration.

Table 2. Percentage of mycelial growth inhibition (MGI) for *Botrytis cinerea* in the presence of LEO and assessment of antifungal effect

LEO (%)	MGI (%)	Biological effect
Control 0	0.00	Vigorous growth
0.25	16.3	Weak fungistatic effect
0.5	48.6	Moderate fungistatic effect
1	74.3	Fungistatic effect
2	90.7	Fungistatic effect
4	100	Fungicidal effect
8	100	Fungicidal effect
10	100	Fungicidal effect
12	100	Fungicidal effect
14	100	Fungicidal effect

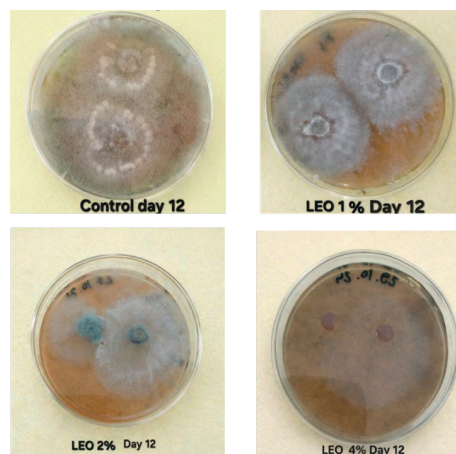


Figure 1. *In vitro* antifungal activity of lavender essential oil (LEO) on the mycelial growth of *Botrytis cinerea* on the MEA medium after 12 days of incubation at 25°C

Effect of treatments on strawberry quality parameters

The effect of treatments with alginate-based films enriched with lavender essential oil on the main quality parameters of strawberries after 12 days of storage is shown in Table 3. Compared to the control, LEO treatments moderately influenced weight loss, fruit firmness, soluble dry matter (TSS) content, pH, and ascorbic acid content. The lowest weight loss was observed in the 4% treatment, suggesting a better water-holding capacity in the fruit. The firmness of the fruits was relatively similar across the variants,

indicating that the treatments applied did not cause major changes in tissue consistency. In terms of soluble dry matter content, treatment with 1% LEO recorded the highest value, suggesting better maintenance of soluble sugars. pH values showed little variation between treatments, indicating the stability of fruit acidity during refrigerated storage. Also, the ascorbic acid content was slightly higher in the variant treated with 1% LEO, suggesting a possible protective effect of the treatment on ascorbic acid stability.

Table 3. The effect of treatments with alginate-based films enriched with lavender essential oil on the quality parameters of strawberries after 12 days of storage

Treatment	Weight loss (%)	Firmness (lb)	SST (°Brix)	pH	Ascorbic acid (mg/100 g)
Control	0.073 ± 0.015	0.218 ± 0.012	6.82 ± 0.24	3.45 ± 0.08	21.46 ± 1.55
LEO 1%	0.076 ± 0.010**	0.230 ± 0.019	7.44 ± 0.35	3.52 ± 0.06**	22.97 ± 2.13
LEO 2%	0.074 ± 0.011	0.200 ± 0.024**	6.70 ± 0.39**	3.48 ± 0.05***	20.66 ± 1.59**
LEO 4%	0.068 ± 0.007***	0.216 ± 0.008***	6.70 ± 0.21***	3.50 ± 0.07	19.94 ± 2.02***

In vivo evaluation of antifungal protection

The degree of fungal spoilage of strawberry fruits, according to Figure 3, significant differences from the control variant for V2 variants with 2% LEO content and for V3 with 4% LEO. For V1, the 1% LEO mixture shows no statistically significant differences.

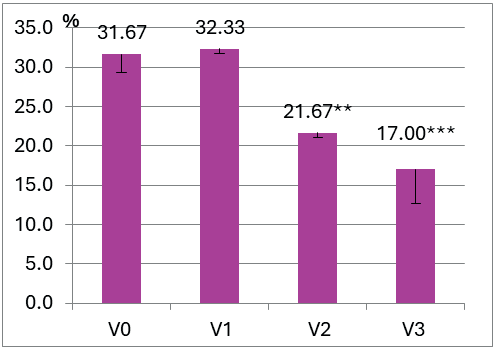


Figure 3. The fungal spoilage degree (FSD %) of strawberry fruits after 12 days since the edible coating treatments were applied

Compared with the literature, the results of the present study are consistent with observations on the use of essential oils and alginate-based edible coatings for controlling phytopathogens

and maintaining the quality of soft fruits. Several studies have demonstrated that essential oils such as lavender, mint, and basil can inhibit the growth of phytopathogenic fungi and help maintain fruit quality during storage (Burt, 2004; López-Reyes et al., 2010; Nazzaro et al., 2013). Reducing weight loss, maintaining firmness, stability of soluble dry matter content, and pH, as well as preserving ascorbic acid content, demonstrate the effectiveness of this approach in delaying post-harvest degradation processes.

The incorporation of essential oils into biopolymer matrices, such as alginate, represents a promising strategy to improve the stability and controlled release of bioactive compounds. However, further studies are necessary to optimize the concentrations used, to evaluate possible effects on the sensory characteristics of fruits, and to assess the effectiveness of such systems under real storage and transport conditions. Future research may contribute to the development of sustainable technologies for postharvest protection and quality preservation of fresh fruits and vegetables.

CONCLUSIONS

The results of this study highlight the potential of lavender essential oil as a natural antifungal agent and the effectiveness of alginate nanoparticles as a vehicle for post-harvest application.

The *in vivo* evaluation of strawberry post-harvest quality indicated that treatments with alginate nanoparticles enriched with lavender essential oil help maintain the fruits' physicochemical parameters during refrigerated storage.

In particular, the 1% concentration of lavender essential oil showed an optimal balance between antifungal efficacy and preservation of nutritional value, confirming the role of alginate in the controlled release of bioactive compounds.

Overall, the proposed strategy can be considered a sustainable and environmentally friendly alternative for post-harvest conservation of strawberries, with the potential to be integrated into modern fresh fruit quality management systems. Further research is needed to optimize technological parameters and assess applicability on an industrial scale.

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RESEARCH ON THE USE OF GREEN CUTTINGS AS AN ALTERNATIVE METHOD OF PROPAGATION OF THE SPECIES *PHLOSTACHYS EDULIS*

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Abstract

Since establishing a moso bamboo plantation represents a real challenge for farmers, both due to the high costs of purchasing planting material and the species' specific gregarious flowering, the low viability of the seeds, and their low germination rate, identifying and implementing alternative propagation methods is a constant concern. The research results highlighted that the techniques used in the propagation process through cuttings, the conditions for collecting cuttings, treatments with rooting biostimulators, and the planting technique of the cuttings had a direct influence on the rooting percentage of the cuttings as well as on the subsequent growth and development of the new bamboo plants. Cuttings with one or two nodes made from the basal area of stems with a diameter of 1 cm and planted horizontally performed the best, providing, within a relatively short period of time and under minimal input conditions, sufficient planting material for establishing new plantations.

Key words: bamboo, biostimulation, cuttings, vegetative propagation.

INTRODUCTION

Since bamboo flowering is gregarious, occurring at intervals of 60 to 100 years, and the plants die after flowering, a gap of approximately 2 to 3 years is created in the availability of raw material. To meet the increasing demand for bamboo as a raw material in the circular economy, there is an urgent need to expand the area occupied by this species, either by establishing new plantations or by promoting natural regeneration (Vitali & Dobrinouiu, 2020). The possibility of establishing large-scale plantations through generative means (from seeds) is limited because most bamboo species produce seeds at long intervals, from 60 to 120 years. Therefore, although this method of producing planting material is considered to be the most common method of multiplying this species, it is recommended only for establishing small-scale plantations. For large-scale production of planting material, it becomes imperative to identify and implement alternative methods of producing bamboo planting material, namely vegetative propagation, with propagation through cuttings being considered by researchers to be a fairly reliable method (Kaushal et al., 2011). Thus, Singh et al. (2004)

reported a rooting percentage during the summer of 98% for stem cuttings without the addition of plant growth regulators in the bamboo species *D. asper*. Rooting in the summer season of 88% was reported for *B. nutans*, compared to the winter season, when the rooting percentage of cuttings was only 53.4% (Singh et al., 2011). Deb et al. (2016) reported that approximately 70.6% of stem cuttings planted in April responded positively, with about 9.4 shoots/nodes and approximately 15.3 roots/nodes within 6 months of planting, whereas planting the cuttings in January had a positive response of about 31%, with approximately 3.1 shoots/nodes and about 2.2 roots/nodes for the species *B. nagalandiana*. Gulabrao et al. (2012) observed that stem cuttings of *B. bambos*, *B. vulgaris*, *B. balcooa*, *D. hamiltonii*, *B. tulda*, *D. strictus*, *D. giganteus* and *B. nutans* rooted best in the spring (56.67%), followed closely by the summer season (54.58%), but less so in winter (36.67%). Thus, it is evident that the interaction between bamboo species and season is a risk factor in vegetative propagation, with summer being considered the most suitable season for propagation through cuttings of most bamboo species (Singh et al., 2006).

The effect of growth regulators also depends on the orientation of the cuttings in the growth substrate (Bhol & Nayak, 2012; Bhol & Parida, 2015). Horizontal planting of cuttings of *D. asper* treated with IBA resulted in higher rooting (65%) than vertical planting of the cuttings (55%) during the summer (Razvi et al., 2011). The present research was undertaken with the inducing rooting of cuttings made from branch portions of the Moso bamboo species *Phyllostachys edulis*, in order to ensure the availability of material intended for the establishment of new plantations on a large scale.

MATERIALS AND METHODS

The experiment was initiated in May (2025) and finished in January (2026). It consisted of evaluating the effect of the biostimulator Radi-Stim-Solution, the type of cuttings, and the method of planting the cuttings on the rooting percentage, the number of roots and lateral branches formed on the rooted cuttings, and the vegetative development of the rooted cuttings over 9 months from the start of the experiment. For this purpose, a three-factorial experiment, in

subdivided plots, with three repetitions, was designed. The experimental factors under study being the following:

Factor A - The biostimulator used, with 2 graduations:

- a₁ - Radi-Stim 3% + TurboRoot 2.5%;
- a₂ - Radi-Stim 3% + Algavell 2.5%.

Factor B - The type of cuttings, with 3 graduations:

- b₁ - cuttings made from the apical zone of the stem;
- b₂ - cuttings made from the middle zone of the stem;
- b₃ - cuttings made from the basal zone of the stem.

Factor C - The method of planting the cuttings, with 2 graduations:

- c₁ - cuttings planted horizontally;
- c₂ - cuttings planted vertically.

By combining the experimental factors included in the study, 12 experimental variants resulted (Table 1), in 3 repetitions, and the analysis and statistical interpretation of the research results were carried out according to the analysis of variance method for the probability P5%, P1%, and P0.1%.

Table1. Experimental variants tested during the research

Experimental factors tested			Experimental variants
Factor A	Factor B	Factor C	
a ₁	b ₁	c ₁	V ₁ (a ₁ b ₁ c ₁) - apical cutting planted horizontally +Radi-Stim 3%+TurboRoot 2.5%
		c ₂	V ₂ (a ₁ b ₁ c ₂) - apical cutting planted vertically +Radi-Stim 3%+ TurboRoot 2.5%
	b ₂	c ₁	V ₃ (a ₁ b ₂ c ₁) - median cutting planted horizontally +Radi-Stim 3%+TurboRoot 2.5%
		c ₂	V ₄ (a ₁ b ₂ c ₂) - median cutting planted vertically +Radi-Stim 3%+TurboRoot 2.5%
	b ₃	c ₁	V ₅ (a ₁ b ₃ c ₁) - basal cutting planted horizontally +Radi-Stim 3%+TurboRoot 2.5%
		c ₂	V ₆ (a ₁ b ₃ c ₂) - basal cutting planted vertically +Radi-Stim 3%+TurboRoot 2.5%
a ₂	b ₁	c ₁	V ₇ (a ₂ b ₁ c ₁) - apical cutting planted horizontally +Radi-Stim 3%+Algavell 2.5%
		c ₂	V ₈ (a ₂ b ₁ c ₂) - apical cutting vertically +Radi-Stim 3%+Algavell 2.5%
	b ₂	c ₁	V ₉ (a ₂ b ₂ c ₁) - median cutting planted horizontally +Radi-Stim 3%+Algavell 2.5%
		c ₂	V ₁₀ (a ₂ b ₂ c ₂) - median cutting planted vertically +Radi-Stim 3%+Algavell 2.5%
	b ₃	c ₁	V ₁₁ (a ₂ b ₃ c ₁) - basal cutting planted horizontally +Radi-Stim 3%+Algavell 2.5%
		c ₂	V ₁₂ (a ₂ b ₃ c ₂) - basal cutting planted vertically +Radi-Stim 3%+Algavell 2.5%

For the preparation of the cuttings, annual stems with a diameter of 1 cm were taken from the bamboo plants, retaining from each stem the basal, median, and apical nodal areas, where a portion of stem branching 15 cm in length, respectively 2 nodes, was preserved, resulting in cuttings 10 cm long with a single stem node, used as biological material for vegetative propagation.

After shaping, the resulting cuttings were treated to stimulate the rooting process by immersing

their nodal point for 12 hours in a Radi-Stim solution of different concentrations, according to the experimental protocol, with 25 cuttings selected for each experimental variant studied.

After the time scheduled for pre-treatment with biostimulants had elapsed, the cuttings were removed from the biostimulant solution, allowed to dry for 3 minutes, and then planted in prismatic vegetation pots with a volume of 3 liters in a nutrient mixture consisting of 3 parts clay to 1 part poultry compost, according to the

pre-established experimental protocol. In order to enhance the growth and vegetative development processes of the rooted cuttings, 3 treatments (immediately after cuttings planting, at 3 months after cuttings planting, and at 6 months after cuttings planting) with growth biostimulants of the TurboRoot and Algavell type were administered by moistening the nutrient substrate at a concentration of 2.5% (Figure 1).



Figure 1. Transfer of biostimulated cuttings into growing pots

Observations and measurements carried out during the study

After planting the cuttings in the growing pots, periodic observations were made regarding their vegetative state, as well as biometric measurements, in order to evaluate the influence of the experimental factors studied on the growth and vegetative development rate of the rooted cuttings obtained through propagation by the green cutting method.

Thus, from each experimental variant, at 6 and 9 months after planting the cuttings, destructive samples of rooted cuttings were taken, which were cleaned of soil, washed, and biometric measurements were performed on them regarding the rooting percentage, the number of roots formed and their average length, as well as the number of shoots differentiated on the rooted cuttings and their average length (Figures 2, 3).



Figure 2. The appearance of bamboo plants after 6 months when planting the biostimulated cuttings



Figure 3. The appearance of bamboo plants after 9 months when planting the biostimulated cuttings

RESULTS AND DISCUSSIONS

Results and discussion regarding the rooting percentage of moso bamboo cuttings

Analyzing the research results regarding the effect of the biostimulator Radi-Stim on the rooting process of branch cuttings used for vegetative propagation of the species *Phyllostachys edulis*, it is observed that there were considerable variations among the experimental variants studied (Table 2).

Thus, the rooting percentage six months after the start of the experiment recorded values ranging from 16.36% to 57.58%, with the rooting process increasing in intensity and reaching values ranging from 29.28% to 66.76% nine months after planting the cuttings in growth pots.

Table 2. The rooting rate of cuttings in the species *Phyllostachys edulis*

Experimental Variants	Rooting rate after 6 month (%)	Difference (%)	Significance	Rooting rate after 9 months (%)	Difference (%)	Significance
V ₁ (a ₁ b ₁ c ₁)	21.73	-12.68	ooo	35.54	-13.68	ooo
V ₂ (a ₁ b ₁ c ₂)	16.36	-18.05	ooo	33.27	-15.95	ooo
V ₃ (a ₁ b ₂ c ₁)	37.92	3.51	***	49.53	0.31	-
V ₄ (a ₁ b ₂ c ₂)	29.33	-5.08	ooo	42.85	-6.37	ooo
V ₅ (a ₁ b ₃ c ₁)	46.57	12.16	***	58.72	9.50	***
V ₆ (a ₁ b ₃ c ₂)	35.13	0.72	**	62.87	13.65	***
V ₇ (a ₂ b ₁ c ₁)	26.74	-7.67	ooo	32.59	-16.63	ooo
V ₈ (a ₂ b ₁ c ₂)	18.39	-16.02	ooo	29.28	-19.94	ooo
V ₉ (a ₂ b ₂ c ₁)	48.93	14.52	***	57.55	8.33	***
V ₁₀ (a ₂ b ₂ c ₂)	33.37	-1.04	oo	55.83	6.61	***
V ₁₁ (a ₂ b ₃ c ₁)	57.58	23.17	***	66.76	17.54	***
V ₁₂ (a ₂ b ₃ c ₂)	40.87	6.46	***	65.88	16.66	***
Average (Control)	34.41	Control	Control	49.22	Control	Control
	LD _{5%} = 0.317; LD _{1%} = 0.685; LD _{0.1%} = 1.119			LD _{5%} = 0.532; LD _{1%} = 0.863; LD _{0.1%} = 1.212		

Compared to the average experience, statistically significant differences were recorded, ranging from very significantly negative (ooo) to very significantly positive (***) among the variants, with the greatest rooting capacity being shown by cuttings made from the basal area of the stems and planted in a horizontal position in both stages of the determinations (V₁₁) (Table 2).

Results and discussions regarding the number of roots formed on bamboo cuttings after planting

The results of biometric determinations related to the number of adventitious roots developed on cuttings after the application of the biostimulation treatment and planting in growth pots highlighted quite a large variability among the experimental variants tested during the research. This biometric parameter was

influenced both by the type of cuttings and the position in which they were planted after treatment with Radi-Stim Solution at a concentration of 3%.

It can thus be observed that, 6 months after planting the cuttings in the nutrient substrate, the biostimulated cuttings differentiated between 4.29 roots/cutting (V₂) and 12.27 roots/cutting (V₁₁), the number of roots increased as the bamboo rooted cuttings progressed in growth; thus, 9 months after planting, the number of adventitious roots differentiated per cutting ranged between 8.51 roots/cutting (V₁) and 14.36 roots/cutting (V₁₁), the best performing were the cuttings made from the nodal areas at the base of the stems, planted in a horizontal plane and treated with the Algavell biostimulator at a 2.5% concentration (V₁₁) in both stages of the determinations (Table 3).

Table 3. Number of roots differentiated on cuttings in the species *Phyllostachys edulis*

Experimental Variants	Roots No after 6 months (No.)	Difference (No.)	Significance	Roots No. after 9 months (No.)	Difference (No.)	Significance
V ₁ (a ₁ b ₁ c ₁)	7.25	-0.50	ooo	8.51	-1.74	ooo
V ₂ (a ₁ b ₁ c ₂)	4.29	-3.46	ooo	8.93	-1.32	ooo
V ₃ (a ₁ b ₂ c ₁)	7.58	-0.17	o	10.71	0.46	**
V ₄ (a ₁ b ₂ c ₂)	6.53	-1.22	ooo	10.52	0.27	**
V ₅ (a ₁ b ₃ c ₁)	9.27	1.52	***	12.38	2.13	***
V ₆ (a ₁ b ₃ c ₂)	7.71	-0.04	-	11.63	1.38	***
V ₇ (a ₂ b ₁ c ₁)	8.23	0.48	***	10.15	-0.10	-
V ₈ (a ₂ b ₁ c ₂)	5.27	-2.50	ooo	9.37	-0.88	ooo
V ₉ (a ₂ b ₂ c ₁)	8.29	0.54	***	12.77	2.52	***
V ₁₀ (a ₂ b ₂ c ₂)	7.58	-0.17	o	12.29	2.04	***
V ₁₁ (a ₂ b ₃ c ₁)	12.27	4.25	***	14.36	4.11	***
V ₁₂ (a ₂ b ₃ c ₂)	8.75	1.00	***	13.68	3.43	***
Average (Control)	7.75	Control	Control	10.25	Control	Control
	LD _{5%} = 0.087; LD _{1%} = 0.195; LD _{0.1%} = 0.433			LD _{5%} = 0.125; LD _{1%} = 0.263; LD _{0.1%} = 0.754		

Compared to the average of the experimental variants taken in the study, it is observed that, 9 months after planting, the cuttings made from

the basal and median parts of the bamboo stems performed the best, showing highly significant positive differences (***) statistically (V₅, V₆,

V₉, V₁₀, V₁₁ and V₁₂), regardless of their planting position, while the cuttings made from the apical part of the stems showed the weakest capacity for adventitious root differentiation after planting in the nutrient substrate, with insignificant (-) (V₇) or highly significant negative (ooo) differences compared to the average of the experiment (V₁, V₂ and V₈) (Table 3).

Results and discussions regarding the length of roots formed on bamboo cuttings after planting

Following biometrical measurements on the length of adventitious roots developed on moso bamboo cuttings after their planting in the nutrient substrate, the experimental variants in

which the cuttings were treated with the biostimulator Radi-Stim Solution (3% concentration) and planted in a horizontal plane have been highlighted. In these variants, the average root length ranged between 8.63 cm (V₁) and 12.66 cm (V₁₁) after 6 months of planting, reaching lengths between 9.53 cm (V₂) and 14.49 cm (V₁₁) after 9 months from planting.

The best performance in terms of root growth rate was observed for cuttings made from the basal and middle nodal regions of bamboo stems, where root length exceeded 10 cm at 6 months after planting (V₃, V₅, V₉ and V₁₁), regardless of the growth biostimulator applied after cutting root formation (Table 4).

Table 4. Root length formed on cuttings rooted in the species *Phyllostachys edulis*

Experimental variants	Roots length after 6 months (cm)	Difference (cm)	Significance	Roots length after 9 months (cm)	Difference (cm)	Significance
V ₁ (a ₁ b ₁ c ₁)	8.63	-0.95	ooo	9.97	-2.62	ooo
V ₂ (a ₁ b ₁ c ₂)	5.58	-4.00	ooo	9.53	-3.06	ooo
V ₃ (a ₁ b ₂ c ₁)	10.73	1.15	***	12.31	-0.28	o
V ₄ (a ₁ b ₂ c ₂)	8.62	-0.96	ooo	12.68	0.09	-
V ₅ (a ₁ b ₃ c ₁)	10.36	0.78	***	12.44	-0.15	-
V ₆ (a ₁ b ₃ c ₂)	8.78	-0.80	ooo	11.67	-0.92	ooo
V ₇ (a ₂ b ₁ c ₁)	9.33	-0.25	o	13.97	1.38	***
V ₈ (a ₂ b ₁ c ₂)	8.81	-0.77	ooo	12.35	-0.24	o
V ₉ (a ₂ b ₂ c ₁)	12.38	2.80	***	14.21	1.62	***
V ₁₀ (a ₂ b ₂ c ₂)	9.27	-0.31	o	13.83	1.24	***
V ₁₁ (a ₂ b ₃ c ₁)	12.66	3.08	***	14.49	1.90	***
V ₁₂ (a ₂ b ₃ c ₂)	9.86	0.28	*	13.74	1.15	***
Average (Control)	9.58	Control	Control	12.59	Control	Control
	LD _{5%} = 0.126; LD _{1%} = 0.313; LD _{0.1%} = 0.725			LD _{5%} = 0.212; LD _{1%} = 0.374; LD _{0.1%} = 0.672		

Biometric measurements carried out 9 months after the moment of planting showed the direct influence of the type of biostimulator administered to the nutrient substrate after rooting the cuttings. With the Algavell biostimulator administered in three treatments, there the growth in length of adventitious roots, whose average length exceeded 13.74 cm, with differences recorded as highly statistically significant (***) compared to the average of the experimental variants (V₇, V₉, V₁₀, V₁₁ and V₁₂), regardless of the planting method practiced (Table 4).

Results and discussions regarding the number of shoots formed on rooted cuttings

The research results regarding the influence of the experimental factors studied on the number Of differentiated shoots on moso bamboo rooted cuttings obtained through the green cutting method demonstrated that, six months after planting, the cuttings treated with the

biostimulator Radi-Stim Solution at a 3% concentration and that received additional treatments with the biostimulators TurboRoot and Algavell differentiated between 1.01 shoots/rooted cutting (V₂ and V₆) and 1.86 shoots/ rooted cutting (V₁₁), with the highest capacity for differentiation of new shoots being shown by the cuttings that benefited from the additional supply of nutrients made available to the rooted cuttings as a result of the administration of the Algavell biostimulator at a 2.5% concentration, regardless of the type of cuttings used for propagation or the planting method practiced (Table 5).

At 9 months after planting cuttings in the nutrient substrate, the number of differentiated shoots on the moso bamboo rooted cuttings ranged between 1.23 shoots/rooted cutting (V₂) and 2.69 shoots/ rooted cuttings (V₁₁), with the cuttings made from the nodal areas at the base of the stems performing the best. These cuttings, further treated with the biostimulators

TurboRoot and Algavell, showed very significant positive differences (***) compared to the experiment average (Table 5), regardless of the

position in which the cuttings were planted (V_5 , V_6 , V_{11} and V_{12}).

Table 5. Number of shoots differentiated on rooted cuttings by species *Phylostachys edulis*

Experimental Variants	Shoots No. after 6 months (No)	Difference (No)	Significance	Shoots No. after 9 months (No)	Difference (No)	Significance
$V_1 (a_1b_1c_1)$	1.11	-0.17	oo	1.48	-0.55	ooo
$V_2 (a_1b_1c_2)$	1.01	-0.27	ooo	1.23	-0.80	ooo
$V_3 (a_1b_2c_1)$	1.12	-0.16	oo	2.27	0.24	ooo
$V_4 (a_1b_2c_2)$	1.03	-0.25	ooo	1.85	-0.18	ooo
$V_5 (a_1b_3c_1)$	1.15	-0.13	oo	2.59	0.56	***
$V_6 (a_1b_3c_2)$	1.01	-0.27	ooo	2.37	0.34	***
$V_7 (a_2b_1c_1)$	1.22	-0.06	o	1.68	-0.35	ooo
$V_8 (a_2b_1c_2)$	1.18	-0.10	oo	1.43	-0.60	ooo
$V_9 (a_2b_2c_1)$	1.62	0.34	***	2.37	0.34	***
$V_{10} (a_2b_2c_2)$	1.41	0.13	**	1.95	-0.08	oo
$V_{11} (a_2b_3c_1)$	1.86	0.58	***	2.69	0.66	***
$V_{12} (a_2b_3c_2)$	1.63	0.35	***	2.47	0.44	***
Average (Control)	1.28	Control	Control	2.03	Control	Control
	LD _{5%} = 0.067; LD _{1%} = 0.094; LD _{0.1%} = 0.209			LD _{5%} = 0.025; LD _{1%} = 0.063; LD _{0.1%} = 0.117		

Results and discussions regarding the height of bamboo rooted cuttings after planting the cuttings

The endogenous reserve substances present in the cuttings taken from the bamboo stems, but especially the exogenous biostimulant substances applied to the cuttings before and after planting, played a determining role in the vegetative growth and development of the bamboo rooted cuttings. Thus, 6 months after planting the cuttings, the height of the rooted cuttings resulting from cuttings planted in a horizontal plane reached average heights

ranging between 19.73 cm (V_1) and 58.57 cm (V_{11}), while, by planting the cuttings in a vertical position, the height of the rooted cuttings ranged between 15.27 cm (V_2) and 54.28 cm (V_{12}), with statistically significant positive (*) and highly significant positive (***) differences compared with the average of the experimental variants tested during the research, in the experimental variants where the growth and vegetative development of bamboo rooted cuttings were stimulated by applying the biofertilizer Algaveel at a concentration of 2.5% (V_7 , V_8 , V_9 , V_{10} , V_{11} and V_{12}) (Table 6).

Table 6. Height of bamboo plants propagated by the green cutting method in the species *Phylostachys edulis*

Experimental Variants	Plants height after 6 months (cm)	Difference (cm)	Significance	Plants height after 9 months (cm)	Difference (cm)	Significance
$V_1 (a_1b_1c_1)$	19.73	-14.62	ooo	43.35	-12.70	ooo
$V_2 (a_1b_1c_2)$	15.27	-19.08	ooo	40.19	-15.86	ooo
$V_3 (a_1b_2c_1)$	21.96	-12.39	ooo	44.37	-11.68	ooo
$V_4 (a_1b_2c_2)$	18.29	-16.06	ooo	41.03	-15.02	ooo
$V_5 (a_1b_3c_1)$	28.57	-5.78	ooo	48.31	-7.74	ooo
$V_6 (a_1b_3c_2)$	24.28	-10.07	ooo	43.18	-12.87	ooo
$V_7 (a_2b_1c_1)$	39.73	5.38	***	56.75	0.70	*
$V_8 (a_2b_1c_2)$	35.27	0.92	*	48.47	-7.58	ooo
$V_9 (a_2b_2c_1)$	50.96	16.61	***	76.49	20.44	***
$V_{10} (a_2b_2c_2)$	45.29	10.94	***	71.43	15.38	***
$V_{11} (a_2b_3c_1)$	58.57	24.22	***	83.29	27.24	***
$V_{12} (a_2b_3c_2)$	54.28	19.93	***	75.76	19.71	***
Average (Control)	34.35	Control	Control	56.05	Control	Control
	LD _{5%} = 0.824; LD _{1%} = 1.062; LD _{0.1%} = 1.326			LD _{5%} = 0.565; LD _{1%} = 1.093; LD _{0.1%} = 1.633		

The height of bamboo plants measured 9 months after planting the cuttings in growing pots reached values ranging from 40.19 cm (V_2) to 75.76 cm (V_{12}) as a result of using the vertical planting method for the cuttings, while under the conditions of planting the cuttings in a horizontal position, the values of this biometric

parameter varied between 43.35 cm (V_1) and 83.29 cm (V_{11}), with bamboo plants obtained from cuttings taken from the basal and median parts of the stems showed the most intense growth and vegetative development rate, in the context of biostimulation with Radi-Stim at a 3% concentration and Alagavell at a 2.5%

concentration, with very significantly positive differences (***) compared to the average of the experimental variants, regardless of the planting method used (Table 6).

CONCLUSIONS

Since bamboo is a species with gregarious flowering, flowering only once during its lifetime and, after which, the plants die. Moreover, the seeds are most often not viable, which is why they cannot be used in the propagation process to produce on a large scale the plants needed for the establishment of new plantations; therefore, it is recommended to use alternative methods of plant propagation, namely the production of planting material through vegetative means.

The present research highlighted the fact that the success of using the bamboo propagation method through green cuttings is influenced by a series of external factors, such as: the type of cuttings, the method of planting the cuttings, treatments with biostimulators, and the nutrient substrate used for planting the cuttings, etc.

The results obtained during the present study demonstrated that single-node cuttings, made from the basal area of the stems, treated with the biostimulator Radi-Stim Solution (3%) and planted horizontally, recorded the highest rooting percentage compared with cuttings planted in a vertical position, both after 6 months and 9 months from the moment of biostimulation and planting in the nutrient substrate.

Against the background of stimulating the rooting process by treating bamboo cuttings belonging to the species *Phyllostachys edulis* with the biostimulator Radi-Stim solution at a concentration of 3% and by stimulating the processes of growth and vegetative development through the administration of the biostimulators TurboRoot and Algavell at a concentration of 2.5%, the cuttings taken from the basal and median nodal regions of the stems and planted in a horizontal plot displayed the highest capacity for adventitious root differentiation and the most intense rate of root length growth at both times of conducting the biometric measurements.

The greatest capacity for differentiation of new shoots was observed in bamboo rooted cuttings

obtained from cuttings that benefited from the additional supply of nutrients as a result of the administration of the biostimulant Algavell at a concentration of 2.5%, regardless of the type of cuttings used for propagation and the planting method practiced, where bamboo rooted cuttings obtained from cuttings taken from the basal and median parts of the stems showed the most intense growth and vegetative development, against the background of enhancing the effect of the Radi-Stim biostimulant at 3% solution concentration, the newly formed shoots exceeded 45 cm in length 6 months after planting and over 71 cm in length 9 months after planting.

Since, to date, there are no studies regarding the propagation of the species *Phyllostachys edulis* (the only bamboo species adapted to the temperate climate) by vegetative means, and the research conducted so far has only targeted tropical bamboo species, which are characterized by much better-developed mechanical tissue, studies evaluating the suitability of this species for propagation through the cutting method can serve as a starting point for future research.

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SECONDARY METABOLITES OF COMMON JUNIPER (*JUNIPERUS COMMUNIS* L.) SUITABLE FOR USE IN AGRICULTURE AND FOOD PRODUCTION. REVIEW

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Abstract

Juniperus communis (common juniper) is a shrub or small coniferous tree with certain botanical characteristics, including its evergreen nature and berries, which mature over a period of 2-3 years and may occur on the plant at different stages of ripeness simultaneously. This enables an extended availability of both needles and berries. This review summarises findings published over the last 20 years in scientific journals and monographs. The needles and berries of common juniper differ significantly in their secondary-metabolite profiles and in their applications in sustainable agriculture and healthy food products. The needles are characterised by elevated monoterpene content, and these compounds exhibit toxicity and repellent activity against certain pests in both field and warehouse conditions. Allelopathic suppression of weed seed germination by needle extracts of *J. communis* is regarded as a promising strategy for the development of natural bioherbicides. Berries accumulate substantial amounts of phenolic compounds and pigments, including proanthocyanidins, which exhibit antioxidant and antimicrobial activity. These properties are used to stabilize food matrices, impart a distinctive flavour, and extend the shelf life of products.

Key words: *Juniperus communis*, needle, berry, secondary metabolite, application, horticulture.

INTRODUCTION

Juniperus communis L. (common juniper) is a perennial, evergreen, slow-growing woody species of the family Cupressaceae, occurring as a shrub or small tree. Common juniper has a circumpolar distribution, occupying the temperate and subarctic regions of the Northern Hemisphere (Thomas et al., 2007; Enescu et al., 2016; Salamon et al., 2023; Stefkov et al., 2025). In Europe, it occurs from the Atlantic coast of Portugal and Ireland across Central and Eastern Europe, including Scandinavia, the Balkans, the Alps, and the Carpathians, to the Urals and Western Siberia. In Asia, populations are found in Anatolia, the Caucasus, the boreal and steppe zones of Western and Eastern Siberia, the mountains of Central Asia, and the Russian Far East. In North America, it is present in Canada, northern USA states, and in the subalpine and alpine zones of the Cordilleras (Enescu et al., 2016). Isolated populations have also been reported in high-altitude regions of Nepal and

northern Pakistan, as well as in the Atlas Mountains of North Africa (Lakušić & Lakušić, 2011; Serebryanaya & Shinkarenko, 2015), but these do not constitute the main circumpolar range. Across these continents, *J. communis* inhabits a broad spectrum of ecosystems, ranging from lowland mixed coniferous-broadleaved forests and boreal forest-steppe to tundra communities, subalpine meadows, and rocky slopes, reflecting its high ecological plasticity and capacity to adapt to diverse edaphic and climatic conditions (Lakušić & Lakušić, 2011).

The exceptionally wide geographic distribution of *J. communis* is closely associated with pronounced intraspecific variability and a high degree of ecological adaptability, which together contribute to the formation of diverse physiological and biochemical response strategies under contrasting environmental conditions (Kerckhoffs et al., 2020; Hantemirova & Bessonova, 2023; Zlámaný, 2024). As an evergreen woody species with long-lived

assimilating organs and an extended reproductive cycle, *J. communis* maintains continuous metabolic activity throughout the year, while simultaneously supporting generative structures at different stages of development (Thomas et al., 2007). This biological organization ensures prolonged and spatially differentiated synthesis and accumulation of secondary metabolites in both vegetative and generative organs, creating a stable and renewable source of phytochemically diverse plant material (Raaijmakers et al., 2022).

SECONDARY METABOLITES PROFILE

The study of the chemical composition of *Juniperus* species began in the mid-20th century, initially focusing on identifying the major compounds of essential oils. Subsequently, essential oils and extracts obtained from berries, needles, shoots, and bark were analysed.

Comprehensive studies have been published examining both the qualitative and quantitative characteristics of the chemical composition of various *J. communis* organs (Filipowicz et al., 2003; Seca & Silva, 2006; Bakkali et al., 2008; Seca et al., 2015; Tavares & Seca, 2018), as well as the proposed biosynthetic pathways for its secondary metabolites (Orhan, 2019).

Like many woody taxa of economic and ecological relevance, *J. communis* is characterized by an intensive, organ-specific secondary metabolism, producing a wide range of low-molecular-weight compounds involved in chemical defence, stress tolerance, and ecological interactions (Bakkali et al., 2008; Belov et al., 2023). The needles predominantly synthesize volatile terpenoids, particularly monoterpenes, which play a key role in protection against herbivores, pathogens, and competing vegetation (Höferl et al., 2014; Raaijmakers et al., 2022), whereas the berry-like cones accumulate phenolic compounds, including proanthocyanidins and related antioxidants, associated with antimicrobial activity and the protection of reproductive tissues (Filipowicz et al., 2003; Belov et al., 2023).

Such a pronounced functional differentiation of secondary metabolites between organs is well documented for woody species, but appears especially distinct in *J. communis*, reflecting its complex life history and ecological breadth.

Since the publication of these reviews, new data on the chemical composition of *J. communis* needles and berries, obtained using modern analytical methods, have accumulated (Butkienė et al., 2015; Judžentienė, 2019; Tobolková et al., 2024). This makes it relevant to review the papers from the past 10-12 years, taking into account geographic and environmental variability in the qualitative and quantitative composition of secondary metabolites. The main classes of secondary metabolites found in needles and berries and their biological activity are presented in the Table.

Terpene. The majority of chemical investigations of different parts of common juniper have focused on the identification of monoterpene and sesquiterpene constituents, such as *sabinene*, *α-pinene*, *β-myrcene*, *caryophyllene*, *γ-terpinene*, *limonene*, and *germacrene D*. However, their relative proportions in essential oil or different extracts vary significantly between plant parts, harvesting time of the plant samples, extraction procedures, and species populations (Judžentienė, 2019; Fejér et al., 2020; Rajčević et al., 2020; Gonçalves et al., 2022; Raaijmakers et al., 2022; Belov et al., 2023).

More than 135 constituents were identified in the essential oil of needles of common juniper from the north-western Balkans, north-western and northern Europe populations, with pronounced predominance of monoterpene compounds (Judžentienė, 2019; Rajčević et al., 2020; Dumitrescu et al., 2022; Raaijmakers et al., 2022). A similar monoterpene dominance in the essential oils of needles, forming the structure of the volatile fraction, was also described for Slovak populations from Central Europe (Dimitrov et al., 2024). However, the relative proportions of some monoterpene constituents, such as *sabinene* and *α-pinene* varied significantly in the essential oil of needles between populations. In particular, the average values for *sabinene* content detected by Rajčević et al. (2020) varied within limits of 19.8-26.4%, while the proportion of *α-pinene* was 15.2-18.5%. Different ratios between these monoterpenes were determined in the needle oil of Lithuanian and Estonian populations: *α-pinene* varied in wide diapason from 21.7 to 76.8%, but mostly, the detected values ranged between 35 and 40%. The same samples of oil contained approximately 0.5-14.2% of *sabinene*.

(Judžentienė, 2019; Raal et al., 2022). The proportion of sesquiterpenes, represented primarily by *germacrene D* and *β-caryophyllene*, reaches approximately 3.0% of the total essential oil profile of needles (Table 1), indicating their significant contribution to the chemical

architecture of vegetative organs. In shoots of *J. communis*, the proportion of sesquiterpenes can increase to 20%. However, such values are characteristic of vegetative tissues and are not intended for direct quantitative comparison with generative organs (Judžentienė, 2019).

Table 1. Secondary metabolites detected in needle and berries of *J. communis* using HPLC-DED and GC-MS methods, and their potential biological activity and nutritional value

Secondary metabolite	Content range	References	Biological activity, nutritional value
Monoterpenes (<i>α</i> -pinene, sabinene, <i>β</i> -pinene, limonene)	5-38% in essential oil	Bajac et al. (2025); Bais et al. (2014); Basha et al. (2024); Butkienė et al., 2015; Çolak et al. (2026); Dimitrov et al. (2024); Dumitrescu et al. (2022); Jojić et al. (2024); Judžentienė (2019); Höferl et al. (2014); Kalaba et al. (2020); Raal et al. (2022); Raina et al. (2019); Rajčević et al. (2020); Salamon et al. (2023); Semerdjieva et al. (2021); Tsitsirigka et al. (2025)	Antimicrobial and repellent activity Aroma component
Sesquiterpenes (<i>β</i> -caryophyllene, <i>germacrene D</i> , pinocarvone)	0.4-3.0%	Bajac et al. (2025); Basha et al. (2024); Belov et al. (2023); Butkienė et al., 2015; Çolak et al. (2026); Dumitrescu et al. (2022); Jojić et al. (2024); Höferl et al. (2014); Kalaba et al. (2020); Raal et al. (2022); Raina et al. (2019); Rajčević et al. (2020); Semerdjieva et al. (2021); Tsitsirigka et al. (2025)	Anti-inflammatory activity Aroma stabilization
Total phenolic compounds	9-68 mg GAE*/g of dry residue	Fejér et al. (2020); Mediavilla et al. (2023); Rawat et al. (2025); Tobolková et al. (2024)	Antioxidant activity
Flavonoids (quercetin, rutin, catechin, kaempferol)	0.2-10 mg/g of dry residue	Bais et al. (2014); Belov et al. (2023); Çolak et al. (2026); Kalaba et al. (2020); Markó et al. (2021); Raina et al. (2019); Tahir et al. (2016)	Antioxidant activity
Biflavonoids (amentoflavone, ginkgetin)	0.02-0.09% of dry residue	Bajac et al. (2025); Butkienė et al., 2015; Dimitrov et al. (2024); Jojić et al. (2024); Rajčević et al. (2020)	Antimicrobial and anti-inflammatory activity
Lignans (podophyllotoxin-like compounds)	0.01-0.05%	Bajac et al. (2025); Bais et al. (2014); Butkienė et al., 2015; Çolak et al. (2026); Jojić et al. (2024); Kalaba et al. (2020); Raina et al. (2019); Rajčević et al. (2020); Tahir et al. (2016)	Antimicrobial and cytotoxic activity
Aldehydes (benzaldehyde, p-anisaldehyde, vanillin)	0.10-0.36% in essential oil	Bajac et al. (2025); Bais et al. (2014); Belov et al. (2023); Dimitrov et al. (2024); Dumitrescu et al. (2022); Jojić et al. (2024); Judžentienė (2019); Kalaba et al. (2020); Popescu et al. (2023); Raina et al. (2019); Tahir et al. (2016)	Aroma component
Ketones (camphor, terpinen-4-one, pinocarvone)	0.10-0.70%	Bais et al. (2014); Çolak et al. (2026); Dimitrov et al. (2024); Dumitrescu et al. (2022); Fejér et al. (2020); Jojić et al. (2024); Judžentienė (2019); Kalaba et al. (2020); Rajčević et al. (2020)	Antimicrobial activity Aroma component
Total organic acids	0.18-0.50%	Dimitrov et al. (2024); Popescu et al. (2023)	Antiseptic activity Food taste improving
Organic acids (citric, malic, tartaric, acetic)	0.10-0.36%	Bajac et al. (2025); Belov et al. (2023); Çolak et al. (2026); Fejér et al. (2020); Jojić et al. (2024); Judžentienė (2019); Raina et al. (2019); Tahir et al. (2016)	Food taste improving
Alcohols (<i>α</i> -terpineol)	0.15-0.45%	Butkienė et al., 2015; Dimitrov et al. (2024); Judžentienė (2019)	Aroma component

*Note. GAE - gallic acid equivalent.

Markó et al. (2021) studied sexual dimorphism in *J. communis*, which includes an analysis of

essential oil from vegetative organs and demonstrates that the qualitative terpene

composition of needles in male and female plants generally retains the same dominant component structure. The monoterpenes *α-pinene* and *sabinene* remain the main constituents of the essential oil, regardless of plant sex, while differences primarily concern essential oil yield and the relative proportions of individual minor components. These data indicate that sexual differentiation has a limited impact on the qualitative terpenoid profile of needles and does not disrupt the overall monoterpene dominance characteristic of the species (Markó et al., 2021).

The data reviewed demonstrate that the terpenoid profile of *J. communis* needles in different European regions is characterized by a consistent predominance of monoterpenes, with high inter- and intra-population variability in the relative proportions of individual components. The monoterpenes *α-pinene* and *sabinene* form the structural basis of the essential oil fraction, while the contribution of sesquiterpenes remains stable but quantitatively secondary, reflecting the adaptive plasticity of terpene metabolism in needles across a wide range of environmental conditions.

The major constituents of berry essential oil remain the monoterpenes: *α-pinene*, *β-pinene*, and *sabinene*, with moderate participation of *limonene* and sesquiterpenes (*β-caryophyllene*, *germacrene D*). However, variability in relative proportions is determined by both local environmental conditions and the stage of berry ripening (Gonçalves et al., 2022; Surso, 2022; Belov et al., 2023; Dimitrov et al., 2024). Thus, ethanol extracts from juniper berries of the second year of ripening contained two times less *α-pinene*, *β-myrcene*, *germacrene D*, and *β-caryophyllene*, and the fractions of *sabinene* and *limonene* found in berries of the first year of ripening were not detected in samples of the second year of ripening. The acetone extracts of the same samples of berries did not have significant differences in detected contents (Belov et al., 2023). However, some authors argue that during berry ripening, a tendency toward an increase in the proportions of *α-pinene* and *β-caryophyllene* is observed, reflecting the dynamics of terpene synthesis during phenological development (Fejér et al., 2020; Markó et al., 2021).

Phenolic constituents. The total content of phenolic compounds in extracts from needles and berries varies widely from 9 to 68 mg GAE (gallic equivalent) per gram of dry residue of the extract (Table 1). The phenolic profile of the juniperus needles is characterized by a consistent predominance of quercetin and kaempferol glycosides with a moderate contribution of phenolcarboxylic acids. Quantitative values of quercetin-3-O-glucoside varied from 0.37 to 1.15%, rutin from 0.21 to 0.54%, and the total phenolic fraction was 0.8-2.3% of dry weight (Judžentienė, 2019; Rajčević et al., 2020; Gonçalves et al., 2022; Tobolková et al., 2024).

The phenolic profile of *J. communis* berries is presented in comparable ranges in a number of European and Eastern European studies (Fejér et al., 2020; Gonçalves et al., 2022; Belov et al., 2023). The total phenolic content in the berries reaches approximately 6.5-18 mg/g dry weight, with the flavonoid and proanthocyanidin fractions, depending on the ripening stage and growing conditions. Among the identified components, quercetin-3-O-rutinoside and isorhamnetin-3-O-glucoside dominated, with their shares fluctuating between 0.25-0.48% and 0.18-0.31%, respectively. Interannual and location variability was manifested in fluctuations in quercetin and rutin concentrations, highlighting the sensitivity of the berry phenolic profile to climatic and ecological conditions, as well as to the stage of fruit ripeness (Fejér et al., 2020; Belov et al., 2023; Popescu et al., 2023). The highest phenolic compound values are consistently recorded in mature berries, which is interpreted as a result of the accumulation of secondary metabolites in the reproductive organs. In addition to terpenes and phenolic compounds, the secondary metabolism of *J. communis* includes a wide range of organic acids, sugars, amino acids, and lipophilic compounds, which can manifest the biological activity and food technological properties.

Other organic constituents. The content of organic compounds of *J. communis* needles, including aldehydes, ketones, organic acids, and alcohols, was analysed (Judžentienė, 2019; Dimitrov et al., 2024).

The aldehydes (p-anisaldehyde, benzaldehyde) were present in the range of 0.10-0.32%, ketones (camphor and terpinen-4-one) - 0.2-0.6%, organic acids - 0.25-0.5%. Among the organic

acids, the authors note citric acid – 0.18-0.25%, malic acid – 0.12-0.21%, malotartaric acid – 0.10-0.18%, which is consistent with the data of Belov et al. (2023). In contrast to needles, berries showed higher interlocal and interannual variability, particularly in the ketone fraction, reflecting the phenological dynamics of fruit metabolism. The organic acids (acetic, malic, citric) in *J. communis* berries were detected in the range of 0.18–0.36%, aldehydes (benzaldehyde, vanillin) - 0.10-0.28%, ketones (terpinen-4-one, pinocarvone) - 0.1-0.32% of dry weight (Fejér et al., 2020; Belov et al., 2023; Popescu et al., 2023).

In addition, the other secondary metabolites are present in the needles and berries of *J. communis* including biflavonoids and lignans, the concentrations of which are generally lower and exhibit organ-specific localization. The several biflavonoids (amentoflavone, ginkgetin) and lignans (podophyllotoxin-like compounds) were identified in needles from European populations at concentrations of 0.02-0.08% and 0.01-0.09% of dry weight, respectively. In berries, the presence of these compounds is minimal (<0.01%), emphasizing the organ-specific localization of secondary metabolites (Dimitrov et al., 2024; Jojić et al., 2024).

Thus, the chemical profile of *J. communis* is designed by a combination of environmental, phenological, and genetic factors. Geographic location and associated climatic conditions (temperature, insolation, humidity, and altitude) have a key influence on the relative proportions of terpenes and phenolic compounds in both needles and berries (Fejér et al., 2020; Rajčević et al., 2020; Tobolková et al., 2024; Rawat et al., 2025).

Analysed data confirm that the chemical profile of *J. "Communis"* needles and berries is a highly variable but structurally organized system of secondary metabolites, in which monoterpenes, sesquiterpenes, phenolic compounds, and organic acids are integrated based on organ specificity, phenology, and geographic context. The influence of sex, age, and reproductive state of plants is an additional factor, introducing moderate quantitative changes but not altering the overall structure of the chemical profile.

APPLICATIONS OF *Juniperus communis* SECONDARY METABOLITES IN AGRICULTURE AND THE FOOD INDUSTRY

Owing to the rich and chemically diverse spectrum of secondary metabolites, including terpenoids, phenolic compounds, flavonoids, and lignans, extracts and essential oils of common juniper (*J. communis*) exhibit considerable potential for application across a wide range of fields, including medicine, pharmaceuticals, nutraceuticals, veterinary science, cosmetics, and plant protection. Of particular practical relevance is their use in agriculture and the food industry as sources of natural antioxidants, antimicrobial and preservative agents, as well as biologically active components capable of enhancing product stability and quality.

Numerous studies demonstrate that *J. communis* extracts and essential oils exhibit pronounced antioxidant activity in standard *in vitro* model assays, with the contribution of individual classes of compounds varying depending on the type of preparation and the extraction method employed (Bais et al., 2014; Höferl et al., 2014; Belov et al., 2023). For juniper berry essential oil, Höferl et al. (2014) reported high scavenging activity of free 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radicals, with more pronounced effects in reactions based on hydrogen atom transfer mechanisms, which the authors attributed to the high content of monoterpenes, particularly α -pinene.

At the same time, several studies have shown that ethanolic berry extracts exhibit high antioxidant activity in fluorescence recovery after photobleaching (FRAP) assays and hydroxyl radical scavenging tests, highlighting the significant role of the polyphenolic fraction in shaping overall antioxidant capacity (Fejér et al., 2020). The values of the inhibitory concentration, which scavenges 50% of free radicals (IC₅₀), determined by ABTS and DPPH assays may vary substantially depending on the plant origin, conditions of growth, extraction procedures, chemical composition of the oil or extracts (Höferl et al., 2014; Basha et al., 2024; Hrytsyna et al., 2024).

Therefore, direct quantitative comparisons between studies are constrained by methodological differences.

However, most conclusions from the analysis of different *J. communis* populations showed that the antioxidant activity of ethanol extracts is closely related to their phenolic compound content (Fejér et al., 2020; Belov et al., 2023).

In addition to studies assessing exclusively the direct free radical scavenging activity of juniper berry essential oil *in vitro*, activation of cellular antioxidant responses has been demonstrated in the *Saccharomyces cerevisiae* model, including increased activity of the primary enzymatic antioxidant defence systems, superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) (Höferl et al., 2014).

Due to the chemical composition of juniper essential oil and extracts, as well as their biological activities, they can serve as sources of antioxidants and functional compounds in food model systems (Ibraimova et al., 2025; Tsitsirigka et al., 2025)

Incorporation of *J. communis* berries into bread formulations resulted in an almost two-fold increase in the antioxidant activity of the final product, as determined by DPPH analysis (Ibraimova et al., 2025). This observation may indicate a transfer of functional properties during food processing, an effect not reported by authors who investigated isolated extracts alone. Essential oils and organic extracts of *J. communis* also exhibit antibacterial activity against a range of Gram-positive bacteria, including *Staphylococcus aureus*, *Bacillus subtilis*, and *Listeria monocytogenes*, with Gram-positive strains generally showing higher susceptibility than Gram-negative bacteria (Bais et al., 2014; Tavares & Seca, 2018; Semerdjieva et al., 2021; Dumitrescu et al., 2022). In the case of Gram-negative bacteria such as *Escherichia coli*, *Salmonella enterica*, and *Pseudomonas aeruginosa*, the degree of growth inhibition varies depending on concentration, the chemical composition of the oil or extract, and the methodological approach applied in antimicrobial testing (Bais et al., 2014; Semerdjieva et al., 2021; Çolak et al., 2026). The authors noted higher efficacy against pathogen bacteria, suggesting the potential applicability of juniper oils in multifarious applications,

including food-related and environmental systems.

These observations are further supported by the study of Čmíková et al. (2024), which demonstrated that essential oil obtained from dried *J. communis* berries reduced the bacterial load of *S. enterica* in a vacuum-packaged carrot model, indicating that antimicrobial activity can be retained within a real food matrix. Kalaba et al. (2020) also reported inhibitory effects of juniper essential oil against *S. aureus* and *L. monocytogenes*, confirming its activity against pathogens of relevance to meat and dairy products.

More recent investigations by Peruč et al. (2021, 2022, 2024) established the ability of juniper essential oil to inhibit biofilm formation and disrupt mature biofilms of non-tuberculous mycobacteria (*Mycobacterium avium*, *M. intracellulare*, and *M. goodii*). In addition, the authors identified synergistic effects between *J. communis* essential oil and selected antibiotics, pointing to the potential relevance of juniper oil for preventing microbial contamination in water supply systems.

Several studies have also confirmed the antifungal activity of *J. communis* essential oils against phytopathogenic fungi, including *Fusarium* spp., *Rhizoctonia solani*, *Botrytis cinerea*, and *Colletotrichum* spp., whereas aqueous extracts generally exhibit weak or negligible activity (Bais et al., 2014; Semerdjieva et al., 2021).

Bajac et al. (2025) demonstrated that microencapsulated forms of juniper essential oil retain antimicrobial activity and enable prolonged release of active terpenes, including α -pinene and sabinene.

Despite substantial variability in the investigated materials (berries, processing by-products, microencapsulated formulations) and methodological approaches, the majority of studies converge on the conclusion that *J. communis* exhibits a broad spectrum of antimicrobial activity (Carroll et al., 2011; Tavares & Seca, 2018; Semerdjieva et al., 2021). Essential oils generally display stronger inhibitory effects than extracts, while the efficacy of extracts is strongly dependent on the solvent used and the resulting chemical composition (Bais et al., 2014; Semerdjieva et al., 2021). Taken together, these findings

indicate the potential of *J. communis* essential oils and extracts as sources of natural antimicrobial agents, with effectiveness demonstrated in laboratory assays and model applied systems (Čmíková et al., 2024; Bajac et al., 2025; Çolak et al., 2026).

Comparative analysis of the published data indicates that the insecticidal and acaricidal activity of *J. communis* is predominantly associated with its essential oils, whereas extracts and powders tend to exhibit more limited or indirect effects (Carroll et al., 2011; Shafaie et al., 2019; Semerdjieva et al., 2021; Hrytsyna et al., 2024).

In the study by Shafaie et al. (2019), berry powders and extracts of *J. communis* exerted ovicidal and reproduction-inhibiting effects on stored-grain pests; however, under comparable experimental conditions, essential oils demonstrated higher efficacy.

The insecticidal activity of *J. communis* essential oil depends primarily on the presence and relative proportions of terpene classes, notably monoterpenes (α -pinene, sabinene) and, to a lesser extent, sesquiterpenes, which contribute to toxic and repellent effects against insects and mites (Garay et al., 2020). The authors accentuated that the efficacy of *J. communis* essential oil against pests, including mosquitoes, aphids, and certain other phytophagous insects, correlated with the concentration of these terpene components. The higher levels of α -pinene and sabinene were associated with increased mortality and reduced feeding activity in arthropods, whereas oils with lower proportions of these compounds exhibited weaker activity. In particular, the monoterpenoids α -pinene and sabinene were shown to interfere with insect nervous system physiology and to suppress vital processes more effectively than many synthetic analogues, especially within integrated pest management systems (Garay et al., 2020).

The nematocidal activity of *J. communis*-based extracts has been attributed by some authors to the presence of totarol, a tricyclic phenolic diterpene with a totarane skeleton (Tavares & Seca, 2018). Using the model nematodes *Caenorhabditis elegans* and *C. briggsae*, Belov et al. (2023) demonstrated that 70% ethanolic and acetone extracts obtained from first-year ripening juniper berries, owing primarily to their

content of monoterpenes (α -pinene, β -myrcene and limonene), sesquiterpenes (germacrene D), phenolic compounds, as well as other constituents such as myo-inositol (a cyclic polyol), may be regarded as potential sources of natural biopesticides, including agents for nematode control.

One of the most consistently reported findings across different studies is the pronounced repellent and deterrent activity of *J. communis* essential oil against arthropods, including ticks (*Ixodes scapularis* and *Amblyomma americanum*) and stored-product insect pests (*Sitophilus granarius* and *Callosobruchus maculatus*). These effects are commonly attributed to the combined action of mono- and sesquiterpenes, particularly the high content of α -pinene, sabinene, and myrcene (Carroll et al., 2011; Shafaie et al., 2019; de Aguiar et al., 2024).

Semerdjieva et al. (2021) demonstrated that *J. communis* essential oil effectively reduces the viability and feeding activity of aphids (*Rhopalosiphum padi* and *Sitobion avenae*), exhibiting antifeedant and insecticidal effects comparable to those of plant-derived biopesticides.

These findings are further extended by de Aguiar et al. (2024), who reported pronounced larvicidal activity of a commercial *J. communis* essential oil against *Aedes aegypti* ($LC_{50} \approx 92.45$ ppm), along with a deterrent effect on oviposition, supporting the high sensitivity of mosquitoes to the monoterpene components of the oil.

The acaricidal potential of *J. communis* has been additionally confirmed by Erdoğan (2026), who demonstrated the toxicity of the essential oil against *Varroa destructor* ($LC_{50} \approx 3.75$ μ L/L), while simultaneously observing a lack of adverse effects on honey bees, a factor of critical importance for practical applications in apiculture.

Earlier work by Carroll et al. (2011) established that the repellent activity of *J. communis* essential oil against *A. aegypti* and ticks is retained at low doses, indicating the high biological activity of volatile terpenes. Subsequently, Bajac et al. (2025) showed that microencapsulated formulations of the essential oil provide prolonged insecticidal and repellent effects through controlled release of active

components, thereby enhancing the stability and applied value of such systems.

Despite differences in target organisms (mosquitoes, ticks, aphids, agricultural insect pests, and store-bought pests) and observed biological effects (insecticide, repellent, antifeedant, ovicidal, and acaricidal), the majority of authors converge on the view that the biopesticidal activity of *J. communis* is multifactorial in nature and is determined by a complex of mono- and sesquiterpenes, particularly α -pinene, sabinene, and myrcene (Carroll et al., 2011; Semerdjieva et al., 2021; de Aguiar et al., 2024). Overall, the literature analysis indicates that *J. communis* essential oils represent a promising source of plant-derived biopesticides and acaricides for integrated plant protection systems, agricultural product storage, and veterinary applications, whereas extracts and powders appear more suitable as auxiliary or complementary agents (Shafaie et al., 2019; Bajac et al., 2025; Erdoğan, 2026).

Owing to their rich chemical composition, particularly the terpenoids and polyphenols present in the essential oil and extracts of *J. communis*, which influence both aroma and flavour as well as functional properties, including antioxidant protection and the stabilisation of biologically active compounds, these juniper-derived materials are widely used in the manufacture of a variety of food products and beverages. Traditionally, essential oil derived from common juniper berries has been employed in the production of strong alcoholic beverages, including gin and various herbal liqueurs (Höferl et al., 2014; Gari et al., 2020; Albrecht & Madisch, 2022; Basha et al., 2024; Čmiková et al., 2024; Stefkov et al., 2025).

Hrytsyna et al. (2024) report that in Slovakia, juniper cones (berries) are used in the production of the national alcoholic beverage “Borovička”. The terpene and oxygenated compound profile of *J. communis* imparts a characteristic “coniferous-citrus” flavour to such beverages.

Despite their traditional use in alcoholic beverages, juniper essential oil may also be applied in other food flavouring systems, such as marinades, sauces, different meat products, beverages and functional foods, where a coniferous-spicy note is desired (Gari et al., 2020; Stefkov et al., 2025).

Gbaguidi et al. (2020) further suggest that essential oils from aromatic plants, including *J. communis*, may be regarded as natural biopreservatives for both liquid and semi-liquid food systems, as well as for meat products. Their unique properties support potential application in sauces and seasonings, where the oil may simultaneously suppress the growth of spoilage microorganisms and slow lipid oxidation, thereby maintaining microbiological stability and the aromatic profile of the product without the use of synthetic preservatives.

In an experimental study, Tomović et al. (2020) incorporated *J. communis* berry essential oil into the formulation of dry fermented sausages as a partial or complete alternative to sodium nitrite. The authors demonstrated that inclusion of the essential oil resulted in a statistically significant reduction in lipid oxidation during ripening and storage, as evidenced by lower thiobarbituric acid reactive substances (TBARS) values compared with control samples without the oil. Simultaneously, the essential oil contributed to the suppression of undesirable microflora, ensuring microbiological stability comparable to that of traditional nitrite-containing formulations. Sensory evaluation indicated that, at optimally selected concentrations, juniper oil did not impair, and in some cases improved, the aromatic profile of the sausages through characteristic coniferous-spicy notes (Tomović et al., 2020). On the basis of combined technological, microbiological, and sensory data, the authors concluded that *J. communis* essential oil may be used as a functional ingredient in the development of “cleaner” and healthier meat products with reduced levels of synthetic additives.

The nanoemulsions of *J. communis* essential oil at concentrations up to 0.75% effectively inhibited the growth of *Listeria monocytogenes* and *E. coli* while simultaneously slowing the increase in lipid oxidation indices (TBARS), making such systems promising for chilled fish fillets and semi-processed products (Çolak et al., 2026).

Bampidis et al. (2023) evaluated the use of *J. communis* berry essential oil and tincture as feed additives for all animal species. The report indicates that, when included in feed at recommended dosages, these products are safe and do not induce toxic effects in animals. Their

pronounced antioxidant potential contributes to the stabilisation of lipid components in feed and prevents rancidity. In addition, biologically active compounds present in the oil exert moderate antimicrobial effects, contributing to a reduction in microbial load in feed mixtures. Collectively, these effects identify *J. communis* essential oil as a promising natural ingredient for improving feed quality and enhancing food safety in animal production. Extracts of *J. communis* included in the diet of fish, Nile tilapia (*Oreochromis niloticus*) have been shown to improve growth performance and feed conversion ratio (Radwan et al., 2024). They exert positive effects on gut health by enhancing the morphological integrity and functional capacity of the intestinal mucosa. Active components of the extract strengthen antioxidant and immune responses, reducing oxidative stress and increasing resistance to infections. Furthermore, inclusion of the extract stimulates the expression of genes associated with immunity and antioxidant defence, supporting its potential as a functional additive in aquaculture feeding strategies.

Essential oils obtained from *J. communis* needles are characterised by a harsher terpene profile, with elevated levels of α -pinene and other monoterpenes, which limits their direct application in food systems due to sensory aggressiveness. The primary applications of needle-derived oils are therefore associated with antimicrobial and disinfectant potential, including possible use in the sanitation of surfaces, water, and air, as well as in veterinary and household products (Albrecht & Madisch, 2022).

CONCLUSIONS

A synthesis of the published data shows that the biological and practical value of *J. communis* is primarily determined by its essential oils and extracts, which are rich in mono- and sesquiterpenes, polyphenols, and other secondary metabolites, with composition and activity strongly dependent on the source material and processing methods. Essential oils exhibit the most pronounced antioxidant, antimicrobial, insecticidal, and acaricidal effects, whereas extracts and powders generally show more selective and less intense activity,

but can be effective in functional food and feed systems. The practical applicability of *J. communis* products has been confirmed in real matrices – meat products, bakery goods, fish-based foods, and aquaculture feeds, where they enhance antioxidant stability, microbiological safety, and, at appropriate dosages, sensory quality. Additionally, by-products of juniper can be effectively integrated into cascade utilisation chains, including the production of biochar with adsorption and agronomic functions. Overall, these findings confirm the high potential of *J. communis* as a multifunctional natural resource for the food, agricultural, veterinary, and related industries, provided that the form, dosage, and application context are scientifically optimized.

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ECONOMIC IMPACT OF AIR POLLUTION ON HEALTH AND PRODUCTIVITY IN MAŁOPOLSKIE REGION, POLAND (2013-2018)

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Abstract

Particulate matter pollution in the Małopolskie region of Poland has been causing serious problems for both human health and the regional economy. Using data from 2013 to 2018 (air quality from the European Environment Agency and health records from Eurostat), we aimed to understand the actual scale of the impact. Cognitive decline was estimated based on exposure-response data from other studies (Zhang et al., 2018). Evidence from the study suggested that WHO guidelines were being exceeded significantly. PM_{2.5} averaged between 5 and 7.7 times the recommended levels, and PM₁₀ was around 2.6 to 3.1 times higher. Deaths from respiratory causes increased by 23% by 2018 compared to 2013, and hospitalizations increased by about 26%. Poland's aging population explains much of this trend. The economic side was striking, though lost productivity due to cognitive impairment amounted to approximately €1.18 billion, which accounted for most of the total economic cost (over 96%). Air quality policy clearly needs to address more than just immediate respiratory effects; the cognitive and productivity dimensions appear substantial and shouldn't be ignored in policy planning.

Key words: air pollution, cognitive decline, economic impact, particulate matter, productivity loss.

INTRODUCTION

Air pollution has become one of the most significant environmental and public health challenges affecting European urban centers today. Among the different pollutants, particulate matter stands out as the most pervasive, exerting widespread effects on millions of individuals across the continent (Khomenko et al., 2021). In the Małopolskie region of southern Poland, particularly in its capital city, Kraków, concentrations of particulate matter have consistently exceeded the air quality thresholds recommended by the World Health Organization (WHO), posing considerable risk to its 3.4 million residents. Although researchers have extensively documented the immediate health effects of particulate matter exposure, such as respiratory disorders, cardiovascular problems, and premature mortality, less attention has been devoted to the broader economic implications that extend beyond direct healthcare expenditures (Brunekreef & Holgate, 2002; Cohen et al., 2017).

Traditional health impact assessments have typically focused on quantifying mortality and

hospitalizations, subsequently estimating the associated healthcare costs. European studies have effectively demonstrated the relationship between particulate matter concentrations and respiratory disease rates, showing that long-term exposure leads to higher rates of emergency visits, hospital stays, and deaths among vulnerable population groups (Atkinson et al., 2014). However, this conventional approach likely underestimates the actual economic burden because it omits indirect costs, such as reduced workplace productivity, cognitive impairment, and overall loss of labor efficiency (Graff Zivin & Neidell, 2012). Recent studies suggest that understanding the full economic impact of air pollution requires paying closer attention to its effects on cognitive performance.

Zhang et al. (2018) reported significant findings from a large-scale study in China, which included nearly 32,000 participants. The study identified measurable declines in both verbal and mathematical test performance linked to higher PM₁₀ exposure; specifically, scores declined by 0.32% (verbal) and 0.19% (mathematical) for every 10 µg/m³ increase in PM₁₀ concentration. These results suggest that

prolonged exposure to polluted air may impair cognitive functioning. Supporting these findings, Cullen et al. (2022) conducted research in France, involving over 61,000 adults, and documented notable declines in semantic fluency among individuals exposed to elevated PM_{2.5} levels. The consistency of results across diverse countries and different approaches to measuring cognitive function strengthens the evidence supporting a connection between particulate matter exposure and cognitive decline (Power et al., 2016; Weuve et al., 2012).

The economic implications of pollution-induced cognitive decline could be substantial, yet this dimension has received limited research attention thus far (Landrigan et al., 2018). Even minor cognitive impairments experienced by workers due to daily exposure to polluted air could, when aggregated at a regional scale, generate productivity losses that significantly outweigh direct healthcare costs. This concern is particularly relevant in economies such as Poland's, where service-oriented and knowledge-based industries represent an increasingly substantial portion of economic output. Cognitive performance influences work productivity, decision-making ability, problem-solving efficiency, and innovation capacity, all of which affect a region's economic competitiveness.

Since joining the European Union in 2004, Poland has undergone notable economic transformations, characterized by steady GDP growth, rising wages, and greater integration into global markets. Małopolskie has been an integral part of this development trajectory, with Kraków emerging as a major center for business services, information technology, and knowledge-intensive industries. However, this economic advancement has occurred alongside persistent air quality challenges, as particulate matter concentrations in the region consistently rank among the highest in Europe (Holnicki et al., 2017). This raises the question of whether air pollution may be limiting productivity gains and constraining economic potential.

Population aging adds further complexity to this issue. As in many European countries, Poland faces demographic shifts marked by a growing proportion of individuals aged 65 and older. This trend is particularly evident in

Małopolskie, where the elderly population increased by 5.2% between 2013 and 2018. Because respiratory diseases disproportionately affect older adults, observed health trends over time may be more strongly influenced by demographic changes than by environmental exposures. Disentangling these overlapping influences requires analytic methods that account for population structure and age distribution (Dominici et al., 2002).

Previous economic assessments of air pollution in Poland have traditionally centered on direct health system costs, using standard methodologies to estimate expenditures related to pollution-associated hospitalizations and outpatient care. Although these studies provide valuable insights for healthcare planning, they likely omit significant components of the total economic burden, such as productivity losses, reduced labor force participation, and diminished economic output. International research indicates that indirect costs can exceed direct medical expenditures by an order of magnitude or more, yet comparable estimates for Poland remain scarce (Quah & Boon, 2003).

Geographic and meteorological factors also contribute to Małopolskie's persistent air quality problems. The region's topography, characterized by surrounding mountain ranges, restricts atmospheric dispersion of pollutants, while winter temperature inversions frequently trap pollutants near ground level. These conditions, combined with continued reliance on coal for residential heating, industrial emissions, and vehicular traffic, contribute to elevated particulate matter concentrations throughout the year (Sówka et al., 2021). Despite the implementation of various policy measures - including low-emission zones, heating system upgrades, and emission standards - the region continues to struggle to attain WHO-recommended air quality levels.

The aim of this study is to conduct a comprehensive assessment of the economic impact of air pollution in Małopolskie between 2013 and 2018, with particular emphasis on productivity losses resulting from pollution-induced cognitive impairment. This study employs an integrative approach, combining air quality monitoring data, health statistics, economic indicators, and demographic

information to generate a more complete understanding of how prolonged exposure to particulate matter contributes to regional economic burdens. By applying validated exposure-response coefficients from cognitive research to local air quality data, the study estimates productivity losses among working-age individuals and compares these indirect effects with direct healthcare costs. The findings contribute to growing evidence that the economic consequences of air pollution extend well beyond healthcare expenditures, highlighting the importance of integrating cognitive and productivity dimensions into environmental policy planning.

MATERIALS AND METHODS

Study Area and Period. This research focused on the Małopolskie region of Poland, with special emphasis on the city of Kraków, the region's most prominent urban center. Małopolskie is situated in southern Poland, covering an approximate area of 15,183 km² and hosting a population of around 3.4 million inhabitants. The study period extended from 2013 to 2018, spanning six consecutive years of air quality monitoring, health outcomes, and socioeconomic indicators.

Data Sources. To ensure data reliability, information was obtained exclusively from authoritative databases. Air quality measurements were retrieved from the European Environment Agency (EEA) Air Quality Database, accessed through its official data portal. Health outcome statistics were obtained from the Eurostat Health Statistics Database (<https://ec.europa.eu/eurostat/>), specifically from causes of death and hospital discharge datasets. Economic and productivity-related indicators were extracted from the OECD Statistics Database (<https://stats.oecd.org/>), including average annual wages, GDP per hour worked, and labor market data. Demographic statistics were obtained from Eurostat Population Statistics. Cognitive decline coefficients were sourced from peer-reviewed literature, primarily Zhang et al. (2018) and Cullen et al. (2022).

Air Quality Measurements. Annual mean concentrations of PM_{2.5} and PM₁₀ were collected from six monitoring stations located

within the Małopolskie region: Kraków Aleja Krasińskiego, Kraków ul. Bulwarowa, Kraków ul. Bujaka, Kraków os. Piastów, Kraków os. Wadów, and Kraków ul. Złoty Róg. Data were recorded using continuous automated monitoring equipment compliant with European Union (EU) reference methods (EN 12341:2014 for PM₁₀ and EN 14907:2005 for PM_{2.5}). Measurements were conducted hourly and subsequently aggregated into daily and annual mean values. Quality assurance procedures included regular equipment calibration, maintenance documentation, and data validation according to EEA guidelines. Excess pollution levels were calculated by subtracting the WHO guideline values (5 µg/m³ for PM_{2.5} and 15 µg/m³ for PM₁₀) from observed concentrations.

Health Outcome Data. Respiratory mortality statistics were acquired from Eurostat causes of death records, classified using ICD-10 codes J00-J99 (diseases of the respiratory system). The extracted data included total annual deaths and age-stratified mortality rates (per 100,000 population) for the Małopolskie region. Hospitalization statistics related to respiratory diseases were obtained from Eurostat hospital discharge records, including total annual admissions (ICD-10: J00-J99), average hospital stay duration, and age group distributions. Healthcare expenditure data were extracted from OECD Health Statistics, including total health spending, hospital treatment costs, and per capita expenditure, adjusted to constant 2018 euros using medical inflation indices.

Economic and Productivity Indicators. Average annual wages for Poland were obtained from Eurostat Earnings Statistics and the OECD Average Annual Wages Database, reported in euros and adjusted for purchasing power parity. GDP per hour worked, representing labor productivity, was sourced from OECD Productivity Statistics. Annual working hours per employee were retrieved from OECD Employment Statistics. All economic variables were standardized to 2018 constant prices using appropriate deflators.

Demographic Characteristics. Population statistics for the Małopolskie region were obtained from Eurostat Population Statistics, including total population, age group distribution (0-14, 15-64, 65+), median age,

and percentage of urban population. For Kraków specifically, population data were drawn from Statistics Poland (Główny Urząd Statystyczny). The working-age population (15-64 years) was identified as the primary cohort for productivity loss calculations. Annual population estimates were used to calculate per capita exposure rates and to estimate the number of individuals affected by elevated air pollution levels.

Cognitive Decline Coefficients. Exposure–response coefficients describing the relationship between air pollution and cognitive decline were extracted from published epidemiological studies. Primary coefficients came from Zhang et al. (2018), who reported declines of -0.32% in verbal test scores and -0.19% in mathematical test scores per $10 \mu\text{g}/\text{m}^3$ increase in PM10 concentration, based on a study of 31,955 individuals in China. Additional validation coefficients were derived from Cullen et al. (2022), documenting a -4.6% decline in semantic fluency per interquartile range increase in PM2.5 exposure among 61,462 French adults. These coefficients were applied to compute population-level cognitive impacts based on observed PM exceedances above WHO guidelines.

Statistical analysis. Descriptive statistics were computed for all continuous variables, including means, standard errors, ranges, and 95% confidence intervals. Temporal trends in air pollution concentrations, health outcomes, and demographic indicators were evaluated using linear regression models. Pearson correlation coefficients were calculated to examine bivariate associations between pollutant concentrations and health outcomes. Multiple linear regression models were employed to control for confounding variables, including demographic age structure, temporal trends, and socioeconomic factors. Statistical significance was set at $\alpha = 0.05$ for all hypothesis testing. Statistical analyses were performed using R version 4.1.2 (R Core Team, 2021) and Python version 3.9.7.

Economic valuation methodology. The economic impact of pollution-related cognitive decline on labor productivity was estimated using a three-step approach. First, mean cognitive decline was calculated by averaging

verbal and mathematical decline coefficients from Zhang et al. (2018), weighted by PM10 exceedances above WHO thresholds. Second, productivity loss was estimated as 50% of cognitive decline, following a conservative assumption that not all cognitive impairment directly translates to measurable workplace productivity reduction (Schmidt & Hunter, 1998). Third, annual economic loss was calculated by multiplying the working-age population (529,598 individuals in Kraków) by the average annual wage (€10,635) and the productivity loss percentage. Direct healthcare costs were estimated by multiplying annual respiratory hospitalizations by the average cost per hospitalization (€4,333, inflation-adjusted). Total economic burden was computed as the sum of indirect productivity losses and direct healthcare costs, accumulated over the six-year study period.

Sensitivity Analysis. Sensitivity analyses were performed to assess the robustness of economic impact estimates in relation to variations in key parameters. Cognitive decline coefficients were varied within their 95% confidence intervals. The productivity translation factor was tested at 25%, 50%, and 75% to reflect uncertainty in the relationship between cognitive decline and productivity reduction. Healthcare cost estimates were varied by $\pm 20\%$ to account for potential differences in treatment patterns and cost structures. A Monte Carlo simulation with 10,000 iterations was conducted to generate probability distributions of total economic impact, with results reported as mean values and 95% credible intervals.

RESULTS AND DISCUSSIONS

Air Pollution Levels in Małopolskie Region (2013–2018). During the study period, concentrations of particulate matter in the Małopolskie region persistently and substantially exceeded WHO air quality guidelines (Figure 1). Annual mean PM2.5 concentrations ranged from $29.98 \mu\text{g}/\text{m}^3$ in 2018 to $38.49 \mu\text{g}/\text{m}^3$ in 2013, with an overall mean of $34.55 \pm 3.25 \mu\text{g}/\text{m}^3$. Annual mean PM10 concentrations varied between $39.00 \mu\text{g}/\text{m}^3$ in 2016 and $46.30 \mu\text{g}/\text{m}^3$ in 2015, with an overall mean of $42.39 \pm 2.76 \mu\text{g}/\text{m}^3$.

A statistically significant decreasing trend was identified for PM2.5 ($\beta = -1.42 \mu\text{g}/\text{m}^3$ per year, $p = 0.018$), representing a 22.1% reduction between 2013 and 2018. PM10 concentrations also exhibited a declining tendency ($\beta = -0.57 \mu\text{g}/\text{m}^3$ per year, $p = 0.402$); however, this trend was not statistically significant. Despite the observed reductions, annual mean concentrations for both pollutants consistently exceeded WHO guidelines, with PM2.5 levels remaining 5.0-7.7 times higher, and PM10 levels 2.6-3.1 times higher, across all years evaluated.

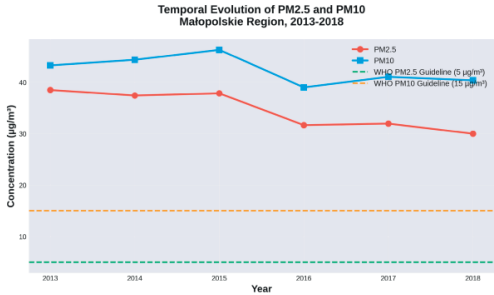


Figure 1. Temporal evolution of PM2.5 and PM10 concentrations in Małopolskie region, 2013-2018. Despite declining trends, concentrations remained 5-8 times above WHO guidelines

Health Outcomes. Respiratory disease mortality demonstrated a sustained upward trend over the study period. Annual deaths increased from 20,020 in 2013 to 24,624 in 2018, representing a 23.0% increase ($\beta = 919.6$ deaths/year, $R^2 = 0.998$, $p < 0.001$). Similarly, respiratory disease-related hospitalizations rose from 88,550 in 2013 to 111,794 in 2018, marking a 26.2% increase ($\beta = 4,646.2$ hospitalizations/year, $R^2 = 0.998$, $p < 0.001$). Unexpectedly, crude ecological correlations revealed negative relationships between particulate matter concentrations and respiratory health outcomes (Figure 2). PM2.5 was strongly and negatively correlated with respiratory mortality ($r = -0.928$, $p = 0.008$) as well as hospitalizations ($r = -0.927$, $p = 0.008$). These paradoxical associations are attributable to confounding effects, particularly the influence of aging population trends, which exerted a stronger impact on mortality and morbidity than pollution alone.

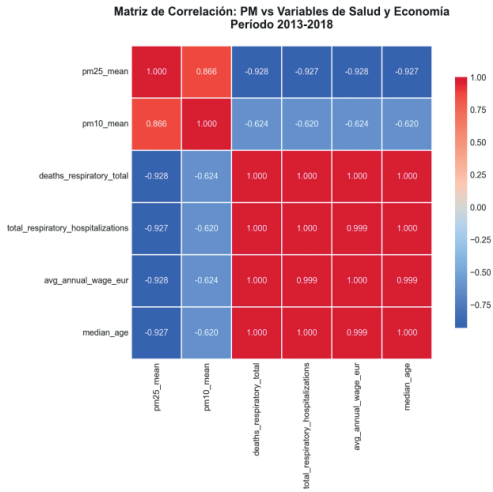


Figure 2. Correlation matrix showing relationships between PM concentrations and health variables. The paradoxical negative correlations reflect confounding by population aging

Demographic Characteristics. The total population of the Małopolskie region remained relatively constant, ranging narrowly from 3.40 million in 2013 to 3.40 million in 2018. However, substantial demographic changes were observed within age distributions. The working-age population (15-64 years) declined by 1.5% (a reduction of approximately 33,000 individuals), while the elderly population (65+ years) increased by 5.2% (an increase of around 32,000 individuals). The median age increased from 39.0 to 40.5 years over the study period, reflecting consistent and progressive population aging.

Economic Impact Assessment. Based on validated exposure-response coefficients from Zhang et al. (2018), the mean PM10 exceedance ($27.39 \mu\text{g}/\text{m}^3$ above the WHO guideline) was associated with an estimated cognitive decline of 6.98% across the exposed population. Specifically, verbal test performance declined by 8.77% (95% CI: 6.85–10.69%), while mathematical test performance declined by 5.20% (95% CI: 3.64–6.76%).

Applying the conservative assumption that cognitive decline translates into a 50% reduction in workplace productivity, the annual economic impact in Kraków was estimated at €196.7 million (Figure 3). Cumulatively,

productivity losses totaled €1,180.2 million over the six-year study period.

In contrast, direct healthcare costs associated with respiratory disease management (including hospitalizations and treatment expenditures) were estimated at €38.3 million across the same period. Accordingly, indirect losses from productivity impairment exceeded direct medical costs by a factor of 30.8:1.

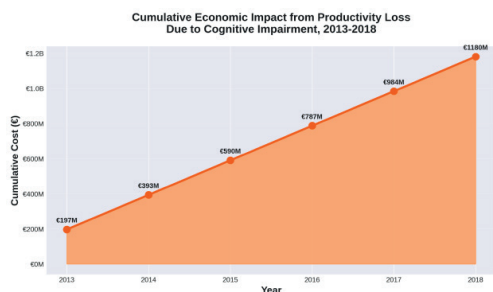


Figure 3. Cumulative economic impact from productivity losses attributable to cognitive impairment, reaching €1.18 billion by 2018

Sensitivity Analyses. Sensitivity analyses examining variations in cognitive decline coefficients within published confidence intervals indicated that lower-bound estimates produced total productivity losses of €886.6 million over six years, whereas upper-bound estimates reached €1,473.8 million. When the productivity translation factor was adjusted to reflect 25%, 50%, and 75% efficiency loss, economic impact results ranged from €590.1 million to €1,770.3 million, with the base case (50%) representing a conservative midpoint estimate.

Analysis and Interpretation. This study provides evidence that particulate matter pollution in the Małopolskie region, particularly in Kraków, remained consistently above the limits recommended by the World Health Organization between 2013 and 2018. Despite a statistically significant 22% decline in PM_{2.5} concentrations during this period, annual mean values continued to exceed WHO guidelines by a factor of five to nearly eight. In contrast, PM₁₀ concentrations exhibited a statistically insignificant decline, suggesting that mitigation efforts were more effective in reducing fine particulates, while coarse particulate matter remained relatively persistent. The region's dependence on coal-

fired heating, vehicular emissions, and industrial activities, combined with unfavorable geographical and meteorological factors (especially winter temperature inversions) likely contributed to sustained pollution levels. One notable observation was the paradoxical negative correlations between particulate matter concentrations and respiratory mortality and hospitalization rates. While statistically significant, this inverse relationship does not suggest a protective effect of pollution. Instead, it reflects the influence of demographic aging, which exerted a stronger effect on respiratory health trends than pollution levels alone. The share of individuals aged 65 and older increased by over 5% during the study period, and the median age rose from 39.0 to 40.5 years. These demographic shifts are known to increase baseline mortality and morbidity rates, a phenomenon documented in previous studies (Dominici et al. 2002). This highlights the importance of adjusting for population age structure when analyzing ecological associations between pollution and health outcomes.

A key contribution of this study is the incorporation of cognitive impairment as an additional pathway linking air pollution and economic consequences. Based on the exposure-response coefficients from Zhang et al. (2018), long-term exposure to PM₁₀ was associated with declines of 8.77% in verbal performance and 5.20% in mathematical skills, calculated for the observed exceedances above WHO thresholds. These results are consistent with findings from Cullen et al. (2022), who reported a 4.6% reduction in semantic fluency associated with elevated PM_{2.5} exposure among French adults. Similar evidence has emerged from multiple contexts, suggesting that chronic exposure to particulate matter may impair cognitive function through biological mechanisms involving neuroinflammation, oxidative stress, and blood-brain barrier disruption (Block & Calderón-Garcidueñas, 2009; Power et al., 2016).

The relationship between cognitive skills and productivity has been well documented in the labor economics literature, particularly in occupations requiring complex problem-solving, sustained attention, and decision-making capacity (Schmidt & Hunter, 1998).

Although cognitive decline is not directly equivalent to productivity loss, the application of a 50% conversion factor represents a conservative estimate for quantifying economic consequences. This factor accounts for the fact that workplace performance is influenced by multiple non-cognitive factors while still recognizing the meaningful impact of cognition on labor output.

Methodologically, this study has several limitations. The ecological design precludes individual-level causal inferences, and the assumption of uniform exposure across the working population likely oversimplifies actual exposure variability. Furthermore, the analysis focused exclusively on particulate matter, without incorporating the potential cognitive and health effects of other pollutants such as nitrogen dioxide or ozone. Additionally, productivity estimates rely on average annual wages and do not capture potential long-term labor market impacts, such as early retirement, educational losses, or intergenerational effects. Despite these limitations, the integration of cognitive, economic, and environmental data represents a valuable step toward more comprehensive assessments of pollution-related burdens.

The findings align with emerging international research emphasizing that the consequences of air pollution extend beyond traditional respiratory and cardiovascular outcomes, encompassing neurocognitive effects that may have significant socioeconomic implications (Graff Zivin & Neidell, 2012). The growing relevance of knowledge-based industries in Kraków and the broader Małopolskie region further underscores the importance of considering cognitive impacts when evaluating the regional consequences of pollution. As labor markets become increasingly reliant on human capital, even modest reductions in cognitive performance may have sizeable economic repercussions.

CONCLUSIONS

This study confirms that particulate matter pollution in the Małopolskie region, especially in Kraków, persistently exceeded World Health Organization air quality standards between 2013 and 2018, with PM_{2.5} levels reaching

five to nearly eight times the recommended thresholds. Despite modest reductions in PM_{2.5} concentrations during the study period, air pollution remained at harmful levels. Concurrently, respiratory-related mortality and hospitalizations increased by 23% and 26%, respectively. However, these health trends were strongly influenced by demographic aging, indicating that population structure plays a relevant role in interpreting pollution-related health outcomes.

The analysis also demonstrates that the economic consequences of air pollution extend far beyond direct healthcare costs. Productivity losses resulting from cognitive impairment associated with chronic exposure to particulate matter amounted to an estimated €1.18 billion over six years, surpassing direct respiratory healthcare costs by more than thirtyfold. These findings highlight that indirect economic losses (especially those linked to reduced cognitive function and workplace efficiency) represent the dominant component of the overall economic burden of air pollution in the region. Sensitivity analyses confirmed the robustness of these results, with alternative assumptions consistently demonstrating that productivity-related indirect costs substantially outweigh direct medical expenses. These results suggest that traditional cost assessments relying primarily on healthcare expenditures significantly underestimate the true economic impact of air pollution.

Therefore, environmental policies should not focus exclusively on mortality and hospitalizations but also consider the broader socioeconomic consequences, particularly the impacts on cognitive performance, productivity, and regional economic competitiveness. Incorporating these dimensions into policy evaluation frameworks is essential to better reflect the real costs of air pollution and to justify broader and more comprehensive intervention strategies.

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YIELD CHARACTERISATION AND PHENOLIC CONTENT OF SAFFRON (*CROCUS SATIVUS* L.) PETALS HARVESTED IN SUCEAVA AND NEAMŢ COUNTIES, ROMANIA

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Abstract

*This study investigates the yield characteristics and polyphenolic content of saffron (*Crocus sativus* L.) petals from Neamț County (own production, 2024-2025) and Suceava County (external source, 2023-2024, Romania), in order to assess their potential for valorization in bakery products. Petal samples were collected using a stratified random sampling approach across multiple harvest days and field sectors. Following controlled drying and milling, were analyzed using standardized methods. Total phenolic content (TPC) was determined using the Folin-Ciocalteu assay. TPC values ranged from 17.5-17.9 mg GAE/g DW in the Suceava 2023 samples to 20.0-21.8 mg GAE/g DW in Suceava 2024. Neamț 2024 petals showed greater variability (15.9-25.0 mg GAE/g DW, with the highest levels in Neamț 2025 samples (27.9 mg GAE/g DW). These results support the potential use of saffron petals as functional ingredients in bakery products with enhanced antioxidant properties.*

Key words: saffron, petals, *Crocus sativus*, phenols, yield.

INTRODUCTION

Saffron (*Crocus sativus* L.) Saffron is one of the most valuable spice crops in the world and has been cultivated for centuries due to its unique sensory properties and medicinal value (Fujii et al., 2022). While the dried stigmas are traditionally used as culinary ingredients, natural colorants, and therapeutic agents (Mzabri et al., 2019), increasing attention has recently been directed toward other floral components, particularly saffron petals as valuable sources of bioactive compounds.

In addition to its economic importance, saffron is recognized for its rich phytochemical composition, including carotenoids, phenolic compounds, flavonoids, and volatile constituents, which contribute to its antioxidant, anti-inflammatory, and pharmacological properties (Aissa et al., 2023; Amanpour et al., 2024; Avila-Sosa et al., 2022; Bakshi et al.,

2022; Butnariu et al., 2022; Gohari et al., 2013; José Bagur et al., 2017; Kabiri et al., 2023; Karimi et al., 2010).

Although these compounds are well documented in stigmas, recent research has demonstrated that saffron petals, often considered an agricultural by-product, are also rich in phenolic compounds and flavonoids, thus representing a promising resource for functional and nutraceutical applications (Ghodrati Shahtouri et al., 2024; Gikas et al., 2021; Hosseini et al., 2018; Maestre-Hernández et al., 2023; Marrone et al., 2024; Mohaqiq et al., 2020; Shanker et al., 2025; Stelluti et al., 2021). The productivity and quality of saffron are strongly influenced by environmental and agronomic factors such as soil characteristics, climatic conditions, irrigation regime, planting density, and crop management practices (Bayat et al., 2016; Ordoudi & Tsimidou, 2004). Variability in these factors within a cultivation

area can lead to spatial differences in plant development, flower production, and stigma yield. Understanding this intra-field variability is essential for the accurate evaluation of crop performance and optimizing cultivation strategies (Maggi et al., 2011)

In agricultural research, appropriate sampling strategies are required to obtain representative and unbiased data. Stratified random sampling is widely used for this purpose, as it involves dividing the field into relatively homogeneous strata, followed by the random selection of sampling units within each stratum. This approach improves the precision of statistical analyses and facilitates the assessment of spatial variability in crop productivity (Cochran, 1977). Beyond agronomic performance, the chemical composition of saffron petals plays an important role in determining their potential use in bakery products as a source of natural antioxidants. Phenolic compounds and flavonoids present in the petals exhibit strong antioxidant activity and contribute to the biological properties of saffron-derived products (Cerdá-Bernad et al., 2022, 2023; Fardaghi et al., 2020). Environmental conditions, geographical origin, and plant physiological status influence the concentration of these compounds, thereby affecting their bioactive properties (Bayat et al., 2016; D'Archivio & Maggi, 2017; Maggi et al., 2011). The increasing interest in sustainable agriculture and waste valorization has highlighted saffron petals as a valuable by-product that can be utilized in food, pharmaceutical, and cosmetic applications (Cerdá-Bernad & Frutos, 2023; Hosseini et al., 2018; Rizi et al., 2024; Turcov et al., 2022). Their high content of phenolic and flavonoid compounds makes them an attractive alternative source of natural antioxidants, thereby contributing to the overall added value of saffron cultivation systems (Hossein Goli et al., 2012; Jadouali et al., 2018; Naim et al., 2022, 2023).

Therefore, combining agronomic evaluation with phytochemical analysis provides a comprehensive approach to understanding both the production and valorization of saffron petals. In this context, the present study applies a stratified random sampling approach to evaluate spatial variability in flower production within a saffron field, as a basis for estimating petal biomass. Additionally, the total phenolic content

(TPC) of saffron petal samples was determined to assess their biochemical quality and antioxidant potential. This integrated approach provides insights into both the agronomic performance and the functional value of saffron petals under the studied conditions.

MATERIALS AND METHODS

Stratified Random Sampling Procedure

To ensure a representative assessment of saffron field variability, a stratified random sampling method was employed. Stratified sampling allows the study area to be divided into relatively homogeneous sub-areas (strata), improving sampling precision and ensuring that all parts of the field are adequately represented. The experimental field covered an area of 1000 m² located in Neamț, Romania (Figures 1 and 2) where *Crocus sativus* L. was cultivated under traditional management practices, with a focus on the conversion to organic farming.

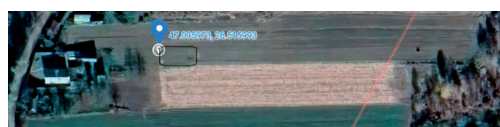


Figure 1. Google maps coordinate of the experimental field in Neamț, Romania

The experimental plot measured 16 m in the west-east direction and 60 m in the south-north direction. A local Cartesian coordinate system (Figure 3) was established to define sampling locations within the field.



Figure 2. *Crocus Sativus* L. experimental field in Neamț, Romania (original photo)

The origin point (0.00, 0.00) was defined at the south-west corner of the plot. The x-axis extended from west to east (0-16 m), while the y-axis extended from south to north (0-60 m).

Sampling locations were expressed as (x, y) coordinates in meters relative to this origin point.

To ensure spatial representation across the entire field, the plot was divided into four equal strata along the north–south axis. Each stratum consisted of a horizontal band measuring 15 m in length in the south-north direction, defined as follows:

- Stratum 1: y = 0-15 m
- Stratum 2: y = 15-30 m
- Stratum 3: y = 30-45 m
- Stratum 4: y = 45-60 m

Within each stratum, five sampling plots were randomly selected, resulting in a total of 20 sampling points across the field. The random coordinates were generated within the boundaries of each stratum and subsequently located in the field using tape measurements relative to the defined origin.

At each sampling location, field measurements were carried out within a 1 m² sampling plot. The coordinates of the randomly selected sampling plots within each stratum are presented in Table 1 and Figure 3.

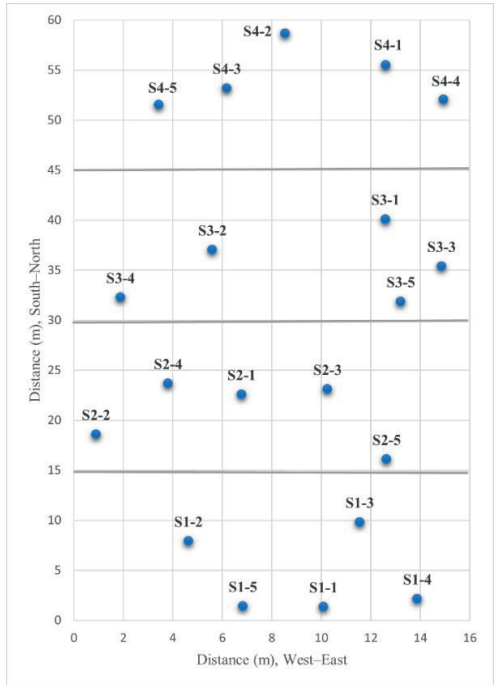


Figure 3. Coordinates of sampling plots across strata within the study field

Coordinates represent local field measurements in meters relative to the south-west corner of the experimental plot, rather than geographic coordinates.

At each sampling location, the following variables were measured:

- number of saffron plants per plot;
- number of flowers per plant;
- stigma yield per sampling point (S1-1, S1-2, etc.).

Table 1. Coordinates of randomly selected sampling plots in the saffron field

Point ID	Stratum	X (m)	Y (m)
S1-1	1	10.09	1.33
S1-2	1	4.63	7.9
S1-3	1	11.55	9.8
S1-4	1	13.88	2.13
S1-5	1	6.83	1.39
S2-1	2	6.78	22.57
S2-2	2	0.9	18.58
S2-3	2	10.25	23.08
S2-4	2	3.81	23.66
S2-5	2	12.64	16.08
S3-1	3	12.59	40.08
S3-2	3	5.6	37.02
S3-3	3	14.86	35.38
S3-4	3	1.89	32.26
S3-5	3	13.21	31.85
S4-1	4	12.61	55.49
S4-2	4	8.54	58.65
S4-3	4	6.18	53.18
S4-4	4	14.94	52.04
S4-5	4	3.43	51.51

All measurements were conducted during the flowering and harvesting period in October–November 2025, which corresponds to the peak saffron production stage. The same measurement procedures and instruments were used across all sampling plots to ensure consistency and comparability of the collected data.

The collected data were subsequently compiled and processed using MedCalc® statistical software (MedCalc Software Ltd., Ostend, Belgium) for statistical analysis.

Total Phenolic Content Determination

Total phenolic content (TPC) in saffron petals (*Crocus sativus* L.) was determined using the Folin-Ciocalteu colorimetric method with slight modifications.

Dried saffron petals were ground into a fine powder prior to analysis.

For phenolic extraction, approximately 1 g of powdered sample was mixed with 10 mL of 60% ethanol.

The mixture was subjected to maceration for 24 h in the dark at room temperature, followed by incubation in a water bath for 3 h at 60°C. The extract was then cooled, filtered, and stored at 4°C until further analysis.

Subsequently, the extract was centrifuged at 4000 rpm for 10 min, and the supernatant was collected for analysis.

An aliquot of 0.1 mL of the extract was mixed with 1.4 mL of distilled water and 0.1 mL of Folin-Ciocalteu reagent. After 3 min of incubation, 0.4 mL of sodium carbonate (Na_2CO_3) solution was added. The reaction mixture was then incubated for 60 min at 25°C in the dark to allow color development.

Absorbance was measured at 765 nm using a UV-Vis spectrophotometer (Papuc et al., 2020; Predescu et al., 2020).

Total phenolic content was calculated from the calibration curve using the corresponding regression equation and expressed as mg GAE (gallic acid equivalents) $\cdot \text{g}^{-1}$ DW (dry weight) (Figure 4).

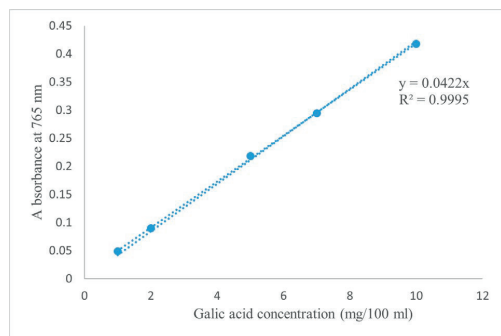


Figure 4. Gallic acid calibration curve used for the determination of the total phenolic content

Statistical Analysis

All measurements for total phenolic content (TPC) were performed in triplicate, and the results are expressed as mean $\pm$ standard deviation.

Statistical analyses were assessed using MedCalc® statistical software version 23.4.9 (MedCalc Software Ltd., Ostend, Belgium). Differences between groups were evaluated using one-way analysis of variance (ANOVA). The assumptions of normality and homogeneity

of variances were assessed prior to analysis using the D'Agostino–Pearson test and Levene's test, respectively.

When the assumptions for ANOVA were satisfied, post-hoc comparisons were performed using the Tukey–Kramer multiple-comparison test to identify significant differences between groups. Statistical significance was considered at $p < 0.05$.

The distribution and variability of the data were further visualized using boxplots generated in MedCalc.

RESULTS AND DISCUSSIONS

Flower Production and Yield Characteristics of *Crocus sativus* L.

A total of 20 stratified random sampling plots were evaluated within the saffron field. The measured agronomic variables included plant density, number of flowers per plant, and stigma yield per sampling point. These parameters were analyzed to evaluate variability in saffron productivity across the experimental field.

Descriptive statistics were calculated to summarize the main characteristics of the sampled data. The results indicated noticeable variability among the sampling plots. The mean plant density across the sampling area was 44.4 plants m^{-2} , based on the planting distance of 15 cm between bulbs. The average number of flowers per plant was 1.41, with a standard deviation of 0.44, indicating moderate variability across the field.

Similarly, the average estimated stigma yield per sampling plot was 0.34 $\text{g} \cdot \text{m}^{-2}$, with a minimum of 0.13 $\text{g} \cdot \text{m}^{-2}$ and a maximum of 0.38 $\text{g} \cdot \text{m}^{-2}$. These variations reflect differences in plant development and local field conditions within the sampled area. The coefficient of variation (CV) was calculated to assess the degree of variability within the field. The CV values of 31.2% for flowers per plant and 31.6% for estimated stigma yield indicate moderate spatial variability across the study field.

The descriptive statistics of the measured variables are summarized in Table 2.

The observed variability suggests that local field conditions, such as soil properties, bulb vigor, bulb caliber, and microenvironmental factors, may influence saffron productivity within the field.

Table 2. Descriptive statistics of the measured variables

Variable	Mean	Std. Dev.	CV (%)	Min.	Max.
Plant density (plants m ⁻²)	44.4	-	-	-	-
Flowers per plant	1.41	0.44	31.2	0.74	2.21
Stigma yield (g·m ⁻²)	0.34	0.11	31.6	0.13	0.38

In addition to agronomic variability among sampling plots, the temporal dynamics of flowering were analyzed to better understand the phenological behavior of the crop during the flowering season.

The temporal pattern of saffron flowering is illustrated in Figure 5, while daily flowering data are presented in Table 3. Flowering began gradually in early October and increased rapidly over the following days.

Table 3. Daily flower counts recorded during the flowering period of *Crocus sativus* L.

Date	Flowers	Cumulative	Cumulative %
6-Oct	9	9	0.03
7-Oct	60	69	0.22
8-Oct	330	399	1.27
9-Oct	207	606	1.92
10-Oct	1103	1709	5.42
11-Oct	2420	4129	13.10
12-Oct	993	5122	16.25
13-Oct	2104	7226	22.92
14-Oct	4732	11958	37.94
15-Oct	2042	14000	44.41
16-Oct	2556	16556	52.52
17-Oct	2088	18644	59.15
18-Oct	2772	21416	67.94
19-Oct	1888	23304	73.93
20-Oct	2355	25659	81.40
21-Oct	1355	27014	85.70
22-Oct	736	27750	88.04
23-Oct	1385	29135	92.43
24-Oct	805	29940	94.98
25-Oct	307	30247	95.96
26-Oct	292	30539	96.88
27-Oct	249	30788	97.67
28-Oct	211	30999	98.34
29-Oct	126	31125	98.74
30-Oct	113	31238	99.10
31-Oct	54	31292	99.27
1-Nov	60	31352	99.46
2-Nov	60	31412	99.65
3-Nov	51	31463	99.82
4-Nov	32	31495	99.92
5-Nov	9	31504	99.95
6-Nov	6	31510	99.97
7-Nov	1	31511	99.97
9-Nov	8	31519	99.99
11-Nov	1	31520	100.00
12-Nov	1	31521	100.00

The flowering intensity reached a peak in mid-October, followed by a progressive decline toward the end of the flowering period.

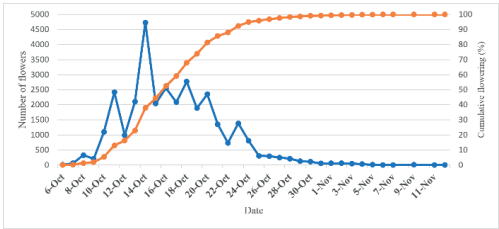


Figure 5. Temporal dynamics of saffron flowering during the 2025 flowering season

The cumulative flowering curve (orange line in Figure 5) further highlights the concentrated nature of the flowering phase. Daily flower counts recorded between 6th October and 12th November 2025 show a rapid increase in flowering intensity, reaching a maximum on 14th October, when 4732 flowers were harvested in a single day. After this peak, flowering gradually declined until early November.

Most flowers were produced within a relatively short time window, with the majority of flowering occurring between 11-20th October. Approximately 50% of the total flowering occurred between 13-16th October, indicating that the peak flowering phase was concentrated within only a few days.

Based on the observed flowering dynamics, seasonal flower production was estimated for each sampling plot to evaluate spatial variability in flower production across the field.

To estimate the total number of flowers produced in each sampling plot during the entire flowering period, flower counts on the sampling dates (12th, 13th, 14th and 18th October) were scaled using the relative contribution of each day to the total seasonal flowering. Based on the total daily flower counts across the field, these observation dates represented 33.6% of the total seasonal flowering.

Considerable variability in flower production was observed among the sampling plots. The estimated seasonal number of flowers per plot ranged from 33 to 98 flowers, with an average value of approximately 61 flowers per plot.

The estimated flower production and corresponding stigma yield for each sampling plot are presented in Table 4.

Table 4. Estimated flower production and corresponding stigma yield per sampling plot

Plot	Saffron flowers observed (4 days)	Estimated seasonal flowers	Estimated Stigma Yield	Estimated Stigma Yield ($\text{g}\cdot\text{m}^{-2}$)
S1-1	19	56	168	0.30
S1-2	11	33	99	0.18
S1-3	17	50	150	0.27
S1-4	14	42	126	0.23
S1-5	16	48	144	0.26
S2-1	13	39	117	0.21
S2-2	22	65	195	0.35
S2-3	33	98	294	0.53
S2-4	29	86	258	0.46
S2-5	27	80	240	0.43
S3-1	14	42	126	0.23
S3-2	16	48	144	0.26
S3-3	28	83	249	0.45
S3-4	24	71	213	0.38
S3-5	32	95	285	0.51
S4-1	16	48	144	0.26
S4-2	17	50	150	0.27
S4-3	24	71	213	0.38
S4-4	21	62	186	0.33
S4-5	28	83	249	0.45

The coefficient of variation for flower production was approximately 31% (Table 2), suggesting that spatial differences in plant performance occurred across the field. The relationship between flower production and estimated saffron yield is shown in Figure 6.

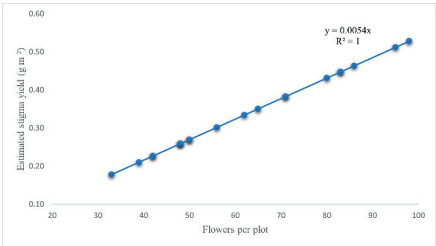


Figure 6. Relationship between the number of flowers per plot and estimated saffron stigma yield

A clear positive linear relationship was observed, indicating that higher flower numbers directly translate into higher saffron yield. Since each saffron flower produces two to six stigmas, the number of flowers serves as a reliable indicator of potential yield. The estimated dry stigma yield averaged $0.34 \pm 0.11 \text{ g}\cdot\text{m}^{-2}$, with values ranging from 0.13 to $0.38 \text{ g}\cdot\text{m}^{-2}$ across the sampling plots. The distribution of estimated saffron yield among the sampling plots is illustrated in Figure 7.

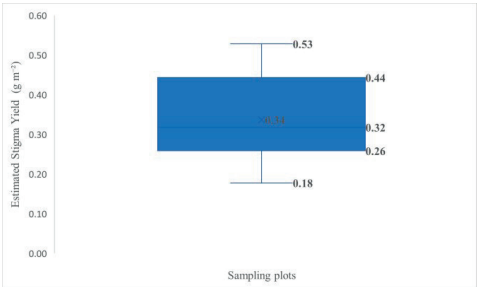


Figure 7. Distribution of estimated saffron stigma yield ($\text{g}\cdot\text{m}^{-2}$) across the sampling plots

The boxplot shows the variability and yield spread values within the field. The median yield was approximately $0.32 \text{ g}\cdot\text{m}^{-2}$, while the mean yield reached $0.34 \text{ g}\cdot\text{m}^{-2}$. In addition to examining the relationship between flower production and saffron yield, the distribution of estimated saffron yield among the sampling plots was evaluated to assess variability within the field. The comparison of flower production and saffron yield among the four field strata is summarized in Table 5 and illustrated in Figure 8.

Table 5. Comparison of flower production and saffron yield among the field strata

Stratum	Mean Flowers	Mean Yield ($\text{g}\cdot\text{m}^{-2}$)
S1	45.8	0.247
S2	73.6	0.397
S3	67.8	0.365
S4	62.8	0.338

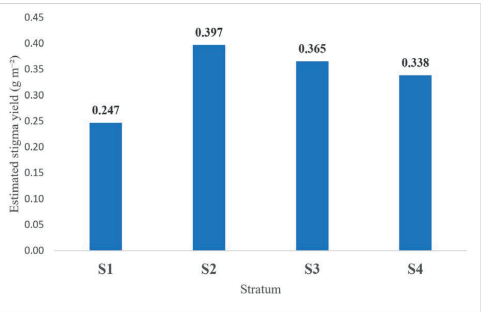


Figure 8. Distribution of estimated saffron stigma yield ($\text{g}\cdot\text{m}^{-2}$) across the strata

Stratum S2 showed the highest mean flower production (73.6 flowers per plot) and the highest estimated saffron yield ($0.397 \text{ g}\cdot\text{m}^{-2}$). In contrast, S1 recorded the lowest flower production (45.8 flowers per plot) and the lowest yield ($0.247 \text{ g}\cdot\text{m}^{-2}$). Intermediate values were

observed in S3 and S4, suggesting that productivity gradually varied across the field. These results indicate that local environmental conditions within different areas of the field influenced saffron productivity.

Total Phenolic Content of Saffron Petal Samples

In addition to agronomic performance, the biochemical composition of saffron petals represents an important indicator of saffron quality. Therefore, the total phenolic content (TPC) of the collected saffron samples was determined to evaluate the antioxidant potential and quality characteristics of the harvested material.

Total Phenolic Content

The total phenolic content (TPC) of saffron petal extracts was determined using the Folin-Ciocalteu method and expressed as milligrams of gallic acid equivalents (GAE) per gram of dry plant material (DW) (mg GAE·g⁻¹). The analyzed samples included petals obtained from two locations, Suceava (S samples) and the Neamț experimental field (N samples), and from different harvesting years (2023-2025). The mean phenolic concentrations of the analyzed samples ranged from 17.64 to 23.25 mg GAE·g⁻¹. The lowest average value was recorded for S23 samples (17.64 ± 0.22 mg GAE·g⁻¹), while the highest phenolic content was observed in N25 samples (23.25 ± 3.67 mg GAE·g⁻¹). Intermediate values were recorded for N24 (19.09 ± 4.41 mg GAE·g⁻¹) and S24 (20.65 ± 0.91 mg GAE·g⁻¹) samples (Table 6).

Table 6. Mean total phenolic content (mg GAE·g⁻¹) of saffron petal samples with standard deviation and Tukey significance letters

Sample	TPC (mg GAE·g ⁻¹)
S23	17.64 ± 0.22 ^a
N24	19.09 ± 4.41 ^a
S24	20.65 ± 0.91 ^{ab}
N25	23.25 ± 3.67 ^b

To evaluate whether the differences between groups were statistically significant, a one-way analysis of variance (ANOVA) was performed. The results indicated a significant effect of sample group on total phenolic content (F = 6.14, p = 0.002). Prior to ANOVA, the assumption of normality was verified using the D’Agostino-Pearson test, which confirmed that

the data followed a normal distribution (p = 0.091). Post-hoc comparisons were conducted using the Tukey-Kramer multiple comparison test. The results showed that N25 samples differed significantly from both N24 and S23 samples (p < 0.05), indicating higher phenolic concentrations in petals collected from the Neamț experimental field in 2025. However, no statistically significant differences were observed between S23, S24 and N24 groups. The distribution and variability of total phenolic content among the analyzed samples are illustrated in Figure 9.

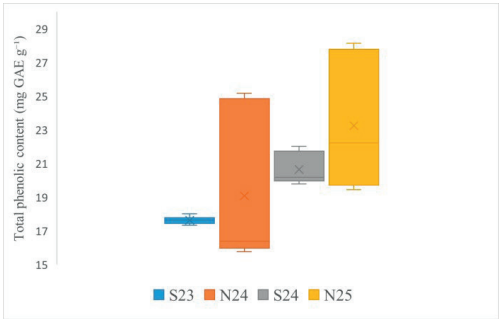


Figure 9. Boxplot showing the distribution of total phenolic content (mg GAE·g⁻¹) in saffron petal extracts collected from Suceava (S) and Neamț (N) during the 2023-2025 harvesting seasons

The boxplot shows the spread of phenolic values within each group. The interquartile ranges and whiskers indicate the variability of the replicate measurements, while the median values highlight differences in phenolic concentration among the analyzed samples. For a clearer comparison of mean values among groups, the average phenolic concentrations are also presented in Figure 10, while the total phenolic content of each sample is presented in Figure 11. The bar charts confirm that samples collected in 2025 from the Neamț experimental field (N25) exhibited the highest mean phenolic content, whereas the samples collected from Suceava 2023 (S23) showed the lowest values. These results suggest that both environmental conditions and harvesting year may influence the accumulation of phenolic compounds in saffron petals.

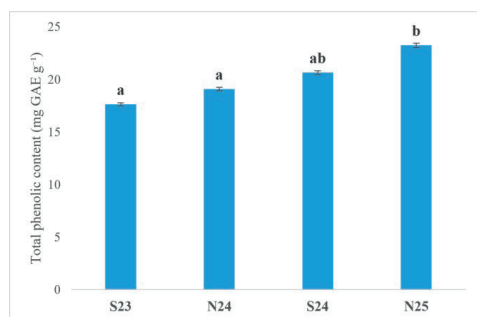


Figure 10. Mean total phenolic content of saffron petal extracts collected from Suceava (S) and Neamț (N) during the 2023-2025 harvesting seasons

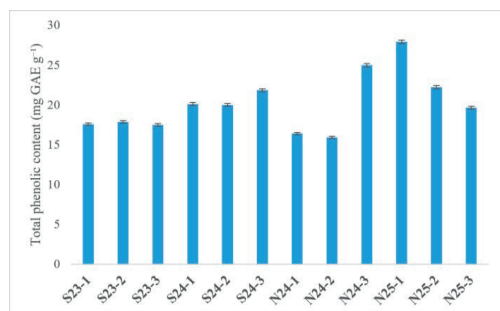


Figure 11. Total phenolic content of saffron petal extracts collected from Suceava (S) and Neamț (N) during the 2023-2025 harvesting seasons.

The statistical analysis demonstrates that phenolic content in saffron petals varies among sample groups, with the highest concentrations observed in the most recent samples collected from the Neamț experimental field.

CONCLUSIONS

The present study provides an integrated evaluation of saffron (*Crocus sativus* L.) cultivation by combining field-based agronomic assessment with phytochemical analysis. The application of stratified random sampling enabled a reliable characterization of spatial variability within the study field, ensuring that differences in plant performance were accurately captured across distinct field strata. The results revealed moderate variability in agronomic parameters, including flower production and estimated stigma yield, indicating that local field conditions such as soil properties, bulb development, and microenvironmental factors can influence saffron productivity. The temporal analysis of

flowering demonstrated a highly concentrated blooming period, with the majority of flowers produced within a short time window, confirming the characteristic phenological behavior of saffron.

Phytochemical analysis showed that total phenolic content varied among the analyzed samples, with significantly higher values observed in samples collected from the Neamț experimental field in 2025. These differences suggest that both environmental conditions and harvest year play an important role in the accumulation of bioactive compounds in saffron petals. The observed variability highlights the sensitivity of secondary metabolite production to cultivation conditions and plant physiological status.

From a practical perspective, the results indicate that spatial variability within the field should be considered when designing management strategies. Uniform agronomic practices may not fully optimize productivity, and site-specific management approaches, including adjustments in planting density, irrigation, and soil management, may improve overall yield performance.

The identification of peak flowering periods provides valuable guidance for optimizing harvesting operations, allowing growers to concentrate labor during the most productive phase and minimize potential losses. In addition, the variation in phenolic content suggests that environmental conditions and harvest timing can influence saffron quality, emphasizing the importance of monitoring cultivation conditions to enhance both yield and biochemical properties.

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CONTRIBUTIONS TO THE STUDY OF THE RARE PLANT SPECIES IN ORHEI NATIONAL PARK FROM REPUBLIC OF MOLDOVA

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Abstract

The territory of Orhei National Park is located in the central part of the Republic of Moldova and represents an area with a valuable, yet still insufficiently studied, natural heritage. Currently, based on the results of systematic research initiated in 2023 and the most recent published works, the spontaneous flora of Orhei National Park includes 822 species of vascular plants. The article presents updated data on 33 species of rare, highly endangered plants included in the 3rd edition of the Red Book of the Republic of Moldova, obtained as part of the research and monitoring of rare species and the conservation status of their populations within the territory of the protected area.

The researched species are protected by law at the national level, and some of them are also protected at the international level: *Cypripedium calceolus* L. and *Galanthus nivalis* L. by the Habitats Directive, and *Cypripedium calceolus* L., *Fritillaria montana* Hoppe by W.D.J. Koch, *Genista tetragona* Besser, *Pulsatilla grandis* Wender, and *Schivereckia podolica* (Bess.) Andrz. are protected by the Bern Convention.

Key words: Orhei National Park, Republic of Moldova, rare tax, conservation.

INTRODUCTION

The *Orhei National Park* is a valuable protected natural area, which was founded in 2013 by the Parliament Decision no. 201/2013 "On the Foundation of the Orhei National Park". It is located in the central area of the Republic of Moldova (Figure 1), on an area of 33792.09 ha, with the main objective of ensuring the conservation of biodiversity.

It is the first national park in the Republic of Moldova, the creation of which also constitutes the country's international commitment, as part of many European conventions. The regulation on the management of the park was developed in accordance with *Law no. 1538-XIII of 25.02.98 on the Fund of natural areas protected by the state*, in order to ensure, maintain, and preserve the rational use of biological diversity, unique natural complexes, special aesthetic and cultural-historical value, their use for scientific purposes, cultural, tourist, and instructive-educational purposes.

The territory of the park is divided into several functional areas (A-C), the core of the park constituting the area - with integral protection (A), which occupies 3.4% of the park's surface and includes sectors of habitats where rare

species are concentrated; only scientific and protection research is allowed.

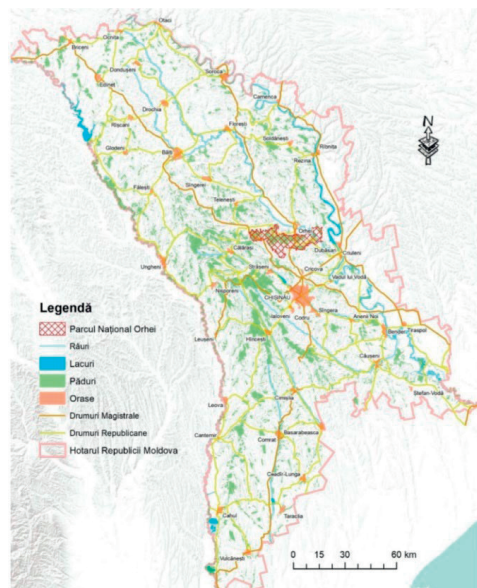


Figure 1. Geographical position of Orhei National Park
(Source: Analele naturii, 2023)

The purpose of the study is to identify and confirm the rare species and objects of the Red

Book of the Republic of Moldova, 3rd edition, on the territory of the Orhei National Park, to highlight the growing conditions, and to assess the current state of the populations of the targeted species, to establish the population parameters, to establish the limiting risk factors that threaten them. It is important to identify the causes of the endangerment of rare species preserved *in situ* conditions in protected areas, where a number of measures are already applied, in order to implement effective approaches to their protection.

MATERIALS AND METHODS

This study includes research on rare species, with a high risk of endangerment included in the Red Book of the Republic of Moldova, 3rd edition, reported on the territory of the Orhei National Park. The field investigations and data collection were carried out during the years 2023-2025, on different itineraries so as to encompass all the sectors where rare taxa vegetate. At the same time, in order to specify the chorology, the herborized collections kept in the Herbarium of the National Botanical Garden (Institute) of the State University were consulted, which contain exsiccata of rare species collected from the area of the Orhei National Park. Field floristic research and critical analysis of the materials were carried out in accordance with the classical comparative-morphological method (Korovina, 1986). The nomenclature of species is the one accepted by current databases such as: *The International Plant Names Index* (IPNI, 2026), *Plants of the World Online* (POWO, 2026), *The World Flora Online* (WFO, 2026), and, in some cases, the nomenclature of the species given in the Red Book of the Republic of Moldova has been preserved. The determination of the species was carried out according to the fundamental works in the field (Gheideman, 1986; Negru, 2007, Pinzaru, 2023).

The assessment of the populations of rare species, the estimation of the degree of threat, and the establishment of the endangered category were carried out according to the requirements of the International Union for Conservation of Nature (IUCN, 2012) and the

legislation of the Republic of Moldova (Environmental legislation, 1999). The photos in colour are original, taken by the authors.

RESULTS AND DISCUSSIONS

Based on the data presented in the substantiation study on the justification for the need to create the national park, the existence of 700 species of vascular plants was found, 52 of which are rare taxa, and 29 species included in the *Red Book of the Republic of Moldova*, 2nd ed., 2001 (Planul de management, 2023).

As a result of the review of scientific publications and the latest research initiated, in 2023 the flora of Orhei National Park included 816 species of vascular plants, of which 92 species are rare plants.

In the last three years, extensive research has been carried out on rare species that occur on the territory of Orhei National Park, focused mainly on the study and distribution of species at high risk of endangerment included in the 3rd edition of the *Red Book of the Republic of Moldova*. The study was driven by the lack of data necessary for the assessment of rare species populations and the need to update information on their conservation status on the Park's territory, both in the context of field research on endangered taxa proposed for the next edition of the Red Book, and for the creation of a scientific basis for the future monitoring system of rare species reported on the territory of Orhei National Park and for mapping them.

As a result of the study, the floristic inventory of Orhei National Park was established, which, by the end of 2025 included 822 species of vascular plants, 98 of which are rare species, representing about 12% of the total taxa registered in the Park's flora.

At the national level, most of the rare species highlighted on the territory of Orhei National Park are protected by the *Law on the Fund of Natural Areas Protected by the State*, 1998 (*Annex D*), and 33 species are included in the Red Book of the Republic of Moldova (2015), which constitutes 20% of the total number of higher plant species (165 species) contained in the last edition (Table 1).

Table 1. Endangered plant species from Orhei National Park included in the Red Book of the Republic of Moldova, 3rd edition

No.	Species	Threat category	Habitat	Locality of distribution
1.	<i>Aconitum lasiostomum</i> Rchb. ex Besser	EN	forest	Ivanca, Teleșeu, Seliște, Vatici, Trebujeni
2.	<i>Allium inaequale</i> Janka	VU	steppes	Mășcăuți
3.	<i>Alyssum gmelinii</i> Jord.	VU	petrophytic	-
4.	<i>Athyrium filix-femina</i> (L.) Roth	VU	forest	Vatici
5.	<i>Cephalanthera damasonium</i> (Mill.) Druce	VU	forest	Ivanca, Vatici, Seliște
6.	<i>Cephalanthera longifolia</i> (L.) Fritsch	VU	forest	Ivanca, Teleșeu, Țigănești
7.	<i>Cephalanthera rubra</i> (L.) Rich.	CR	forest	Ivanca, Vatici, Seliște
8.	<i>Cotoneaster melanocarpus</i> Fisch. ex A. Blytt	EN	petrophytic	Trebujeni
9.	<i>Cypripedium calceolus</i> L.	CR	forest	Ivanca, Seliște
10.	<i>Dentaria glandulosa</i> Waldst. and Kit	VU	forest	Ivanca, Vatici, Bravicea
11.	<i>Dentaria quinquefolia</i> M. Bieb.	EN	forest	Ivanca, Vatici, Bravicea
12.	<i>Dryopteris carthusiana</i> H.P. Fuchs	EN	forest	Seliște, Vatici
13.	<i>Dryopteris filix-mas</i> (L.) Schott	VU	forest	Ivanca, Teleșeu, Seliște, Vatici, Bravicea
14.	<i>Epipactis purpurata</i> Smith.	CR	forest	Ivanca, Teleșeu, Seliște, Vatici, Bravicea
15.	<i>Fritillaria montana</i> Hoppe ex W.D.J. Koch	VU	forest	Trebujeni, Ivanca
16.	<i>Galanthus nivalis</i> L.	VU	forest	Ivanca, Teleșeu, Seliște, Vatici, Bravicea
17.	<i>Genista tetragona</i> Besser	VU	petrophytic	Mășcăuți
18.	<i>Gymnocarpium robertianum</i> (Hoffm) Newman	CR	petrophytic	Trebujeni
19.	<i>Helianthemum canum</i> (L.) horn.	VU	petrophytic	Trebujeni, Butuceni, Morovaia, Mășcăuți
20.	<i>Jurinea stoechadifolia</i> (M. Bieb.) DC.	VU	petrophytic	Mășcăuți, Morovaia, Trebujeni
21.	<i>Koeleria moldavica</i> M. Alexeenco.	VU	steppes	Ivanca
22.	<i>Majanthemum bifolium</i> F.W. Schmidt	CR	forest	-
23.	<i>Orchis purpurea</i> Huds	CR	meadow	Seliște, Morozeni, Teleșeu, Donici, Vatici, Bravicea, Codreanca
24.	<i>Ornithogalum boucheanum</i> (Kunth) Asch.	EN	meadow	Trebujeni, Brănești
25.	<i>Paronychia cephalotes</i> (M.Bieb.) Besser	CR	petrophytic	Mășcăuți, Morovaia
26.	<i>Pulsatilla grandis</i> Wender.	EN	steppes	Trebujeni
27.	<i>Saxifraga tridactylites</i> L.	CR	petrophytic	Trebujeni, Mășcăuți
28.	<i>Schivereckia podolica</i> (Bess.) Andrz.	VU	petrophytic	Trebujeni, Mășcăuți
29.	<i>Scopolia carniolica</i> Jacq.	VU	forest	Bravicea, Țigănești
30.	<i>Sempervivum ruthenicum</i> W. D. J. Koch Schnittsp. and C. B. Lehm.	CR	petrophytic	Trebujeni
31.	<i>Seseli peucedanifolium</i> Besser	VU	petrophytic	Trebujeni, Butuceni, Morovaia
32.	<i>Sorbus domestica</i> L.	EN	forest	Ivanca
33.	<i>Thelypteris palustris</i> Schott	EN	meadow	Seliște

From the inventory of floristic taxa from the Red Book found in the Park, five species are included in the Red Book of Vascular Plants in Romania (Dihoru and Negrean, 2009), and 14 species are listed in the Red Book of Ukraine (Chervona kniga Ukraïni, 2009). At the same time, the Park's list of endangered species includes floristic taxa protected internationally

as well. Thus, the species *Cypripedium calceolus* and *Galanthus nivalis* (Figures 3, 4) are protected by the *Habitats Directive* (Interpretation... 2013), *Cypripedium calceolus*, *Fritillaria montana*, *Genista tetragona*, *Pulsatilla grandis* and *Schivereckia podolica* are protected by the *Bern Convention* (Convention... 1979). Seven taxa: *Cephalanthera damasonium*, *C. longifolia*,

C. rubra, *Cypripedium calceolus*, *Epipactis purpurata*, *Orchis purpurea* and *Galanthus nivalis* are also listed in Annex II of the

Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) (Figure 2).

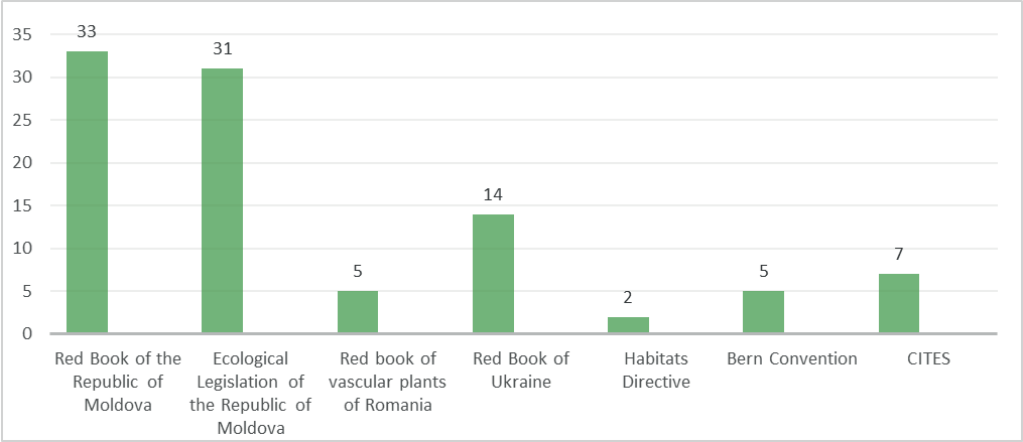


Figure 2. Number of endangered species in Orhei National Park, included in the Red Book of the Republic of Moldova and in other International documents, Conventions and Directive



Figure 3. *Cypripedium calceolus*



Figure 4. *Galanthus nivalis*

Previously, the list of Red Book species reported for the park territory included the critically endangered species *Ophioglossum vulgatum* L., but this was excluded because the species' habitat was completely destroyed by full-scale tillage, a fact recorded both in the specialized literature (Gheideman, 1988; Pinzaru, 2019) and confirmed by recent research.

Although mentioned in the list, the presence of the species *Majanthemum bifolium* F.W. Schmidt on the Park's territory remains uncertain. Currently, there is no proven data published or documented by the herbarium that would confirm the taxa for the researched territory; most likely, it was included erroneously. At the same time, in our field study, which included a

review of the distribution points cited in the Red Book, the species *Alyssum gmelinii* Jord. was not identified on the territory of the Park.

As a result of the research and monitoring activities for rare species carried out during the years 2023-2025, **new breeding sites** were identified for 8 species of endangered plants from the Red Book - *Cephalanthera damasonium*, *C. longifolia*, *C. rubra*, *Epipactis purpurata*, *Jurinea stoechadifolia*, *Dryopteris filix-mas*, *Orchis purpurea* (Figure 5), *Saxifraga tridactylites*, thus updating and completing their chorology on the park territory.

At the same time, in the period 2023-2025 the list of species of Orhei National Park was supplemented with rare floristic taxa documented **for the first time** for this territory, such as: *Centaurea x pulberiana* Pinzaru, *Centaurea ruthenica* Lam., *Diplotaxis cretacea* Kotov, *Epipactis muelleri* Godfery, *Petasites hybridus* (L.) G. Gaertn. and *Senecio macrophyllus* M. Bieb.

Increased attention is also required to be directed toward the endangered species highlighted in the Park's flora, which, for the time being, are not included in the Red Book nor in the *List of species protected by law*. Out of these, the most important are: *Centaurea ruthenica* Lam., *Epipactis muelleri* Godfery, *Gentiana cruciata* L., *Iris aphylla* L., and *Senecio macrophyllus* M.Bieb.

It is important to note that, at the time the start of the research, for some rare species there was recent information with data regarding the spread and the results of their monitoring on the territory of the Park, while for others it was necessary to identify the breeding sites in the field or to specify and confirm the information regarding their chorology, the assessment of their conservation status, as well as the establishment of the **"zero"** moment of their monitoring.



Figure 5. *Orchis purpurea*

These findings confirm both the scientific importance of the investigations carried out within the framework, as well as the efficiency of monitoring activities, which allow periodic updating of knowledge of local biological diversity.

The **high floristic diversity** of Orhei National Park is due to the varied geographical, climatic, and pedological conditions, which have determined the formation of diverse plant communities, which belong to several types of vegetation: forest, meadow, aquatic and marshy, petrophyte, and anthropic.

Of the total endangered species included in the latest edition of the Red Book of the Republic of Moldova (2015), 16 species are characteristic of forest habitats, 11 of petrophytic habitats, 3 of steppe habitats, and 3 of meadow habitats (Figure 6).

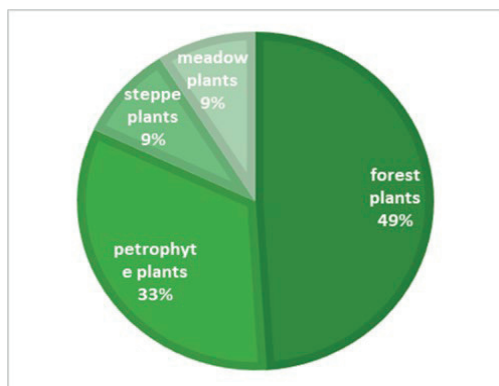


Figure 6. Spectrum of ecotopological elements in Orhei National Park

Forest habitats in Orhei National Park play a key role, as they host approximately half (49%) of the number of Red Book taxa reported here, providing favourable and relatively stable conditions for endangered species.

Even so, the populations of some species are very small, with an extremely low number of individuals, dominated by specimens in the vegetative phase (*Cephalanthera rubra*, *Cypripedium calceolus*), in addition, flowering

and fruiting, under the conditions of our country, do not occur annually. The pronounced climate changes of the last decade have been the main cause of the reduction in the areas occupied by the populations of endangered floristic taxa.

Calcareous slopes of Orhei National Park represent a unique habitat in the region, very valuable, with exclusive vegetation, made up of plants with strict ecological requirements, vulnerable to changes in environmental conditions and anthropogenic actions. The bare limestone, or limestone covered with typical rendzinic soil, rich in the calcareous skeleton of the rocks, includes primary, xerophilic, calciphilic, and endemic phytocenoses (Pinzaru, 2023). Among the taxa taken in the study, important calciphilous species, endemic to Ponto-Sarmatic, such as *Genista tetragona*, *Schivereckia podolica*, *Sempervivum ruthenicum*, and *Seseli peucedanifolium* Besser (Figure 7), as well as rare Ponto-Balkan species such as *Jurinea stoechadifolia* and *Paronychia cephalotes*, are characterized by extensive populations, with a stable number of individuals, which bloom and bear fruit abundantly every year.



Figure 7. Fragment of a calcareous slope with *Genista tetragona*

Paronychia cephalotes is a critically endangered species with a fragmented area, which vegetates only on the limestones of the Middle Sarmatian, currently, it has a certain presence only within the territory of the Orhei National Park (Figure 8).

The endangered species, characteristic of **steppe and meadow** habitats, reported in the Park, include taxa with restricted ecological amplitude and reduced specific habitats, threatened by

various degradation factors. Among them is *Koeleria moldavica*, a Podolian endemic species. *Pulsatilla grandis* is found at the southern border of the area and is strongly threatened with extinction due to its high decorativeness. *Thelypteris palustris* - taxon characteristic of humid meadows and marshy areas, strongly affected by changes in habitat conditions (Figure 9).



Figure 8. *Paronychia cephalotes* in the natural habitat

CONCLUSIONS

Both previous and current studies highlight a high potential for conserving rare species in Orhei National Park, which represents a unique and diverse object. The periodic monitoring of rare species carried out in this protected area provides updated data on antatus of populations and their trends, contributing to an understanding of the biology and ecology of rare species, which are extremely important for the application of effective conservation strategies based on the identification of specific threats that endanger their existence and on the promotion of scientifically based conservation practices.

As a result of anesearch, an inventory of rare plants of the Orhei National Park was drawn up,

which counts 98 species, constituting 12% of its total flora. The list of plant species included in the Red Book of the Republic of Moldova (3rd ed.) reported in the natural habitats of the Park totals 33 species, 9 of which are critically endangered (CR), 8 endangered (EN) and 16 vulnerable (VU).

The *in situ* protection of threatened species within Orhei National Park is a leading objective, included in the Biodiversity Conservation Strategies, contributing to the maintenance of viable populations in their natural environment and protecting habitats from destruction, taking into account the ecological complex in which they vegetate, in order to quickly understand the direction of change of rare floristic elements.



Figure 9. *Thelypteris palustris*

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AN OVERVIEW OF THE DIVERSITY AND VARIABILITY OF SPONTANEOUS FLORA IN VITICULTURAL REGIONS

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Abstract

At the global level, studies concerning the variability of spontaneous flora in viticultural areas demonstrate the decisive influence of ecological, pedological, and anthropogenic factors. Monitoring spontaneous flora can provide valuable information regarding the ecological status of viticultural areas and the effectiveness of management methods. The composition of spontaneous vegetation contributes to the protection of soil against erosion, the optimization of soil structure and fertility, as well as the modification of soil moisture. The diversity and variability of spontaneous flora represent important indicators of ecological balance and ensure sustainable management measures for viticultural areas. The objective of the present study is to analyze the diversity and variability of spontaneous flora in viticultural areas from several countries, in order to understand its contribution to maintaining ecosystem health, controlling climatic factors, conserving soil, and supporting biodiversity. The methodology applied is based on the review of specialized literature, using relevant bibliographic sources and scientific articles concerning spontaneous flora in viticultural areas. The data were consolidated and interpreted in order to highlight its ecological role, variability, and diversity.

Key words: biodiversity, ecosystem, spontaneous vegetation, viticultural region.

INTRODUCTION

Spontaneous vegetation is characterized by high diversity and variability, resulting from the complex interaction among climatic conditions, soil characteristics, geographical factors, and applied management practices. Floristic composition varies significantly among viticultural regions, highlighting the adaptation of spontaneous species to specific ecological conditions (Luglio et al., 2024).

In recent years, spontaneous flora associated with vineyards has received increasing attention due to growing awareness of its benefits for agroecosystems, soil health, and the provision of multiple ecosystem services (Aguar et al., 2024).

Within vineyard systems, interactions between grapevine and spontaneous plants may generate positive effects, contributing to ecological balance and ecosystem health. Steiner et al. (2021) demonstrated that spontaneous vegetation in viticultural areas can enhance biodiversity and soil stability. Appropriate management of spontaneous vegetation supports the sustainability and equilibrium of

vineyard ecosystems (Ramírez-Pérez et al., 2024).

Spontaneous flora contributes to soil moisture retention, enhances biodiversity within vineyard ecosystems, and does not significantly affect grape yield and quality when properly managed (Virili et al., 2025).

Viticultural areas often induce moderate stress in grapevines through limitations in water and nutrient availability, thereby emphasizing soil and topographic characteristics. A moderate level of stress may be beneficial, and interactions with spontaneous vegetation can support the balance between vegetative growth and production (Reeve et al., 2016; Kesser et al., 2023). Vineyards also contribute to the conservation of agroecosystem biodiversity, as they represent relatively stable and structurally layered systems (Mania et al., 2015).

At the global scale, the intensification of viticulture has increased pressure on spontaneous flora, leading to ecological imbalances. In this context, modern sustainability-oriented strategies aim to restore the ecological role of spontaneous vegetation in maintaining biodiversity and supporting

vineyard ecosystem functioning (Zhao et al., 2021).

Managing spontaneous flora represents an effective alternative to chemical control, supporting sustainable viticultural practices (Tscharntke et al., 2005; Sáenz-Romo et al., 2019). The use of cover crops in vineyards improves soil structure, enhances water retention and nutrient availability, and promotes microbial diversity and beneficial soil microorganisms (Finney et al., 2017; León et al., 2021). Spontaneous vegetation positively influences the abundance and diversity of beneficial arthropods, supporting natural pest control and contributing to ecological balance within vineyards (Rusch et al., 2017; Fiera et al., 2020).

Mediterranean vineyards are particularly vulnerable to soil degradation due to slope and rainfall intensity. The transition to modern soil management systems has intensified soil and mineral nutrient losses (Arnaez et al., 2007).

Protection regimes for spontaneous flora influence biodiversity, while grape and wine quality remain comparable between organic and conventional systems (Döring et al., 2019). The expansion of vineyard plantations and land-use conversion have reduced native spontaneous plant communities, altering natural vegetation patterns (Iriarte-Chiapusso et al., 2017).

The improvement of grapevine varieties and expansion of cultivated areas significantly modify natural ecosystems, often leading to reduced native spontaneous flora and simplified vegetation structure under specialized cropping systems (Droulia & Charalampopoulos, 2022). Ecological agricultural techniques can protect ecosystems, improve soil health, and provide economic advantages due to increasing demand for organic products (Marcelino et al., 2024).

Particular attention has been given to spontaneous vineyard plants, with research focusing on species composition, diversity, variability, and response mechanisms to environmental conditions, resulting in resilience and adaptation to anthropogenic disturbances (Ilie & Cosmulescu, 2023).

Monitoring climate change enables assessment of future impacts and supports proactive adaptation strategies aimed at sustaining viticulture and managing spontaneous

vegetation under changing climatic conditions (Chalak & Ghorra Chamoun, 2024). Therefore, the objective of this study is to provide an updated overview of the importance of floristic composition associated with vineyard ecosystems.

MATERIALS AND METHODS

The study was conducted using a documentary research method complemented by comparative analysis of data available in the scientific literature. The scientific analysis was based on bibliographic sources, peer-reviewed scientific articles, botanical monographs, and specialized reports.

Bibliometric analysis and visualization of keyword relationships were performed using the VOSviewer platform, which enabled the identification of major research directions and connections within the literature.

The methodological approach consisted of a synthetic review of studies addressing spontaneous flora in vineyards, focusing on species diversity and ecological variability. Data regarding the role of spontaneous vegetation in maintaining biodiversity, ensuring agroecosystem stability, and shaping ecological interactions between spontaneous plants and grapevine were also evaluated.

The integration and comparison of collected information allowed the identification of key trends concerning floristic biodiversity conservation and supported the formulation of evidence-based conclusions regarding the importance of spontaneous flora in sustainable vineyard management.

RESULTS AND DISCUSSIONS

The study of spontaneous flora in viticultural areas has attracted interest across multiple continents (Figure 1). The selected countries represent reference viticultural regions characterized by significant ecological and floristic diversity. Their inclusion aims to highlight relationships between viticultural practices, biogeographical conditions, and spontaneous flora structure at a global scale. The map was created using the MapChart platform for illustrative purposes.

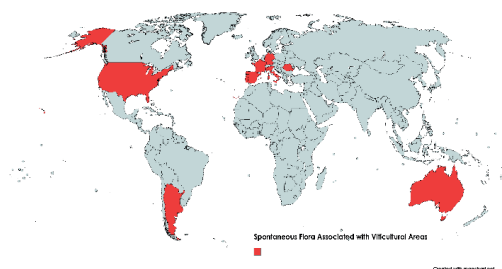


Figure 1. Global distribution of the main traditional viticultural countries included in the analysis of spontaneous flora associated with vineyard areas

According to Bettoni et al. (2016), spontaneous flora plays a crucial role in conserving and improving the physical, chemical, and biological properties of soil, reducing erosion and increasing organic matter. It may influence grape production and quality, particularly when soil cover is implemented during the vine's dormant period, although competition for water and nutrients may occur.

Perennial species adapted to edaphic conditions and producing high biomass can reduce vegetative growth and increase anthocyanin and polyphenol content in wine.

The hydric regime is a fundamental factor underlying these processes, as water availability directly influences physiology and soil-vegetation interactions. According to Costea (2006), vine water relations determine the balance between vegetative growth and reproductive development, with adequate soil moisture being essential for optimal growth and fruit formation. Costea emphasizes that insufficient water negatively affects vegetative growth, yield components, and bud differentiation, whereas excessive moisture can also disturb vine balance and increase disease susceptibility. Research on water availability effects is inconclusive, as some studies report increased soil moisture while others do not. Comprehensive evaluation requires analysis of water dynamics and root activity. Celette et al. (2008) showed that integrated water management in mixed grapevine-cover crop systems allows intercrop integration without compromising grape quality. These outcomes resonate with Costea et al. (2025) conclusions that the vine's response to water availability is nonlinear: slight water deficits can reduce

vegetative vigour and improve fruit quality, while severe hydric stress disrupts metabolic processes

Rotchés-Ribalta et al. (2023) emphasize the importance of spontaneous plants in attracting pollinators and supporting agroecosystem functioning.

Although spontaneous species are often viewed as competitors, biodiversity constitutes an essential component of natural heritage. Spontaneous flora influences vine growth through water competition while promoting an optimal balance between vegetative and reproductive growth. Reduced canopy density improves cluster exposure and fruit-zone microclimate, thereby enhancing grape quality (Xi et al., 2011).

Further studies conducted in Canada highlighted that greater abundance of certain species is influenced by seed availability, establishment capacity, biomass production, soil cover, and suppression of competing vegetation. Species used for cover cropping included red clover, pearl millet, and yellow clover (Sharifi et al., 2021). While spontaneous vegetation within cover crop systems increases vineyard biodiversity, inappropriate seed density or composition may reduce vine yield (Miglécz et al., 2015; Donkó et al., 2017).

In viticultural areas of Madrid, soil management practices have contributed to soil erosion, affecting both spontaneous flora and productivity; however, yields remain strongly dependent on irrigation in semi-arid regions (Dorado et al., 2016). Management strategies must therefore balance crop protection with environmental conservation, as outcomes depend on soil and climate conditions, training systems, and grower practices (Pires, 2022).

Mechanical practices are frequently preferred for managing under-row vegetation, using tools such as hoes, cultivators, or discs, which differ in efficiency and degree of soil disturbance (Gagliardi et al., 2023).

A study conducted in Şanlıurfa (Türkiye) identified and documented 184 spontaneous plant taxa present in vineyard areas, based on morphological characteristics and comparison with previously recorded local flora (Balos, 2023). The floristic classification is presented in Figure 2.

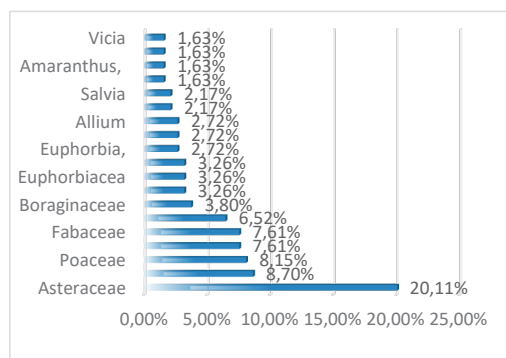


Figure 2. Floristic classification of spontaneous plant taxa identified in the study

Vegetation analysis revealed several botanical families, including some with invasive or potentially harmful species for grapevine cultivation. The most represented family was Asteraceae (37 taxa), followed by Apiaceae (16 taxa) and Poaceae (15 taxa), while other families contributed to overall floristic diversity at lower proportions.

Annual plants provide rapid soil cover, whereas perennial species contribute to nitrogen fixation and soil stabilization, facilitating integration with spontaneous vineyard vegetation (Sharifi & Zolfaghari, 2025).

Incorporating spontaneous flora into agricultural management highlights ecosystem services provided by native species while adaptive control strategies help limit competition for resources (Garcia et al., 2018). Soil management practices in vineyards often leave partial ground cover, influencing vine growth, nutrient dynamics, soil water content, grape quality, and soil properties. The impact of these practices depends on climate, soil type, and irrigation regime. Studies indicate that leguminous cover crops increase total and mineral nitrogen through biological nitrogen fixation, whereas spontaneous plant communities may reduce total nitrogen levels compared to other plant families (Peoples et al., 2009; Abad et al., 2021).

In Uruguayan vineyards, optimal temperature, water, and soil fertility conditions may promote excessive vegetative growth, creating microclimatic conditions unfavorable for fruit maturation and disease management. Growers commonly use vigorous rootstocks to maintain vegetative balance. In fertile soils and humid

climates, limiting resources is challenging. Coniberti et al. (2018) demonstrated that integrating cover crops with supplementary irrigation can regulate vegetative development and improve vineyard sustainability while maintaining wine quality.

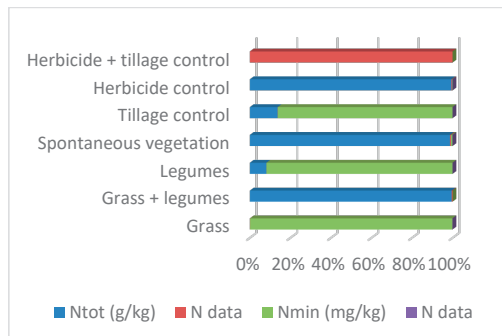


Figure 3. Distribution of mean soil N_{tot} and N_{min} values according to cover crop type

Conversely, Guerra et al. (2022) investigated mulching as an ecological weed-control method in irrigated Mediterranean vineyards. Their three-year study showed that certain management practices reduce invasive species density and biomass without negatively affecting vine growth and productivity. Vizitiu et al. (2023) describe vineyard ecosystems as simplified anthropogenic systems dominated by grapevine, characterized by reduced ecological stability, which may increase vulnerability to biological imbalances. In this framework, spontaneous flora is often regarded as a competitive factor potentially exacerbating water and nutrient stress.

Permanent vegetation between vine rows provides soil protection, enhances fertility, and contributes to vegetation management; however, tillage may stimulate rhizome expansion, indicating the need for integrated management approaches. Abad et al. (2020) and Recasens et al. (2023) highlighted the influence of cover crops on spontaneous vegetation composition, demonstrating effective weed control without compromising grape yield or quality. Sowing cover crops prior to vineyard establishment represents an efficient strategy for managing spontaneous flora and promoting balanced vine development (Bordelon & Weller, 1997).

Vegetation control methods have evolved from intensive tillage to herbicide application. Although herbicides may reduce species diversity, different management approaches can maintain balance between vegetation control and biodiversity conservation (Baumgartner et al., 2007; Fried et al., 2019).

Environmental factors shaping spontaneous plant communities in French vineyards are illustrated in Figure 4. Strong interconnections among abiotic variables highlight their decisive influence on species distribution and density. These relationships facilitate identification of ecological zones where spontaneous flora abundance may affect vineyard ecosystem functioning.

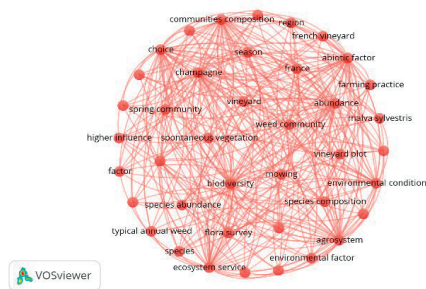


Figure 4. Conceptual representation of factors and interactions within the vineyard ecosystem

The second conceptual model (Figure 5) emphasizes interactions among biodiversity, plant communities, and specific viticultural regions. Close correlations underscore the importance of regional characteristics and management decisions in structuring spontaneous vegetation communities and shaping ecological stability.

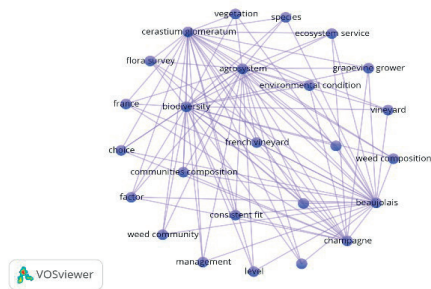


Figure 5. Connections between biodiversity, crop management, and plant communities in French vineyards

Jáuregui et al. (2025) reported that cover crops provide multiple benefits, including improved soil quality, enhanced organic matter content, increased aggregation, better infiltration and water retention, and reduced erosion. They also assist in nutrient cycling and vegetative growth regulation. However, they may compete for water and nutrients, harbor pests, and complicate maintenance procedures. Nevertheless, by occupying ecological niches and competing for resources, cover crops can help regulate spontaneous species and promote ecological balance.

Freund et al. (2013) demonstrated that vegetation diversity between vine rows stimulates arthropod biodiversity, whereas Tesic et al. (2007) highlighted potential competition for water and nutrients under reduced herbicide regimes, emphasizing the importance of balanced vegetation management.

Heavy rainfall can cause soil particle disintegration, erosion, runoff, and excessive vegetative growth. Studies indicate that permanent under-vine vegetation improves soil stability, reduces erosion and nutrient leaching, and enhances soil biological activity, particularly under climate change scenarios (Vanden & Centinari, 2021). While spontaneous flora can prevent erosion and support pest control, it is often removed due to competition for mineral resources. Extensive vegetation management generally favors biodiversity and ecosystem services, although responses depend on species, climate, and soil type (Winter et al., 2018).

Conventional monoculture practices reduce biodiversity and soil fertility, potentially increasing susceptibility to pathogens. Certain perennial plants improve soil structure, diversify microbial communities, and enhance ecosystem functioning (Quintarelli et al., 2022). Results vary: cereals frequently suppress spontaneous flora, while some legumes may stimulate it due to nitrogen enrichment. Reduced light may inhibit germination, whereas increased soil moisture may promote it. Seasonal dynamics and soil operations further influence outcomes (Fernando & Shrestha, 2023).

Mattiello et al. (2023) investigated copper accumulation in five spontaneous species from Mediterranean vineyards across 11 sites. Species such as *Lolium perenne*, *Plantago*

lanceolata, *Rumex obtusifolius*, and *Taraxacum officinale* demonstrated phytoremediation potential, contributing to sustainable vineyard management by reducing soil copper concentrations.

The influence of ground cover depends on climate, soil depth, organic matter, and percentage of coverage. Delpuech and Metay (2018) examined how spontaneous flora affects soil cover, water consumption, vine growth dynamics, and productivity, emphasizing the need for context-specific management to prevent yield losses. Several cover crops tested in vineyards showed positive ecological effects, particularly in tilled soils (Ingels et al., 2005). Although spontaneous flora may reduce vegetative growth and yield, it can enhance biodiversity and soil health; thus, even moderate yield reductions may support long-term sustainability (Karl et al., 2016).

Functional trait-based approaches help explain species responses to agricultural practices and their influence on ecosystem processes, offering valuable tools for diagnosing vegetation community organization and guiding sustainable vineyard management (Kazakou et al., 2016). The climatic and hydric context, including water balance and stress periods, emerges as a defining constraint on vineyard performance, as described by Costea et al. (2023) in his comprehensive analysis of vine water relations

In dry Mediterranean regions, water regulation is essential to prevent excessive vegetative growth and quality decline. Moderate water limitation induced by spontaneous flora may benefit grapevine performance (Tomaz et al., 2017). Soil structure and nutrient availability remain crucial during growth periods (Susaj et al., 2013). Studies in Merlot vineyards showed that spontaneous flora reduced soil moisture but did not significantly affect yield or grape quality, with annual variations largely driven by rainfall rather than soil management (Steenwerth et al., 2016).

A six-year study in central Italy reported that spontaneous vegetation consistently reduced vine vigor and productivity while maintaining must sugar levels. During dry periods, it enhanced polyphenol concentration and berry coloration, accelerated ripening, and reduced disease risk (Palliotti et al., 2005). In Argentina,

traditional agricultural practices support biodiversity and organic matter recycling, although transferring such practices to higher-altitude vineyards with distinct flora may alter ecological balance (Abbona et al., 2007).

Monteiro and Lopes (2007) observed that cover crops in Mediterranean vineyards reduced soil moisture and vine vigor without significantly compromising yield, while contributing to soil conservation and improved growth–fruiting balance. Muscas et al. (2017) further reported that spontaneous flora across vineyard surfaces may reduce vine vigor and influence pest dynamics, potentially supporting more sustainable plantation management.

CONCLUSIONS

The reviewed specialized literature demonstrates that the spontaneous flora in viticultural regions is characterized by high diversity and floristic variability, being directly influenced by climatic factors and vineyard management practices. Its capacity to adapt to local conditions is highlighted by the dynamics and predominance of species, emphasizing the complex nature of these ecosystems.

In a balanced approach, it can be observed that spontaneous flora fulfills a dual role: on one hand, it contributes to the preservation of biodiversity and the maintenance of ecological stability, and on the other hand, it influences competitive relationships for resources with the grapevine.

Overall, spontaneous flora represents an important element for the proper functioning of the viticultural ecosystem and for the development of sustainable viticulture, which manages to harmonize production objectives with environmental protection.

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UPDATE ON WHITEFLIES OF ROMANIA, INCLUDING TWO NEW SPECIES FOR THE ROMANIAN FAUNA (HEMIPTERA: ALEYRODIDAE)

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Abstract

This paper presents the results of whitefly findings in parks and urban areas from Bucharest and Ilfov County between 2024 and 2025. *Aleuroclava aucubae* (Kuwana), native to the East Palearctic is recorded for the first time in Romania. Individuals of the aforementioned species were found on different ornamental plants, including *Celtis occidentalis*, *Cotoneaster* spp., *Kerria japonica*, *Morus alba*, *Morus* spp., *Pyracantha coccinea*, and *Syringa vulgaris*. The other newly found species is *Tetraleurodes hederæ* Goux, a pest that feeds exclusively on *Hedera helix* and has been recorded in Europe, in France, Italy, and Malta. Data are also presented on other native whitefly species observed in the same period on different host plants. This communication contributes to Romania's whitefly fauna and insect biodiversity.

Key words: *Aleuroclava aucubae*, first record, non-native, *Tetraleurodes hederæ*, whiteflies.

INTRODUCTION

Whiteflies (Hemiptera: Sternorrhyncha: Aleyrodidae) are a small group of insects related to the scale insects (Coccoidea) and the aphids (Aphidoidea). The group is one of the economically most important groups of insects found on crops and ornamental plants. The purpose of this study was to record further details of the whitefly fauna of Romania. In the past, Dobreanu and Manolache (1969) researched the Aleyrodidae in Romania, resulting in the first inventory of these species, consisting of sixteen species.

MATERIALS AND METHODS

Faunistic investigations on whitefly species were conducted between 20-25 September 2024 and 20-23 October 2025. Samples were collected by both authors from parks and urban areas located in Bucharest municipality and Ilfov County. The specimens were slide-mounted according to published methods and identified using the diagnostic keys provided by Martin, Mifsud, and Rapisarda (2000) and Dobreanu et al. (1969). Morphological descriptions and illustrations of slide-mounted puparia of all the species listed below are provided by Martin et al. (2000). The nomenclature used follows Martin and Mound (2007).

Slide-mounted specimens have been deposited at Fera Science Ltd (UK) and the National Phytosanitary Laboratory (Romania).

RESULTS

Aleuroclava aucubae (Kuwana) (Fig. 1, 2, 3, 4)



Figure 1. *Aleuroclava aucubae* puparia (Malumphy, 2024)



Figure 2. *Aleuroclava aucubae* puparium (Malumphy, 2024)



Figure 3. Microscopic view of *Aleuroclava aucubae* puparium (Cean, 2025)



Figure 4. Detail of the posterior part of the abdomen of *Aleuroclava aucubae* (Cean, 2025)

Collection data: Bucharest city centre on *Celtis occidentalis*, *Morus alba*, *Syringa vulgaris*, and unidentified shrubs and trees, 20.IX.2024; Grădina Botanică Dimitrie Brândză, on *Pyracantha coccinea*, 21.IX.2024; Parcul Kiseleff, on *Morus* sp., *Pyracantha coccinea*, and unidentified shrubs, 23.IX.2024; Muzeul Național al Satului "Dimitrie Gusti", on *Morus* sp., 23.IX.2024; Parcul Lumea Copiilor, on *P. coccinea*, 25.IX.2024; and Chitila, path along river, on several *P. coccinea*, 24.IX.2024; Chitila path along river, on *Morus* sp., *Prunus* sp., 20.X.2025; Parcul Operei, on *Morus* sp., *Celtis* sp., *Kerria japonica*, 23.X.2025; Muzeul Național al Satului Dimitrie Gusti, on *Morus* sp.,

20.X.2025; Parcul Kiseleff, on *Cotoneaster* sp. and *Syringa vulgaris*, 20.X.2025; Parcul Izvor, on *Morus* sp., 21.X.2025.

Adults, early nymphal instars, and puparia were present in 2024; only puparia were present in 2025.

***Aleurochiton acerinus* Haupt (Figures 5, 6)**

Collection data: Grădina Botanică "Dimitrie Brândză", on *Acer campestre*, 21.IX.2024; Mogoșoaia-Chitila, on *Acer campestre*, 21.IX.2024; Parcul Kiseleff, on *Acer* spp., 23.X.2025.



Figure 5. *Aleurochiton acerinus* summer puparium (microscopic view) (Cean, 2025)



Figure 6. Detail of the posterior part of the abdomen of *Aleurochiton acerinus* (Cean, 2025)

***Aleurochiton aceris* (Modeer) (Figures 7, 8, 9)**

Collection data: Grădina Botanică "Dimitrie Brândză", on *Acer platanoides*, 21.IX.2024; Chitila path along river, on *Acer platanoides*, 21.IX.2024; Parcul Izvor, on *Acer platanoides*, 21.X.2025.



Figure 7. *Aleurochiton aceris* winter puparium (microscopic view) (Cean, 2025)

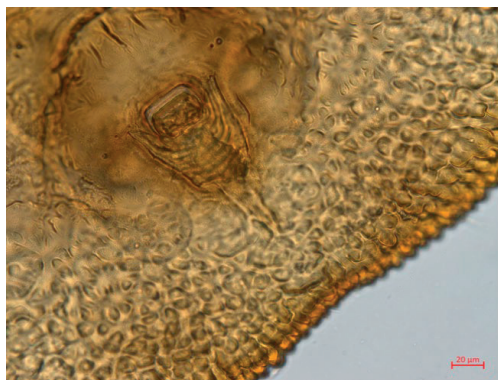


Figure 8. Detail of the posterior part of the abdomen of *Aleurochiton aceris* (Cean, 2025)



Figure 9. *Aleurochiton aceris* winter puparium (Cean, 2025)

***Aleurochiton pseudoplatani*, Visnya**

Collection data: Parcul Operei, on *Acer pseudoplatanus*, 20.IX.2024.

***Aleyrodes proletella* (L.)**

Collection data: Mogoşoaia-Chitila, on *Chelidonium majus*, 24.IX.2024.

***Bemisia tabaci* (Gennadius) (Fig. 10)**

Collection data: Bucharest city centre, on *Ipomea* sp., 22.IX.2024, 25.IX.2024; Parcul Lumea Copiilor, on *Pelargonium* sp., 25.IX.2024; Chitila, on *Vitis* sp., 20.X.2025. Adults and puparia were present in 2024, only puparia in 2025.



Figure 10. Detail of the posterior part of the abdomen of *Bemisia tabaci* (Cean, 2025)

***Siphoninus phillyreae* (Halliday) (Figures 11, 12)**



Figure 11. *Siphoninus phillyreae* puparium (microscopic view) (Cean, 2025)

Collection data: Grădina Botanică Dimitrie Brândză, on *Fraxinus excelsior*, 21.IX.2024;

Mogoșoaia-Chitila forest, on *Crataegus monogyna*, 24.IX.2024; Parcul Național Văcărești, on *Fraxinus angus*, 25.IX.2024; Mogoșoaia on *Crataegus* sp., 20.X.2025.



Figure 12. Detail of the posterior part of the abdomen of *Siphoninus phillyreae* (Cean, 2025)

***Tetraleurodes hederae* Goux (Figure 13)**

Collection data: Mogoșoaia (near station), on *Hedera helix*, 24.IX.2024.



Figure 13. Empty pupal case of *Tetraleurodes hederae* (Malumphy, 2024)

DISCUSSIONS

Aleuroclava aucubae is native to the East Palearctic, including China, Japan, and Taiwan. It has been introduced to the USA (Evans, 2007) and Europe, including Azerbaijan (Gavrilov-Zimin & Borisov, 2020), Croatia (Šimala et al., 2014), France (Streito et al., 2014), Hungary (Hári et al., 2021), Italy (Pellizzari & Šimala, 2007), Montenegro (Malumphy et al., 2015), Russia (Russian Caucasus) (Gavrilov-Zimin & Borisov, 2020), and Slovenia (Seljak, 2012). It is a polyphagous species, being recorded on host

plants in 15 families (Mound & Halsey, 1978). It overwinters in the puparial stage (Seljak, 2012). The puparia usually occur at low densities; they do not egest large quantities of honeydew, and they do not appear to have a limited impact on the host plant.

The species has been recently intercepted in England on many occasions on plants for planting, mostly *Photinia*, imported from Italy. This demonstrates that it is moving in trade, and this is probably how it was introduced to Romania. It is small and occurs on the lower leaf surface; it has a negligible impact and is therefore easily overlooked.

Aleurochiton acerinus

The species is reported by Dobreanu et al. (1969) in the counties of Ilfov, Brăila, Prahova, Caraș-Severin, Brașov. The mentioned host tree is *Acer campestre*. The adults appear in April-May from the overwintered puparia (Dobreanu et al.). There is puparial dimorphism, with puparial characters differing between summer, with a pale derm, and winter, with a sclerotised form (Malumphy). We record here the species in the Bucharest municipality.

Aleurochiton aceris

The species was reported in Romania by Dobreanu et al. (1969) under the name *Aleurochiton complanatus* in the counties of Ilfov, Prahova, Caraș-Severin, Harghita, and Gorj. The mentioned host tree is *Acer platanoides*, and damage is induced more frequently by the autumn generation.

Puparia of the species *A. aceris*, like those of *A. acerinus*, exhibit seasonal dimorphism, with puparial characters differing between summer, with a pale derm, and winter, with a sclerotised form and covered in a dense layer of wax. We record here the species in the Bucharest municipality.

Aleyrodes proletella

The species is native to the Palearctic and is broadly polyphagous, feeding on herbaceous plants in numerous families, showing a preference for Cruciferae and Compositae (Martin et al., 2000). In Romania, the insect was recorded all over the country, especially on *Chelidonium*, *Brassica* and *Euphorbia* genera (Dobreanu, 1969). It can be a pest of different varieties of cabbage, such as cauliflower,

cabbage, kale and Brussels sprouts, in both private and commercial crops (Jansen, 2011).

Bemisia tabaci

The species is regulated as a quarantine species, and it is mandatory to eradicate populations originating from non-European countries because of the risk of introduction of plant virus diseases, which can be transmitted by the silverleaf whitefly, although the population without a direct link to imports are considered to be native (Jansen, 2011). It is broadly polyphagous, feeding on plants belonging to more than 80 plant families.

Siphoninus phillyreae

This species occurs in the Nearctic, Neotropical, Western Palearctic, Afrotropical, Eastern Palearctic, and Australasian regions. It is polyphagous on woody host plants. It was recorded from Romania by Dobreanu (1969) in Ilfov, Caraș-Severin, Prahova, Brașov, Constanța counties. It is frequently found on *Crataegus* and *Fraxinus*, and it is of minor economic importance.

Tetraleurodes hederæ

A rarely recorded European species found in France, Italy, and Malta that feeds exclusively on *Hedera helix*. The finding in Romania is a significant increase in its geographical range. The puparia are highly conspicuous due to the white wax, so it is surprising that there are so few records when the host plant is so common and widespread.

CONCLUSIONS

The investigations carried out only outdoors revealed 8 whitefly species from 6 different genera. Two species are recorded for the first time from Romania, namely *Aleuroclava aucubae* and *Tetraleurodes hederæ*. This study contributed to updating the Romanian whitefly fauna and insect biodiversity.

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CURRENT STAGE AND FUTURE PERSPECTIVES OF *EX SITU* PLANT GERMPLASM CONSERVATION IN ROMANIA

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Abstract

Human-induced pressure significantly accelerates the species extinction rate. It is estimated that 27% of European plant species are threatened with extinction. This highlights the urgent need for the conservation of plant genetic resources, which can be achieved through in situ and ex situ methods. Although in situ conservation is preferred, it's not always feasible due to habitat destruction or other environmental or anthropic stresses. Ex situ conservation methods, including germplasm collections in gene banks, are essential for preserving plant genetic diversity. Romania's ex situ conservation efforts are conducted mainly for crop plants and are concentrated in only two gene banks. For wild flora, ex situ conservation is limited to only a few botanical gardens and research institutes. This work aims to assess the current state of ex situ plant conservation in Romania and discusses the opportunities and advantages associated with establishing a National Germplasm Bank for both wild and cultivated species.

Key words: anthropic pressure, ex situ conservation, gene banks, germplasm collections.

INTRODUCTION

It is estimated that the current human-induced extinction rate of species is up to 700 times higher than the natural rate (Christenhusz & Govaerts, 2025). Accordingly, recent reports have shown that 19.4% of assessed European species are threatened with extinction, and 27% of European plant species are threatened (Hochkirch et al., 2023).

With the increased risk of species loss, conservation of plant genetic resources becomes a major global challenge. In general, biodiversity loss is minimized by *in situ* and *ex situ* conservation. *In situ* conservation refers to the protection and efficient management of species populations in their natural habitats. Efficient management results in long-term preservation of species' genetic diversity. Under major stress caused by habitat destruction (mainly due to the conversion of natural habitat into farmland), invasive species, pollution, etc., *in situ* conservation, which should be the first choice, is not always possible.

A valuable alternative is the conservation of plant genetic resources outside their natural habitat through *ex situ* conservation. Through *ex situ* conservation, species could be preserved away from the major threats that affect their

natural habitats. The techniques and methods of *ex situ* conservation allow species preservation by translocation in botanical gardens or arboreta, or by maintaining them in germplasm collections as seeds and/or living collections (e.g., vitrocollections). A germplasm collection is defined as a collection of genetic material that captures a maximum genetic variability within a species. Usually, a germplasm collection for plant species consists of a collection of seeds, different types of propagules, cells, and tissues. Gene banks store germplasm collections under specific conditions for long-term conservation and sustainable use of genetic resources.

Gene banks were established all over the world, mainly for crop plants and their wild relatives, to maintain the genetic basis needed for breeding. For wild plants, the main purpose of gene bank establishment is to reduce species extinction risk. Until now, more than 354,200 of the world's wild plant species are preserved in gene banks established in botanic gardens (BGCI, 2025).

In Romania, *ex situ* conservation of plant genetic resources is limited to a few botanical gardens, research institutes and only two gene banks. In this context, the aim of the present work was to gather, summarise, and analyse information concerning the current stage of *ex situ*

conservation of plant genetic resources in Romania. Moreover, in light of EU environmental policies, the opportunity of establishing a National Germplasm Bank for both cultivated and wild species is discussed.

MATERIALS AND METHODS

The main research method was to collect and synthesise the available data from publicly available digitised materials, scientific publications, and direct material from the institutions involved in plant conservation. To review the main literature in the field of *ex situ* plant conservation in Romania, systematic research was carried out using the Web of Science and Google Scholar engines. In order to gain updated and reliable data, the main national structures involved in *ex situ* plant conservation efforts were identified from accessible public resources, contacted, and asked for specific information. In addition, a comprehensive screening of available institutional reports, gene bank databases, and *Index Seminum* catalogues was undertaken.

RESULTS AND DISCUSSIONS

Current status of ex situ conservation in Romania

In order to evaluate the *ex situ* conservation status of plant germplasm in Romania, we focused on seed and *in vitro* collections maintained in seed banks, botanical gardens, and research institutes (Tables 1 and 2):

- **Gene banks** - according to publicly available data, there are only two in Romania:
 1. *Vegetal Genetic Resources Bank “Mihai Cristea” Suceava* holds an important collection of spontaneous flora and wild relatives of cultivated plants in Romania, preserved as seeds or propagules under medium (+4°C) or long-term (-20°C) storage conditions, and *in vitro* collections, totaling 428 species. The medium-term collection includes 844 samples of 186 species from 131 countries, while the long-term collection is smaller, including selected genotypes totaling 36 samples representing 14 species from four countries. Specifically, for

Romanian spontaneous flora, there are 627 samples belonging to 141 species maintained mainly in medium-term storage, and only 28 samples from 11 species are preserved in long-term storage. Wild relatives of cultivated plants (CWR) are also conserved, totaling 295 samples of 45 species stored medium-term, and only 36 samples from 14 species are stored long-term. Notable genera include *Amaranthus*, *Dactylis*, *Avena*, and *Aegilops*. An extensive study on CWR was conducted in 2021 by Şandru, presenting a checklist of 525 wild plant species native to Romania, from which a National Priority List of 275 species was compiled, with ca. 10% preserved in the Suceava Gene Bank. These collections are vital for conservation and research purposes, ensuring the preservation of plant biodiversity from Romania and beyond.

In conclusion, from a total of 18,883 accessions, only 3.32% are wild species and 1.56% are CWR, the main collection representing crops (95.12%).

2. *The Plant Genetic Resources Bank for Vegetable, Floriculture, Aromatic and Medicinal Plants Buzău* has 296 registrations in its collections, mostly of crops.

• **Botanical Gardens:**

1. “*Dimitrie Brandza*” *Botanical Garden, Bucharest*, maintains a seed collection (890 taxa; *Delectus seminum*, 2025) of spontaneous and cultivated species in the botanical garden or collected from other areas of the country, at room temperature, for short-term storage of about 2-3 years.
2. “*A. Borza*” *Botanical Garden, Babeş-Bolyai University, Cluj-Napoca*, holds seeds from 879 taxa collected from plants cultivated in the botanical garden and from 53 taxa collected from the spontaneous flora (*Delectus Seminum*, 2019).
3. *The Botanical Garden of the University of Agricultural Sciences and Veterinary Medicine (USAMV), Cluj-Napoca*, has a seed collection for annual exchanges with other botanical gardens. For 2025, there are 879 accessions, all with international IPEN codes and all registered in Botanic Gardens Conservation International (BGCI)'s Plant

Search database. Some samples originate from plants collected from their natural habitats.

4. “Alexandru Buia” Botanical Garden, Craiova, maintains in its collections spores and seeds from 497 taxa of cultivated plants in the botanical garden and seeds from 45 taxa collected from various Romanian regions, totaling 542 taxa, including species, subspecies, and varieties (*Index Seminum*, 2023).
5. “Anastasiu Fătu” Botanical Garden, Iași (BGI), is a member of the Association of Botanical Gardens of Romania (AGBR). The collection comprises seeds from 1281 taxa of cultivated plants in the Botanical Garden, 91 taxa from spontaneous plants within the garden, 771 taxa from various Romanian regions, and 18 taxa from global locations – all collected in 2025 – totaling 2,161 taxa (*Index Seminum* of the “Anastasiu Fătu” Botanical Garden, Iași, 2025). The collection is maintained short-term and is intended for exchange and research. Within the international seed exchange, BGI sends ca. 3,500 seed samples yearly to more than 80 countries.
6. Botanical Garden “Vasile Fati”, Jibou, (Sălaj), is a member of BGCI and the AGBR. The seed collections are kept for at most four years, which is the good viability period for most seeds, but updated and replaced each year for the international seed exchange with other botanical gardens. The seed catalogue for the year 2025 (No. 51) contains 1,646 taxa. The “cultivated spontaneous plants” in the botanical garden represent 881 species and are part of the spontaneous flora of Romania. The “species from the spontaneous flora of the garden” number 718 species and are also considered part of the garden's collections, but they reproduce on their own, and most are remnants of the indigenous flora of the land where the botanical garden is currently located (forest flora, meadows, and valleys near watercourses). The *in vitro* collections currently held include the species *Stevia rebaudiana*, *Pinguicula gigantea*, *Vanilla planifolia*, and *Cymbidium* sp. A detailed analysis of the species from this botanical garden was conducted by Szatmari & Căprar (2015).

7. Botanical Garden, Galați, maintains in its collection the seeds harvested from cultivated plants in the botanical garden and from those growing spontaneously in the garden area, as well as from spontaneous species in various counties of Romania (Galați, Tulcea, and Constanța), totaling 819 taxa (*Index Seminum* of the Botanical Garden, Galați, 2025).
8. Macea Botanical Garden is affiliated with “Vasile Goldiș” University, Arad, a member of AGBR, and cultivates over 2,000 tree and shrub species and ca. 500 herbaceous species.

In addition, botanical gardens make a significant contribution to *ex situ* wild plant conservation through the establishment of outdoor collections (living collections) of endangered plant species. Recent estimates showed that a number of 524 native endangered taxa are grown in botanical gardens across Romania, but the total number of threatened and (sub)endemic taxa from the Romanian flora held by botanical gardens nationwide is significantly higher, totaling 925 taxa (Hurdu et al., 2022). Thus, to date, botanical gardens are the most important structures that contribute to *ex situ* conservation of wild Romanian flora.

• Research Institutes:

1. Institute of Biology Bucharest of the Romanian Academy (IBB) advanced *in vitro* plant conservation and biotechnology research through four stages between 1975 and 2005, evolving from fundamental studies on cytodifferentiation, protoplasts, and genetic transfer to applications in clonal propagation, stress tolerance, and secondary metabolite production (Brezeanu & Cogălniceanu, 2016). Currently, IBB focuses on *ex situ* conservation of endangered, medicinal, or economically important phytotaxons.

Through the PeatRO Projects (PeatRO2, PeatRO3, and PeatRO4), IBB took its first steps toward establishing a National Germplasm Bank by including 29 peatlands' plant species in seed collections (Figure 1) and 14 species in *in vitro* collections (Figure 2) – a total of 32 different species conserved *via* both techniques.

Currently, IBB maintains *in vitro* collections of 21 vascular plant species from Romania's spontaneous flora, 1 bryophyte species (25

species were maintained for varying periods between 2005 and 2010, but only six remained by 2013; Cogălniceanu, 2014), and 5 pteridophyte species, totaling 27 plant species. Over time, regeneration and maintenance protocols have been developed for many additional species, comprising a valuable knowledge database for future conservation efforts.

2. *Institute of Biological Research Cluj-Napoca* developed *in vitro* protocols for both wild and cultivated species, totaling ca. 24 species. Seed collection was established as a common effort with the “Alexandru Borza” Botanic Garden (Cluj-Napoca) and is focused on the wild, threatened and (sub)endemic flora of Romania, totaling 321 samples from 285 taxa (Hurdu et al., 2022).
3. *National Research and Development Institute (NARDI) Fundulea*, currently maintains in the active collection 17 wild species of the genus *Helianthus*.
4. *Vegetable Research and Development Station Buzău* (SCDL Buzău) holds a germplasm collection with ca. 103 species and 2,641 accessions - crops and wild relatives of crops (CWR).
5. *The State Institute for Testing and Registration of Varieties (ISTIS)* holds seeds for 11,717 varieties from 134 species - only from crop plants.
6. *National Institute of Research and Development for Potato and Sugar Beet Braşov* (NIRDPSB) conserves, within the Laboratory for Plant Tissue Culture Research, an *in vitro* germplasm collection that includes both Romanian (32) and foreign potato varieties (25), as well as wild species (2). Additionally, alongside potatoes, there are five sweet potato genotypes.

- **Associations:**

1. *EcoRuralis* - the database initiated in 2014, with entries made until March 2023, has a total of 841 entries, from ca. 100 species - crops and crop wild relatives (CWR). Currently, the seeds are stored in “seed

houses” from 14 counties, dedicated more to a seed system that encourages seed circulation and production.

2. *The Community Seed Bank “Seed House”* - developed with mentoring and support from the International Association of Seed Savers (Austria) - is dedicated to saving, preserving, and multiplying the old, traditional, and rare Romanian seeds. Currently holds in its collection ca. 500 samples from over 250 varieties of legumes, ornamental, and medicinal plants.

Another valuable means of *ex situ* conservation is the establishment of *in vitro* collections. It was estimated that a total number of 64 native, endangered species are maintained in *in vitro* collections of the Institute of Biology Bucharest, Alexandru Borza Botanic Garden, and Institute of Biological Research Cluj-Napoca (Hurdu et al., 2022).

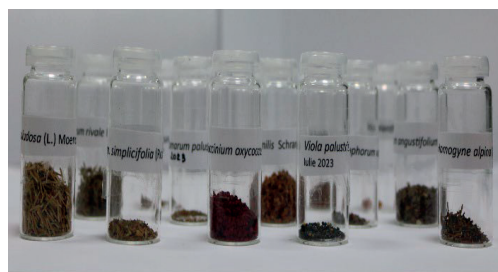


Figure 1. Seed collections of wild flora maintained at the Institute of Biology Bucharest of the Romanian Academy (Photo by Anca Manole)



Figure 2. *In vitro* collections of wild flora maintained at the Institute of Biology Bucharest of the Romanian Academy (Photo by Anca Manole)

Table 1*. Relevant national institutions housing seed collections

No.	Institution name	Number of accessions / species / taxa	Wild species	Crops	Crop wild relatives (CWR)	Link
Gene Banks						
1.	<i>Vegetal Genetic Resources Bank "Mihai Cristea" Suceava – BRGVSV</i>	18,883 accessions 428 species	627 accessions 141 species from Romania	x	295 accessions 45 species 21 countries	https://svgenebank.ro/
2.	<i>The Plant Genetic Resources Bank for Vegetable, Floriculture, Aromatic and Medicinal Plants [BRGV] Buzău</i>	296 species	-	x	x	https://brgv.ro/
Botanical Gardens						
1.	<i>"Dimitrie Brandza" Botanical Garden, Bucharest</i>	890 species	92 species collected from wild flora	-	x	https://gradina-botanica.unibuc.ro/
2.	<i>"A. Borza" Botanical Garden, Babeş-Bolyai University, Cluj</i>	879 species (2019)	53 species collected from wild flora	x	x	https://gradinabotanica.ubbcluj.ro/
3.	<i>The Agro-Botanical Garden of the University of Agricultural Sciences and Veterinary Medicine (USAMV) Cluj-Napoca</i>	879 accessions	X	x	x	https://www.usamvcluj.ro/universitatea/gradina-botanica/
4.	<i>"Alexandru Buiu" Botanical Garden, University of Craiova</i>	542 species (2023)	ca. 45 species collected from wild flora	x	x	https://www.ucv.ro/campus/puncte_de_atractie/gradina_botanica/prezentare.php
5.	<i>"Anastase Fătu" Botanical Garden, Iaşi</i>	2,161 species	ca. 900 species originate from wild flora	-	x	https://www.botanica.uaic.ro/home.html
6.	<i>"Vasile Fati" Botanical Garden, Jibou</i>	1,646 species		-	-	https://cjbj.ro/
7.	<i>Botanical Garden, Galaţi</i>	819 species	ca. 120 species collected from wild flora	-	x	https://cmsngl.ro/
Other Institutions						
Research Institutes						
1.	<i>Institute of Biology Bucharest of the Romanian Academy</i>	27 species	x	x	-	https://ibiol.ro/
2.	<i>National Agricultural Research and Development Institute (NARDI) Fundulea</i>	17 wild species of the genus <i>Helianthus</i>	-	x	x	https://incda-fundulea.ro/index_en.html
3.	<i>Vegetable Research and Development Station Buzău SCDL</i>	ca. 103 species 2,641 accessions	-	x	x	https://scdlbuzau.ro/en/home
4.	<i>The State Institute for Testing and Registration of Varieties (I.S.T.I.S.)</i>	11,717 varieties from 134 species	-	x	-	https://istis.ro/
5.	<i>National Institute of Research and Development for Potato and Sugar Beet Braşov (NIRDPSB)</i>	ca. 59 accessions	x	-	x	https://potato.ro/en/
Universities						
1.	<i>University of Life Sciences "King Mihai I" from Timisoara</i>	166 species (Crop production)	-	x	x	https://www.usab-tm.ro/en
Associations						
1.	<i>"Eco Ruralis" Association</i>	841 accessions from ca. 100 species	-	x	x	https://www.ecoruralis.ro/despre-noi/asociatia/
2.	<i>The Community Seed Bank "Seed House"</i>	over 500 accessions from 250 varieties	-	x	-	https://www.casa-semintelor.ro/en/

Table 2*. Summary table of national institutions housing seed collections

Institution	Accessions / species (approximately)	~% wild species	Main focus
Gene banks	19,179	<5%	Crops + CWR
Botanical Gardens	7,816	<20%	Ornamental species
Other institutions	15,941	<5%	Crops

*Data limitations: Data relies on accessible public sources - institutional reports, gene bank databases, Index Seminum catalogues and data provided by collection holders; unreported holdings may exist.

Grounds for Romanian Flora Conservation

Romania is noted for its rich biodiversity, particularly in the Carpathian Mountains and the Danube Delta. For example, the Carpathians are considered the northernmost diversity hotspot in temperate Europe, featuring rich biodiversity and a significant percentage of endemic phytotaxa (Figure 3) (Breman et al., 2020). Unfortunately, biodiversity hotspots correlate with high extinction rates (Le Roux et al., 2019).

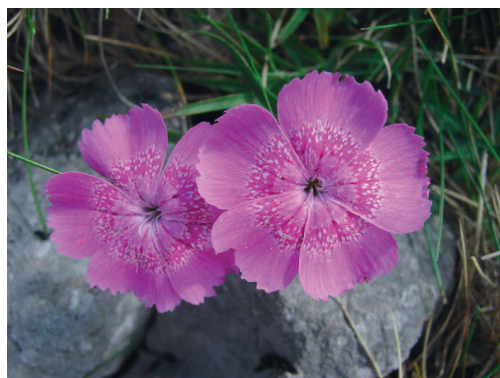


Figure 3. *Dianthus callizonus* Schott & Kotschy, an emblematic endemic species for Romanian Flora (Photo by Anca Manole)

This is documented by the available national red lists of wild plant species, which include more than one-third of Romanian vascular plants. For example, in the most cited national red list of vascular plants, published more than 30 years ago (Oltean et al., 1994), 1,235 species out of a total of 3,795 of Romanian flora were included in different endangered categories (Dihoru & Negrean, 2009). The current number of plant species included in national Red Lists is significantly higher, being estimated at 1,453

threatened and (sub)endemic plant taxa, representing almost 38% of the total native vascular flora of Romania (Hurdu et al., 2022).

Future perspectives to strengthen and integrate national *ex situ* conservation actions

While Romanian gene banks preserve germplasm under medium (+4°C) and long-term (-20°C) storage conditions, most botanical gardens store short-term collections, underscoring the need for a centralised conservation unit and facilities for long-term seed storage. Moreover, the main institutions involved in *ex situ* plant conservation, with the exception of botanical gardens and a few research institutes, prioritise crops, leaving Romania's rich wild flora under-preserved. Taking into account the current stage of plant conservation in Romania, a necessary requirement to integrate and strengthen the national efforts for *ex situ* conservation of plant genetic resources is to establish a National Germplasm Bank. The advantages of establishing a National Germplasm Bank include:

- filling the gap of wild flora conservation by establishing a comprehensive *ex situ* collection of spontaneous species;
- facilitate the implementation of national and international environmental policies (e.g., the EU's Biodiversity Strategy for 2030, a key component of the EU Green Deal);
- provide a unitary framework for *ex situ* plant conservation;
- ease the integration in international conservation programs and networks;
- ensure a national reserve of plant genetic resources as a quadrant for sustainable development.

CONCLUSIONS

Ex situ plant germplasm conservation in Romania is predominantly crop-orientated, totalling 42,936 accessions. From these, approximately half is concentrated in the *Vegetal Genetic Resources Bank "Mihai Cristea" Suceava*. *Ex situ* conservation of wild flora germplasm in Romania is scarce and represents less than 10% of the total number of preserved species and less than 15% of the native wild species. Moreover, the existing *ex*

situ seed collections, with very few exceptions, are for short-term storage and are established mainly for seed exchange (botanical gardens) or improvement (National Institutes). Establishment of the National Germplasm Bank is a reliable means to integrate and centralize conservation efforts to preserve plant diversity from Romania and will ensure long-term preservation and sustainable use of native plant germplasm.

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MORPHOLOGICAL ANALYSIS OF DAFFODIL POPULATION FROM THE NATURAL RESERVE “FÂNATELE CU NARCISE NUCȘOARA – 2.506”

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Abstract

In Romania, there are over 20 places with wild daffodils (*Narcissus poeticus* subsp. *radiiflorus* (Salisb.) Baker), most being part of the Natura 2000 network. This paper presents morphological data on daffodils from Nucșoara, a botanical reserve within the community importance site Strei-Hațeg (ROSCI0236). The daffodil population is considered a post-glacial relict, coexisting with other remarkable species, such as *Peucedanum rochelianum* Heuff. Biometric measurements indicated an aerial stem height ranging from 14 to 36.5 cm and a flower diameter between 1.9 and 5 cm. Statistical analysis (Pearson's R) revealed a weak correlation between the perigon and the coronule diameter. Compared with other populations in Romania, the Nucșoara daffodils display distinct morphological traits: the plants are smaller, and the length of the leaves and the diameter of the flower are also smaller. These variations may be attributed to the population's isolation and specific local environmental conditions, highlighting the scientific importance of this reserve.

Key words: botanical reserve, Nucșoara, *Narcissus poeticus* subsp. *radiiflorus* (Salisb.) Baker, biometric measurements, morphological traits.

INTRODUCTION

In Romania, significant natural heritage resources are distributed across a continuum of scales, from large-scale biosphere reserves to fragmented local ecosystems. Western Romania is characterized by a pronounced diversity of habitats and a high degree of taxonomic variety. (Coste & Arsene, 2000; Arsene et al., 2005, 2006, 2015; Turcean et al., 2014; Bătea et al., 2014; Neacșu et al., 2023 etc.).

By implementing the European Network NATURA 2000 in Romania, the prevention, conservation, preservation, or rehabilitation of important species and habitats is pursued, and the status of natural areas is regulated. In this context, our study provides information about a small protected area that preserves a valuable population of wild daffodils.

This is the Nucșoara Daffodil Meadows Nature Reserve (“Fânatele cu narcise Nucșoara” – national code 2.506), which is located at the foot of the Retezat Mountains, in the Nucșoara river meadow, between the villages of Sălașu de Sus-Mălăiești-Nucșoara, Hunedoara County, and which extends over approximately 20 ha (Figures 1, 2). There are over 20 glades, meadows, and hayfields with wild daffodils in

Romania. By Law No. 5/2000, the following 17 natural reserves with wild daffodils are declared: The Negrileasa daffodil meadow, Bucium commune, 5 ha, Alba County; The Tecșești daffodil meadows, Întregalde commune, Tecșești village, 2 ha, Alba County; Rovina daffodil meadow, Ineu town, 0.10 ha, Arad County; Negrăși daffodil meadow, Negrăși commune, 4.10 ha, Argeș County; Oșorhei daffodil forest, Oșorhei commune, 2 ha, Bihor County; Goroniște daffodil meadow, Tinca commune, 1 ha, Bihor County; Mogoșenilor plain daffodil meadow, Nimigea commune, 6 ha, Bistrița-Năsăud County; Saca massif daffodil meadow Rodna, Valea Vinului village, 5 ha, Bistrița-Năsăud County; Daffodil meadow on the Buda Valley Plain, Cetate commune, Orheiu Bistriței village, 6 ha, Bistrița-Năsăud County; Daffodil meadows in Dumbrava Vadului, Șercaia commune, Vad village, 394.9 ha, Brașov County; Zervești daffodil meadow, Turnu Ruieni commune, 40 ha, Caraș-Severin County; Daffodil meadow from Vlăhița, Vlăhița town, 20 ha, Harghita County; Nucșoara daffodil meadows, Sălașu de Sus commune, 20 ha, Hunedoara County; Tomnatec-Sehleanu daffodil meadow, Repedea commune, 100 ha, Maramureș County; Gurghiu daffodil meadow,

Gurghiu commune, 3 ha, Mureş County; Racâş-Hida daffodil meadow, Hida commune, Racâş village, 1.5 ha, Sălaj County; Daffodil meadow Bateşti, Făget Town, 20 ha, Timiş County. Nuşoara Daffodil Meadow 2.506 is part of the ROSCI0236 Strei-Haşeg site of community importance and is a botanical reserve, IUCN category IV. The reserve represents a vestige of ancient hydrophilic associations with the endemic *Peucedanum rochelianum*. In addition to daffodils, other important species grow here, such as *Iris sibirica*, *Peucedanum rochelianum*, *Gladiolus imbricatus*, and wild orchid species: *Anacamptis morio*, *Dactylorhiza incarnata*, *Dactylorhiza sambucina*.



Figure 1. Location of the Narcissus Meadows Nature Reserve in Nuşoara (<https://maps.google.com/>)

The provenance of the daffodil population is sometimes contested. Certain local accounts dispute its natural and relict character, positing instead that the population was initially established by a Dutch national. For over 20 years, the local administration has organized an annual festival dedicated to these plants. However, numerically, the daffodil population is in decline. Daffodils are ornamental plants, commonly grown in parks and gardens, as well as for cut flowers. Most species are toxic because they contain the alkaloid narcissine; their toxic and narcotic properties have been known since antiquity. It is believed that the etymology of daffodils starts from the Greek *narkissos*, the name of the plant in Homer and other ancient authors, or from the *narkao*, which means to

produce drowsiness, alluding to the intoxicating scent of the flowers or their narcotic properties. According to other versions, daffodils appeared on the tomb of Narcis, known in mythology as the young man in love with his own face, a personality disorder characterized by excessive self-admiration.

In religious symbolism, daffodils have a profound meaning. It is said that daffodils were the first flowers to bloom in the Garden of Gethsemane when Jesus was resurrected, symbolizing purity and rebirth through their presence, and the bowed stem signifying deep gratitude for the Savior's suffering.



Figure 2. The boundaries of the Narcissus Meadows Reserve in Nuşoara (Google Earth image)

The genus *Narcissus* includes about 40 species spread mainly in the Mediterranean region, a few in the Caucasus and in Asia, up to China and Japan.

Table 1. Variability of *Narcissus*

R.S.R. flora (1966)	Ciocărlan, 2009	Sârbu, 2013	Plants of the World Online *
9 species	8 species 2 subspecies	1 species	76 Accepted Species (37 Synonyms)

In the old flora (R.S.R. Flora, volume XI) 9 species are described: **Narcissus bulbocodium*, **Narcissus pseudonarcissus*, **Narcissus incomparabilis*, **Narcissus odoratus*, **Narcissus tazetta*, **Narcissus jonquilla*, **Narcissus biflorus*, *Narcissus poeticus*, *Narcissus angustifolius*. Of these, Ciocărlan (2009) considers only 8 species: **N. jonquilla*, *N. poeticus* (with subspecies *poeticus* and *radiiflorus*), **N. x medioluteus*, **N. tazetta*, **N. bulbocodium*, **N. pseudonarcissus*, **N. x incomparabilis*, **N. odoratus*.

Comparing the 2 references, we found that Ciocărlan mentions **Narcissus bulbocodium*, **Narcissus pseudonarcissus*, **Narcissus*

incomparabilis, **Narcissus odorus*, **Narcissus tazetta*, **Narcissus jonquilla*, *Narcissus poeticus* and considers synonymous **Narcissus biflorus* for **N. x medioluteus* and *Narcissus angustifolius*, for *N. poeticus* subsp. *radiiflorus*. On the other hand, he presents the hybrid **N. x medioluteus*. Another important reference - Sârbu et al. (2013) - it only points to *N. poeticus* subsp. *radiiflorus*, the only spontaneous species in our country (Table 1). Digital database Plants of the World Online (POWO) (<https://powo.science.kew.org/results?q=narcissus>) accepts 76 species.

The daffodils from the Nucșoara meadow are mentioned in most information materials under the name *Narcissus stellaris*, but now this is a synonym and the valid name is *Narcissus poeticus* subsp. *radiiflorus* (Salisb.) Baker.

Narcissus poeticus L. (wild daffodils, coprines) belongs to the *Amaryllidaceae* family and is a 25-60 cm geophyte, with an ovoid-spherical bulb covered in blackish tunics. The leaves are glaucous, flat, linear, obtuse or sub-obtuse at the tip, about 30 cm long, 5-10 mm wide, more or less keeled. The aerial stem is compressed, acute, thin, 20-40 (60) cm. The pedicels of the flowers are 15-18 mm. The flowers are white, fragrant, and solitary, rarely 2-3 (Figure 3), sometimes extended; white at first, often greenish or yellowish, large, 50-65 mm in diameter.

Below the flower, there is a membranous spathe/bract. The perigone tube is 20-30 mm, with 6 tepals expanded into a star, elliptical, oblong-elliptic, or obovate, narrowed cuneately at the base, rounded or acuminate at the apex, with non-overlapping edges. The coronule is campanulate, very short, 2-4 mm high, yellowish, with a reddish, slightly crenulated edge. $2n=14$. The currently used synonyms are: *Narcissus radiiflorus* Salisb., *Narcissus angustifolius* Curtis ex Haw., *Narcissus poeticus* L. subsp. *angustifolius* Hegi. Reference Handb. Amarull. 12 (1888). *Narcissus poeticus* has 2 subspecies, *radiiflorus* and *poeticus*. The distinction between the subspecies is made at the level of the tepals / perigonal lacinia: the subspecies *radiiflorus* has non-overlapping perigonal lacinia of obovate-cuneate shape (Figure 3), and the subspecies *poeticus* has overlapping lacinia of obovate-elliptic shape. Wild daffodils are of European origin, occurring

in the Alps, Jura, Tyrol, Carpathians, Istra, the former Yugoslavia, and Northern Greece. According to Flora Europea, the species is endemic to southern and central Europe and the western Pannonian Peninsula and prefers habitats such as meadows, glades, and orchards in low and humid places, from the mountainous region to the subalpine. The subspecies *radiiflorus* is found in moist, slightly to moderately acidic meadows. From the point of view of autecological factors, it is a mesophilic species (U=3), mesothermic to moderately thermophilic (T=3.5) and amphotolerant to soil pH (R=0).



Figure 3. *Narcissus poeticus* L. subsp. *radiiflorus* (Salisb) Baker (original 2024, 2025)

The plant associations in which wild daffodils grow belong to the order *Molinietalia caeruleae* Koch 1926 (Sanda et al., 2003).

MATERIALS AND METHODS

During 2021-2025, we conducted observations on the daffodil population in Nucșoara. We noted the accompanying species, and last year (2025), during the anthesis period, we performed morphological measurements. We identified the species using current plant identification guides and floras. For measurements, we crossed the reserve diagonally, considering a horizontal transect; from the starting point to the end of the transect, we established 10 observation points. Every 10 m, we analysed the group of plants. We counted the individuals in each group, noted the flowering specimens, and measured the height of the aerial stem, the number of leaves, the length and width of the leaves, and the diameter of the flower and coronule. To measure the

length, width, and height, we used a ruler and a tape; to estimate the diameter of the flowers, we also used a disk made of paper.

RESULTS AND DISCUSSIONS

Floristic ambience. The daffodils from Nucșoara (Figure 4) grow in a meadow, edified by red fescue (*Festuca rubra*) and colonial bentgrass (*Agrostis capillaris*), *Festuco rubrae-Agrostetum capillaris* association. In this meadow, we noted the following 55 species: *Achillea millefolium* L.; *Agrostis capillaris* L.; *Alchemilla vulgaris* L.; *Anacamptis morio* (L.) R.M. Bateman, Pridgeon & M.W.Chase; *Anthoxanthum odoratum* L.; *Betonica officinalis* L.; *Betula pendula* Roth.; *Carex hirta* L.; *Carex pallescens* L.; *Centaurea kerneriana* subsp. *kerneriana* (*Centaurea nervosa* Griseb.); *Colchicum autumnale* L.; *Dactylorhiza incarnata* (L.) Soó; *Dactylorhiza sambucina* (L.) Soó; *Deschampsia cespitosa* subsp. *cespitosa* (*Deschampsia caespitosa* P.Beauv.); *Dryopteris filix-mas* (L.) Schott; *Erica spiculifolia* Salisb. (*Bruckenthalia spiculifolia* (Salisb.) Rchb.); *Erigeron annuus* (L.) Desf.; *Eriophorum vaginatum* subsp. *fauriei* (E.G. Camus) Á. Löve & D. Löve (*Eriophorum vaginatum* L.); *Euphorbia amygdaloides* L.; *Festuca rubra* L.; *Fragaria vesca* L.; *Fragaria viridis* Weston; *Galium verum* L.; *Genista sagittalis* L.; *Genista tinctoria* L.; *Gentiana pneumonanthe* L.; *Gladiolus imbricatus* L.; *Iris sibirica* L.; *Juncus effusus* L.; *Lotus corniculatus* L.; *Luzula campestris* (L.) DC.; *Luzula luzuloides* subsp. *luzuloides* (*Luzula albida* (Hoffm.) DC.); *Narcissus poeticus* subsp. *radiiflorus* (Salisb.) Baker; *Ononis pusilla* L.; *Peucedanum rochelium* Heuff.; *Pilosella officinarum* F.W. Schultz & Sch. Bip. (*Hieracium pilosella* L.); *Poa pratensis* L.; *Polygala amarella* Crantz; *Potentilla recta* subsp. *recta* (*Potentilla erecta* Maiden); *Pulicaria dysenterica* (L.) Bernh.; *Ranunculus bulbosus* L.; *Rosa canina* L.; *Rumex acetosa* L.; *Scabiosa ochroleuca* L.; *Serratula tinctoria* L.; *Stellaria palustris* Ehrh. ex Hoffm.; *Taraxacum* sect. *Taraxacum* F.H. Wigg. (*Taraxacum officinale* F.H.Wigg.); *Trifolium pratense* L.; *Trifolium repens* L.; *Vaccinium myrtillus* L.; *Verbascum phlomoides* L.; *Verbascum nigrum*

L.; *Veronica chamaedrys* L.; *Vicia pannonica* Crantz; *Viola canina* L.

In floristic studies from Țara Hațegului, we found that in the swampy meadows of Sălașu de Sus, Mălăiești and Pui (localities near the daffodil reserve), Boșcaiu et al. (1965) identified 93 species. Of these, we found inside the reserve: *Anthoxanthum odoratum*, *Betonica officinalis*, *Carex pallescens*, *Galium verum*, *Genista sagittalis*, *Genista tinctoria*, *Gentiana pneumonanthe*, *Gladiolus imbricatus*, *Lotus corniculatus*, *Luzula campestris*, *Peucedanum rochelium*, *Rumex acetosa*, *Serratula tinctoria*, *Scabiosa ochroleuca*, *Viola canina*.

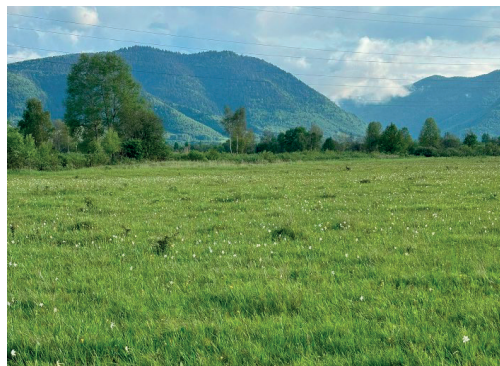


Figure 4. Overview of the daffodil population in Nucșoara (original 2024)

Considering the short distance between the localities and the specifics of the soil (swampy meadows), from Boșcaiu's list of species, we consider many to be likely in the Nucșoara meadow and therefore list them below. These are: *Achillea ptarmica*, *Anthericum ramosum*, *Anthyllis vulneraria*, *Asperula cynanchica*, *Cerastium triviale*, *Chrysanthemum leucanthemum*, *Cirisium rivulare*, *Cirsium canum*, *Lathyrus pratensis*, *Leontodon hispidus*, *Linum catharticum*, *Lysimachia vulgaris*, *Lythrum salicaria*, *Molinia caerulea*, *Myosotis palustris*, *Nardus stricta*, *Oenanthe peucedanifolia*, *Orchis coriophora*, *Orchis elegans*, *Orchis morio*, *Orchis ustulata*, *Ornithogalum flavescens*, *Peucedanum cervaria*, *Peucedanum oreoselinum*, *Pimpinella saxifraga*, *Plantago lanceolata*, *Platanthera bifolia*, *Prunella grandiflora*, *Prunella vulgaris*, *Thalictrum lucidum*, *Trifolium alpestre*, *Trifolium dubium*, *Trifolium hybridum*,

Trifolium montanum, *Trifolium ochroleucum*, *Veratrum album*.

Morphological analysis of the daffodil population. Following measurements carried out in the 10 points from the reserve, we analysed 114 daffodil specimens. The number of individuals (bulbs) measured varied between 6-15/point. The height of the aerial stem varied between 14-36.5 cm. The number of leaves/bulbs was between 2-8. In two points, we found an individual with a single leaf. The length of the leaves varied between 4-32 cm, and the width between 1-8 mm.

Table 2. Statistical values of measured parameters

	Plant height (cm)	Number of leaves / plants	Leaf length (cm)	Leaf width (mm)	Number of flowers / plants	Perigon diameter (cm)	Coronule diameter (cm)
Minimum	12	1	4	1	1	1.9	0.5
Maximum	36.5	8	30	8	2	4	1.3
Average	25.39	2.81	15.94	3.513	1.009	2.62	0.986
Amplitude	24.5	7	26	7	1	2.1	0.8
Standard deviation	4.87	0.93	4.358	1.473	0.096	0.35	0.14

We noticed that there are no very large differences between them. The plants measured at points 1, 5, 7, 8 and 10 have taller stems, exceeding 25 cm (for example, 27.69 cm at point 8), compared with the others, which have values below 25 cm (22.52 cm at point 4). The length of the leaves clearly exceeds 15 cm in the individuals at points 1, 6, 7, 8 (18.66 cm), while in the plants measured at points 2 (13.2 cm) and 9 the leaves are below 15 cm. Regarding the leaf width, a large difference is observed in the measurements at point 4-9.07 mm, compared with the others,, which are between 2.91-3.86 mm. The diameter of the flowers is similar at all poatts, the values being between 2.47-2.89 mm. The coronule exceeds 1 mm at the plants measured at points 1, 7, 8, 9 and is below 1 mm in the other points, without significant variations (0.89 mm - point 5 and 1.05 mm - point 10).

The total averages of all individuals measured in the 10 points are as follows (Table 2): average stem height = 25.39 cm; average number of leaves/plants = 2.81; average leaf length = 15.87 cm; average leaf width = 4.04 mm; average number of flowers/plants = 1.009; average

flower diameter = 2.62 cm; average coronule diameter = 0.97 cm.

The Pearson correlation coefficient calculated between the perigon diameter and the coronule diameter has a value between 0.3165. The weak correlation of the two parameters is reinforced by the small value of the coefficient of determination ($R^2 = 0.1002$, Figure 5).

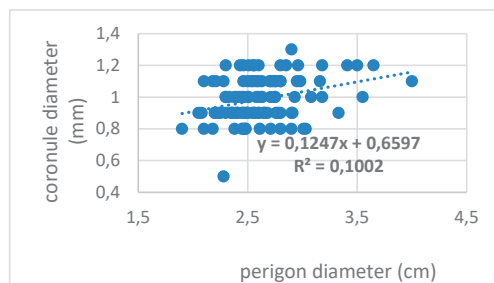


Figure 5. Correlation between coronule diameter and perigon diameter (107 data pairs)

The same conclusions are valid for the correlation between plant height and perigon diameter ($r = 0.1025$, $R^2 = 0.1005$) - Figure 6.

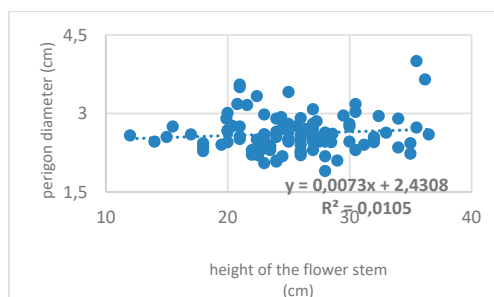


Figure 6. Variation of perigon diameter as a function of floral stem height (107 data pairs)

In contrast, there is a better correlation between leaf length and leaf width, with a Pearson correlation coefficient value of 0.566, calculated for 298 data pairs (measurements); in this case (Figure 7), the value of the coefficient of determination is also higher ($R^2 = 0.3207$). In order to have a situation to refer to, we compared our data with the measurements made by Drăgulescu & Rica (2000) on the daffodil population in the Dumbrava Vadului Botanical Reserve, Braşov County. This is the largest wild daffodil reserve in our country, occupying 394.9 ha, the population being estimated at tens of millions of individuals. As the authors describe

it, the population is vigorous and is found in an oak forest in the pre-mountain plain of Făgăraș at about 500 m altitude.

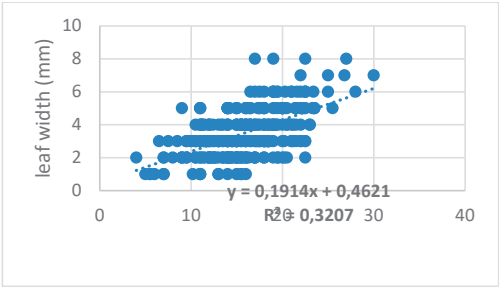


Figure 7. Variation of leaf width depending on leaf length (298 data pairs)

Comparing the measurements, we found that the plants measured by us are shorter and have smaller leaves, and the flower diameter is obviously smaller. The number of leaves and flowers, respectively the diameter of the coronule, have similar values (Table 3). We believe that the size of the population at Dumbrava, the area of the reservation, and the climatic conditions explain these morphological differences. Regarding the population from Nucșoara, following our field observations, we found that *Narcissus poeticus* L. subsp. *radiiflorus* does not have a favorable conservation state. Although the species grows in a protected area, anthropogenic pressures are significant. The picking of flowers and the removal of bulbs by some locals or visitors, especially during the festival, which is attended by hundreds of people, seriously affect the perpetuation and regeneration of the species, leading to a significant decline. On the other hand, failure to respect the information panels and ignoring the marked trails for visiting the reserve led to the destruction of the surface of the vegetation. Many specimens are trampled by both people and cars/ATVs/motorcycles that enter the reserve. Excessive grazing also creates problems, with animals digging the bulbs deep by trampling them. Sometimes the clearing is also burned. A negative imprint is also left by the storage of garbage in the vicinity of the reserve. The lack of civic spirit, ecological education, and low interest in implementing monitoring, control, and protection measures for this species are major causes of the decline in the daffodil population.

Table 3. Statistical values of measured parameters

Parameter / Population	Height of stem (cm)	Number of leaves	Leaf length (cm)	Leaf width (mm)	Number of flowers	Flower diameter (cm)	Coronule diameter (cm)
Nucșoara	14-36.5	2-8	4-30	1-8	1 (2)	1.9-4	0.5-1.2
Dumbrava Vadului	20-54	2-6	15-44	4-10	1 (2)	4-8.4	0.6-1.4

CONCLUSIONS

The Nucșoara daffodil meadow is of remarkable importance from a scientific, botanical, and landscape point of view. The meadow hosts the species *Narcissus poeticus* subsp. *radiiflorus*, but also other important cormophytes such as *Peucedanum rochelianum*, *Iris sibirica*, *Gladiolus imbricatus*, *Dactylorhiza incarnata*, and *Dactylorhiza sambucina*.

The minimum and maximum values recorded following biometric measurements in this daffodil population are the following: the number of individuals in the studied points varied between 6-15/point; the height of the aerial stem was between 14-36.5 cm; the number of leaves/bulb was 2-8; the length of the leaves had values between 4-32 cm; the width of the leaves was 1-8 mm; the diameter of the flowers varied between 1.9-4 cm; and the diameter of the coronule was between 0.5-1.2 cm.

From the calculation of the Pearson correlation coefficient (r) and the coefficient of determination (R^2), we found a weak correlation between the variables perigon diameter and coronule diameter ($r = 0.3165$, $R^2 = 0.1002$), and between plant height and perigon diameter ($r = 0.1025$, $R^2 = 0.1005$) and a stronger correlation between the variables leaf length and leaf width ($r = 0.566$, $R^2 = 0.3207$).

Comparing our measurements with those made by Drăgulescu & Rica (2000) in the Dumbrava Vadului reserve (Brașov County), we found that the daffodils from Nucșoara have smaller dimensions.

In order to preserve this area of outstanding value, we propose and recommend: limiting and monitoring tourism, prohibiting the burning of the meadow as a maintenance measure, creating a grazing calendar, prohibiting the breaking and picking of flowers and severely punishing those who do not respect the law, respecting marked trails and information boards, installing trash

cans, and supporting environmental education campaigns.

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EVALUATION OF THE AGRONOMIC POTENTIAL OF THE NEW *SIDERITIS HYSSOPIFOLIA* CULTIVAR DEVELOPED THROUGH BREEDING AT BRGV BUZĂU

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Abstract

Since 2019, researchers at BRGV Buzău have intensified acclimatization and breeding efforts within the genus *Sideritis*, with a particular focus on *Sideritis hyssopifolia*. Genetic resources from native areas were introduced and evaluated in comparative field trials to assess adaptability, genetic stability, and uniformity. Among the six cultivars studied, genotype G17 showed superior ecological plasticity and excellent adaptation to Romania's climatic conditions. The breeding program was completed in 2024, and G17 was proposed for official registration under the provisional name *Enescu*, with certification expected in 2026. The *Enescu* cultivar demonstrated a high green biomass yield of 6.46 t/ha and the absence of pathogens, recommending it for organic farming systems. Biomorphological evaluations highlighted valuable traits, with maximum plant dimensions recorded in the fourth year of cultivation (49.4 cm diameter and 44.02 cm height). Further studies will focus on biochemical characterization to assess the medicinal and therapeutic potential specific to *Sideritis* species. In the long term, the *Enescu* cultivar is intended for introduction into cultivation and promotion as a medicinal, aromatic, ornamental, and entomophilous species.

Key words: acclimatization, genotype, germplasm, medicinal, entomophilous

INTRODUCTION

The genus *Sideritis* belongs to the family *Lamiaceae*, widely recognized as a family of medicinal plants, and comprises aromatic annual or perennial herbaceous species extensively cultivated for medicinal, perfumery, cosmetic, culinary, and ornamental purposes (Baydar, 2025). The subgenus *Sideritis* includes approximately 125 continental endemic species distributed along the western Mediterranean coast, particularly in southeastern Spain and northwestern Africa (Obón de Castro and Rivera-Nunez, 1994; Dülgeroğlu, 2017). A shared ecological trait of *Sideritis* species is their preference for high-altitude mountainous habitats with calcareous soils, low precipitation, and high temperatures. They are perennial herbs, woody at the base, simple or branched, with silver-green stems and leaves covered by glandular trichomes that confer a woolly, velvety appearance (Negoșanu et al., 2022). *Sideritis* species are recognized as important medicinal plants, and global commercial

demand is steadily increasing. The threat to wild populations due to overharvesting has necessitated the introduction of *Sideritis* species into cultivation in countries such as Greece, Bulgaria, and Turkey. Over the last decade, efforts have focused on optimizing cultivation conditions to maximize the content of bioactive secondary metabolites, thereby enhancing pharmacological potential (Chrysargyris et al., 2019). Samples of *Sideritis scardica*, *S. raeseri*, and *S. syriaca* cultivated in different regions of Greece were analyzed for essential oil content, and the results showed that qualitative and quantitative differences were attributable to abiotic factors such as soil, temperature, humidity, rainfall, and altitude (Kaparakou et al., 2023). In Romania, the only naturally occurring species is *S. montana*, locally known as “încheietoare” or “urechea-șoarecului,” first documented in 1906 by Zach C. Panțu and later by Eugen Ghișă in 1961 in the botanical reserve of Zaul-de-Câmpie. *S. montana* is also found in Dobrogea. Recent climate change - primarily rising temperatures and prolonged drought periods, in

Romania (Ceccarelli, 2010) has increased the demand for new species adapted to hot and arid environments (Bradley et al., 2011). Romania shows heightened vulnerability to the frequency and intensity of extreme meteorological events, with drought representing a major limiting factor affecting plant growth over extensive areas and causing annual agricultural yield reductions of at least 30-50% (Hurduzeu et al., 2014).

Research on *Sideritis* species in Romania began after 2000 at the Laboratory of Genetics and Biodiversity Conservation within the Vegetable Research and Development Station Buzău, under the coordination of researcher Costel Vinătoru, aiming to enrich the acclimatized plant collection with new, valuable species of multiple uses. This work resulted in the development of the first Romanian cultivar of *Sideritis scardica*, officially registered in the National Catalogue of Cultivated Plants under the commercial name 'Domnesc' (Vinătoru et al., 2021) (Figure 1).



Figure 1. Aspects of *S. scardica* cultivation in an experimental field

As a direct consequence of climate change and biodiversity decline, the Plant Genetic Resources Bank (BRGV) Buzău integrated four *Sideritis* species into its acclimatization and breeding program starting in 2021: *Sideritis scardica* L., *S. scardica* L. var. Ossa, *S. syriaca* L., and *S. hyssopifolia*, with a fifth species, *S. cypria*, added in 2022.

MATERIALS AND METHODS

The research was conducted at BRGV Buzău (45°09'N, 25°05'E, 95 m altitude) in southeastern Romania, an area characterized by a continental climate, steppe-type vegetation, and leached chernozem soil. The study period covered 2021-2024. Genetic resources of *Sideritis hyssopifolia* were acquired from its area of origin (Spain). Five cultivated genotypes (G13, G14, G15, G16, and G17) were analyzed

and were spatially isolated from other *Sideritis* species in order to prevent genetic contamination (Figure 2).



Figure 2. *Sideritis hyssopifolia* seedling: a) before and b) after transplanting

The sowing was done in the first decade of March in protected spaces. Seeds were placed in 70-cell black plastic trays filled with commercial peat under nursery conditions, with vegetation factors monitored (Figure 3).



Figure 3. Aspects of culture technology: a) filling pallets with peat; b) thermo-hygrometer for monitoring T°C and U%

Seedlings were transplanted after 45 days (07 May 2023) at the 7-9 leaf stage and 8-10 cm height. No fertilizers, insecticides, or other agrochemicals were applied during seedling production.

The breeding methods included repeated individual selection from the first year to accumulate valuable traits, followed by negative mass selection in the second and third years to eliminate unsuitable forms and conserve superior phenotypic characteristics. At the time of the observations, no UPOV descriptor existed for the genus *Sideritis*; therefore, the basil (*Ocimum basilicum*) descriptor was provisionally adopted, given that both belong to *Lamiaceae*. Subsequently, a new evaluation descriptor for *Sideritis* species was developed and approved by BRGV for future studies.

Weighing was conducted in the BRGV laboratory on fresh plant material using a Kern 572-33 balance (± 0.01 g precision). Statistical

analysis was performed using IBM SPSS v29.0. Data normality was verified prior to ANOVA. One-way ANOVA was applied per genotype, and when significant differences were observed, means were compared using Duncan's multiple range test at $p \leq 0.05$.

RESULTS AND DISCUSSIONS

S. hyssopifolia plants introduced from Spain showed high adaptive capacity, being the only species that flowered in the first cultivation year. Typical *Sideritis* traits include verticillate inflorescences (characteristic of *Lamiaceae*) and leaves resembling those of hyssop. Temperature and precipitation are key climatic determinants for *Sideritis* species. The 2021-2024 period was marked by extremely hot summers with thermal stress and low rainfall, and springs with high precipitation. Elevated December temperatures and short frost periods in January-February did not affect root systems. In 2021, conditions favored optimal rooting and rosette formation in *S. hyssopifolia*, while July soil drought and reduced precipitation stimulated growth. High temperatures and low rainfall during May-July, coinciding with flowering and harvest phenophases in *Sideritis*, favor intensive accumulation of bioactive compounds (Sarrou et al., 2022; Mansinhos et al., 2024). Among all *Sideritis* species acclimatized at BRGV Buzău, *S. hyssopifolia* showed the greatest tolerance to torrential rainfall, with resistance to stem breakage due to basal lignification. Reduced pubescence on stems, leaves, and inflorescences prevents excessive water retention, reducing floral stem bending and breakage (Negosanu et al., 2023).

According to Negosanu et al. (2024), first-year traits included rapid flower stalk formation (one month after transplanting), stem lignification, and strong growth and branching. In the second and third years, plants exhibited increased stem number, higher vegetative biomass (Table 1), lignification extending to tertiary branches, and two flowering waves (summer and autumn). Plant habit varied from fan-shaped to spherical, with erect, hyssop-like stems. Leaves were oval-lanceolate with serrated margins and intense green coloration due to sparse glandular trichomes. Inflorescences were terminal (Figure 4); flowers were small with oval bracts, a tubular calyx with 4-5 mm teeth, and a light greenish-yellow corolla exceeding the calyx. Fruits were ovoid nutlets, typically four per flower, usually all reaching maturity. Pollination was entomophilous and allogamous. Oil glands were present on all aerial organs, producing a characteristic strong aroma.

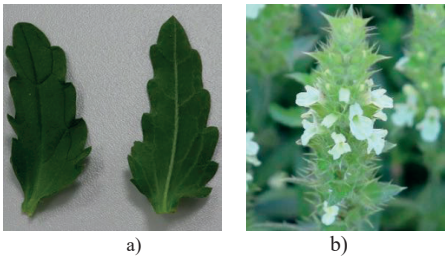


Figure 4. *Sideritis hyssopifolia*: a) jawed leaf; b) inflorescence detail

During the study period, no diseases or pests causing significant crop damage were recorded. Through the applied breeding methods, five genotypes were advanced: G13, G14, G15, G16, and G17.

Table 1. Influence of year of study on the average vegetative mass by genotype

Genotyp / Year	Vegetative mass (g) 2021	Vegetative mass (g) 2022	Vegetative mass (g) 2023	Vegetative mass (g) 2024
	Average±SD	Average±SD	Average±SD	Average±SD
G13	31.1±2.81 ^b	111.9 ±8.63 ^c	163±7.54 ^b	173.2±6.85 ^{ab}
G14	39.4 ±4.18 ^c	117.8±5.04 ^c	131.58±8.78 ^a	157.89±7.78 ^a
G15	35.6 ±7.04 ^{bc}	92.74±6.58 ^a	134.14±9.14 ^a	159.18±8.45 ^a
G16	24.9±1.66 ^a	101.02±2.3 ^c	126.5±3.94 ^a	149.79±4.52 ^a
G17	22.4±2.48 ^a	96.88±3.76 ^{ab}	173.76±7.70 ^c	207.60±6.95 ^c
Annual vegetative mass	30.22±3.63	104.23±5.26	145.78±7.42	163.02±6.90
Coefficient Pearson	$p<0,0001$	$p<0,0001$	$p<0,0001$	$p <0,0001$

Note: SD = standard deviation; p = Pearson coefficient; Duncan Test: Mean values followed by different letters are significantly different at the $p < 0.05$ level.

Monitoring of quantitative traits revealed important morphological and biometric differences among the studied genotypes over the four years, with high relevance for the acclimatization and breeding of *S. hyssopifolia*. Plant height is an essential biometric component for phenotypic evaluation, and its monitoring was conducted by tracking the growth dynamics of the *S. hyssopifolia* genotypes during 2021-2024. All five genotypes showed strong development in 2021; the percentage increase in height was 47.89% in 2022, 6.26% in 2023, and 20.19% in 2024. Recorded value ranges were as follows: in 2021, minimum 20.4 cm (G16) and maximum 27.5 cm (G15); in 2022, minimum

32.1 cm (G16) and maximum 38.4 cm (G15); in 2023, minimum 32.6 cm (G16) and maximum 38.4 cm (G13); and in 2024, minimum 39.1 cm (G16) and maximum 46.9 cm (G15). One-way ANOVA followed by post hoc tests showed no significant differences among genotypes for plant height in 2021 and 2023, whereas significant differences were detected in 2022 and 2024. These findings are consistent with literature data: Santayana et al. (2005) describe *S. hyssopifolia* in its native area as a perennial species not exceeding 40 cm in height, while Mateo-Sanz and Pisco-García (2000) report a height range of 15-35 cm.

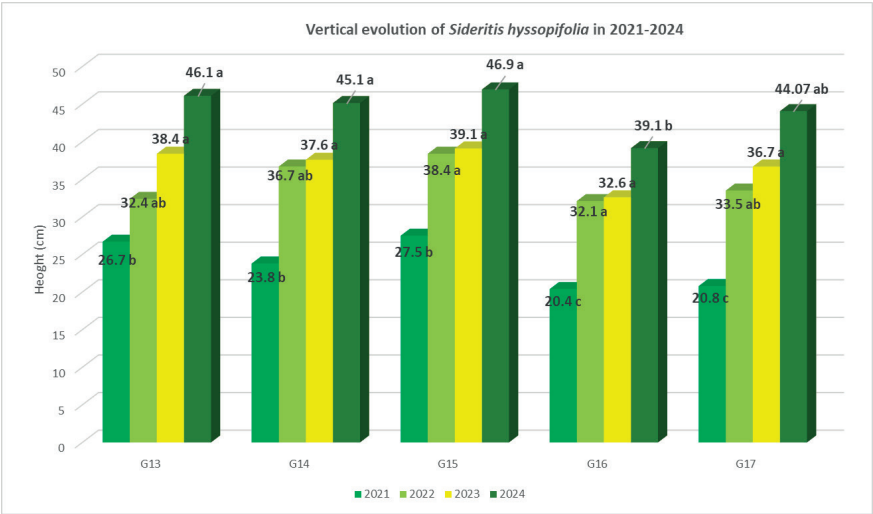


Figure 5. Vertical development of *Sideritis hyssopifolia* genotypes

The evolution of bush diameter over the four cultivation years is presented in Figure 5. One-way ANOVA with Duncan's test indicated no significant differences among the five genotypes within the same year. Value ranges were: in 2021, minimum 20.8 cm (G14) and maximum 24.8 cm (G15); in 2022, minimum 31.7 cm (G15) and maximum 43.4 cm (G17); in 2023,

minimum 36.3 cm (G15) and maximum 44.82 cm (G17); and in 2024, minimum 43.6 cm (G15) and maximum 49.4 cm (G17). Although interannual growth dynamics were pronounced, within-year genotype development was relatively uniform, without major deviations (Figure 6).

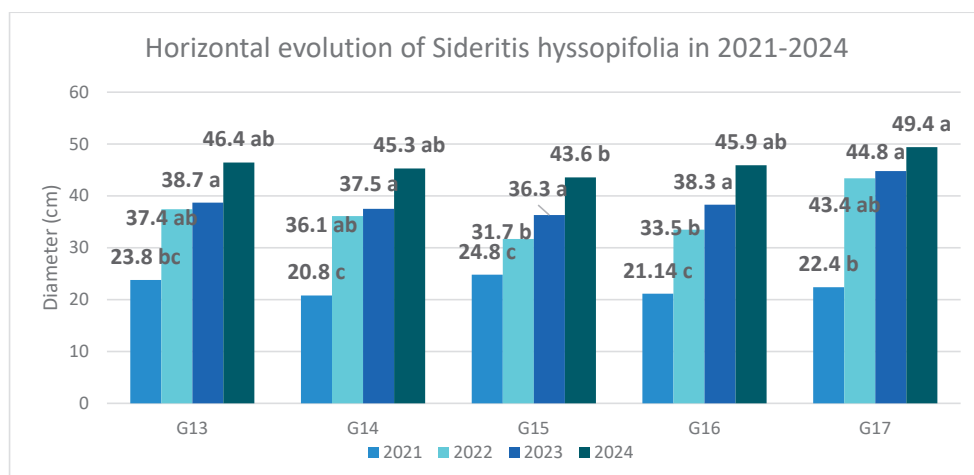


Figure 6. Horizontal development of *Sideritis hyssopifolia* genotypes

From the first cultivation year, *S. hyssopifolia* showed strong branching, producing first-, second-, and third-order inflorescences. Their distribution was dominated by third-order (tertiary) spikes (67.01%), followed by second-order (secondary) spikes (23.66%), while first-order (primary) spikes had the lowest proportion (9.33%) (Figure 7).

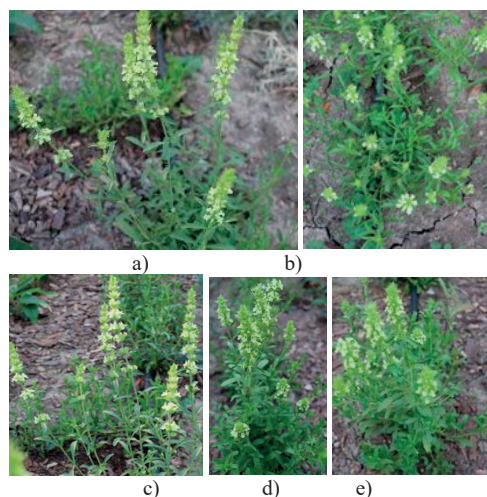


Figure 7. Images from the first year of cultivation: a) G13; b) G14; c) G15; d) G16; e) G17

Quantitative inflorescence descriptors included mean inflorescence length and the number of verticillasters per spike order.

Maximum values for primary inflorescences were recorded in genotype G13 (15.9 cm in

length, 15 verticillasters). Secondary inflorescences reached maximum values in G17 (13.4 cm in length, 7 widely spaced verticillasters). For tertiary inflorescences, genotype G13 again showed the highest values (11.2 cm in length, 11 compact verticillasters). Notably, in the first cultivation year, G17 was distinguished by a compact and well-balanced bush architecture, both horizontally and vertically.

The productivity of *S. hyssopifolia* (Table 2) was calculated based on fresh biomass expressed as g plant and t ha for each year, assuming a planting density of 32,000 plants ha. In 2022, vegetative biomass values for the genotypes were similar, within a narrow range of 92.74–117.8 g plant. Genotype G16 recorded the highest productivity per plant (117.8 g plant) and per hectare (3.77 t/ha), while G14 showed the highest production expressed as the number of flowering stems (2,304 thousand stems/ha), due to the maximum number of stems formed in the second cultivation year (72 stems/plant). In 2023, genotype G16 again exhibited the highest productivity per plant (173.7 g), resulting from a 42.62% increase in the number of flowering stems compared to 2022, and achieved a productivity of 5.56t/ha. The highest productivity, expressed as the number of stems, was recorded for genotype G14, with 3,584 thousand stems/ha, this genotype also showed the highest number of flowering stems per plant, 112.

Table 2. Evolution of production elements in *S. hyssopifolia* genotypes in the 2nd, 3rd and 4th year of culture

Year	2022					2023					2024				
Elements	Productivity/ plant		Productivity/ha			Productivity/ plant		Productivity/ha			Productivity/ plant		Productivity/ha		
Genotyp	NFS	FSM (g)	P (g)	no of 1.000 rods/ha	t	NFS	FSM (g)	P (g)	no of 1.000 rods/ha	t	NFS	FSM (g)	P (g)	no of 1.000 rods/ha	t
G13	67	1.45	96.88	2,144	3.1	93	1.41	131.5	2,976	4.21	102.3	1.55	158.56	3,273	5.07
G14	72	1.4	101	2,304	3.23	112	1.13	126.5	3,584	4.05	113	1.24	140.12	3,616	4.48
G15	56	1.66	92.74	1,792	2.97	90	1.49	134.1	2,880	4.29	99	1.63	161.37	3,168	5.16
G16	61	1.93	117.8	1,952	3.77	87	2	173.7	2,784	5.56	95	1.70	161.5	3,040	5.16
G17	69	1.62	111.9	2,208	3.58	103	1.58	163	3,296	5.22	118	1.72	202.9	3,776	6.46

Note: NFS = number of flowering stems; FSM = flowering stem mass; P(g) = productivity/plant; t = tons.

Inflorescences are located in the upper third of the flowering stem, arranged in verticillasters (4-18), which flower sequentially. Flowering onset occurs in the second half of May, followed by the full flowering phase after 10-12 days. The onset of seed maturation takes place in mid-June, while maximum seed maturity is reached by the end of the first decade of July, when harvesting can be performed. *Sideritis hyssopifolia* requires approximately 69 days for seed maturation and is therefore considered a late-maturing species.

As is characteristic of *Lamiaceae*, the flowering and seed maturation phenophases overlap (Negosanu et al., 2022); thus, the onset of seed maturation in the basal verticillasters coincides with the flowering of the apical verticillasters. This overlap requires increased attention from breeders, as manual seed harvesting must be carried out in two or more stages. When harvesting flowering stems, the optimal moment is when approximately 75% of the inflorescence is open (Figure 8).

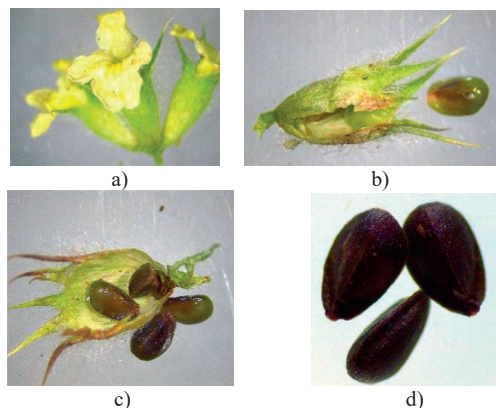


Figure 8. Phenological stage presentation: a) flowering stage; b) and c) onset of seed maturation; d) entirely matured seeds, optimal for harvesting

CONCLUSIONS

Sideritis hyssopifolia demonstrated excellent adaptability to the pedoclimatic conditions of Romania, being distinguished by abundant flowering from the first year of cultivation, the formation of a high number of flowering stems, and the production of viable seeds.

Breeding activities conducted at BRGV Buzau led to the identification of valuable genotypes, with G17 standing out for its vigor, stability, and highest productivity; this genotype has been proposed for official registration under the name 'Enescu'.

S. hyssopifolia shows high potential as a medicinal, aromatic, ornamental, and melliferous plant and can be successfully cultivated under organic farming systems. Dissemination of the obtained results, together with the availability of seed material, creates favorable premises for the expansion of this crop into commercial production.

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ONE HEALTH TO REFRAME THE SYSTEMIC PATHOLOGICAL STATE: RADICALLY QUESTIONING ECOLOGICAL AND SOCIAL ISSUES, WITH FOOD SYSTEMS AT THE FOREFRONT

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Abstract

One Health thinking reveals an inconvenient truth: the accumulation of global crises constitutes a systemic societal pathology - the combined overexploitation of nature and a substantial proportion of humanity, resulting in intertwined ecological and social debt. The 2014 Lancet-University of Oslo Commission stated that, in a society based on social justice and ecological responsibility, health is a precondition, an outcome and an indicator of sustainability, and should be adopted as a universal value and a shared political objective. Health must therefore be understood as a triple and reciprocal condition encompassing ecosystems, societies and individuals; so conceived, it is non-negotiable. This article argues that societies embracing systemic health create the conditions required to reverse the logic of business as usual, a transition from which nature, food systems and rural communities stand to benefit first. Systemic health thus provides a unique instrument for defining indicators that account for and amortize degraded social and ecological capital. Healthy agriculture and resilient food systems are essential to closing the gap between humans and nature. Legal scholarship bears an urgent responsibility in this transformation.

Key words: food systems, planetary boundaries, social-ecological debt, soil-water-biomass, systemic health.

1. SEVENTY YEARS IN A SCHIZOPHRENIC WORLD

When crises overlap and accumulate, and the language of health is invoked to interpret them, the conclusion is inescapable: our societies are profoundly unwell. Yet health - understood in systemic terms - remains a distant horizon for many people and their environments. While scientific reflection is intellectually vibrant, sometimes to the point of conceptual dispersion, political action - frequently reduced to economic metrics - lacks strategic vision.

1.1. Semantic and Operational Confusion

Over recent decades, calls for societal transformation have invoked a wide array of concepts: sustainable development, planetary boundaries, the Anthropocene and its Great Acceleration, the Great Transformation, metamorphosis, One Health and its variants, and ecological civilization (Negrutiu, 2024; Pincemin & Negrutiu, 2024). Although expressed through differing vocabularies and theoretical grammars, these narratives converge on a common premise:

societies cannot exist outside nature; they are embedded within social-ecological systems.

These frameworks collectively challenge the excesses of a world (Flahault, 2008) that resists integration with ecological limits. However, their proliferation has not necessarily clarified public debate. Instead, conceptual fragmentation has often generated semantic confusion, weakening the coherence and resonance of initiatives undertaken locally (Figure 1). Such initiatives require synergy to reach critical mass in the face of entrenched institutional and economic structures.

The Sustainable Development Goals (SDGs) exemplify this difficulty. Comprising 17 goals and approximately 250 indicators, they present formidable challenges of conceptual integration and contextual implementation. Furthermore, positioning “green growth” as their foundational premise is increasingly problematic. The accelerating global demand for finite resources is incompatible with the ecological imperatives embedded in the SDGs (UNEP, 2015). Modelling approaches - including carbon budgeting, resource efficiency strategies,

technological innovation, and incentive frameworks - indicate that absolute decoupling of GDP growth (at annual rates of 2-3 per cent) from resource use remains unattainable under

current growth paradigms (Dittrich et al., 2012; UNEP, 2016), despite partial relative decoupling of CO₂ emissions (Wang et al., 2021).

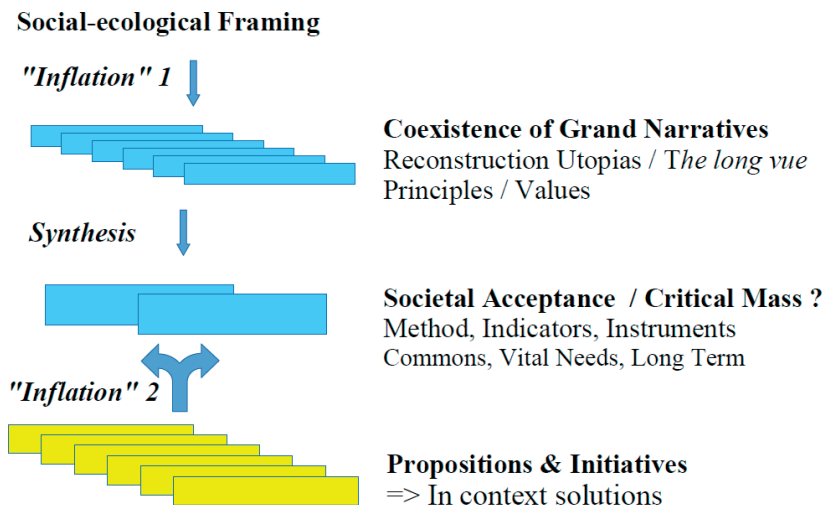


Figure 1. Schematic overview of the diversity of concepts, visions, programs, initiatives, and alternatives within the broader social-ecological approach spanning several decades after the Second World War. Among the proposals and initiatives, we can mention the Sustainable Development Goals (SDGs, 250 indicators) or the Pact for the Power to Live (66 organizations, <https://www.pactedupouvoirdevivre.fr>). These two categories are complementary. To operationalize the overarching narratives, a synthesis is necessary (i.e., harmonization, formatting, comparisons, and testing) to produce frameworks for implementation: methodology, dashboards/indicators, etc. The proposals and alternative solutions can then be deployed in context

Eisenmenger et al. (2020) demonstrate that the SDG architecture remains biased towards economic growth metrics-resource productivity, efficiency, and intensity—while neglecting absolute resource throughput. Ecological objectives are structurally subordinated, and implementation largely relies upon institutions historically associated with unsustainable resource exploitation. In short, the SDGs lack a coherent systemic understanding of social-ecological dynamics. Consequently, their capacity to address interconnected global crises remains limited.

1.2. Societal Pathology

Over the past seventy years (1948-present), this evolving conceptual landscape has unfolded alongside successive geopolitical crises (Table 1; Pincemin & Negrutiu, 2024). A longitudinal perspective reveals the syndrome of a patient unwilling to acknowledge illness. Many core diagnoses were articulated as early as the 1970s, notably in the Stockholm Declaration and the

Club of Rome's Limits to Growth. Yet subsequent diplomatic agendas have multiplied, often reflecting conceptual diversification rather than systemic consolidation.

A comparison between evolving political discourse (Table 1, column 2) and geopolitical realities (Table 1, column 3) reveals a persistent disjunction. Scientific knowledge has progressed significantly, yet political imagination appears increasingly detached from biophysical constraints.

Several structural features characterize this systemic pathology:

First, political and diplomatic agendas that were relatively coherent and ecologically grounded in the 1970s have gradually evolved into narratives increasingly disconnected from material realities. This shift parallels the financialization of economic activity and the expansion of speculative logics (Hickel, 2019; Felli, 2016; Varoufakis, 2024). The pathology has thus become systemic.

Table 1. Based on the geopolitical transformations following the end of the Second World War and the Trente Glorieuses (1945-1972), the reality of the last 70 years is placed in historical perspective through the lens of institutional and diplomatic agendas and rhetoric (column 2) and of crises (column 3), which were primarily financial, energy-related, and epidemiological. The selection of examples is not exhaustive

Time and significance	Political and diplomatic agendas	Geopolitical crises
1945-1948 Foundations that are faltering today.	The Roosevelt years, implementation of post-World War II institutions and regulations.	WHO; ILO; IMF/WB; WTO / ex-GATT; Havana Charter; Human Rights Declaration; United Nations.
1970-1980 1972 - a pivotal year.	Stockholm Declaration 1972; Endorsed by U.N. General Assembly Resolutions 2994/XXVII, 2995/XXVII, and 2996/XXVII, 15 December 1972. Meadows Report 1972; Common Program of the Left in France 1972. Meadows Report: the social-ecological thinking is modeled and quantified.	Two oil shocks (1973 OPEC embargo; 1979 Iranian Revolution). Deterioration of economic indicators in OECD countries: inflation, growth rate, unemployment (1974-75). Rhetoric of the Davos Forum (1970-2026).
1981-1990 From one Earth to one world. China is launching its “world’s factory.” Economic and financial liberalization is beginning in the West.	World Charter for Nature, UN 1982; UNEP Recommendations on Chemical Pollutants 1984–85; Brundtland Report 1987; First IPCC Report 1990. Brundtland Report: Legal Principles for Collective Action and Institutional Change. Managing the Commons.	Structural adjustment programs and the crisis in developing countries in Africa and Latin America (1982-89). Famines in Ethiopia and the Sahel (1984-85). Interest rate and stock market crash, “Black Monday” 1987. Gorbachev launches Perestroika 1985; Fall of the Berlin Wall 1989.
1991-2000¹ Earth Summit. The right to development must equitably meet developmental and environmental needs of present and future generations.	Resource Management Act, New Zealand 1991; Rio Summit 1992 (MDG followed by SDG); Agenda 21. Rio - Global Partnership based on the principles of General Assembly resolution 44/228 of 22 December 1989.	The Gulf War 1990; Economic crisis (1990-93) and Monetary System crisis 1993. After 1993, new economy and economic stabilization. Asian and Russian crises (1997–98).
2001-2010² Consider the SDGs as an unfinished extension of the MDGs. Guarantee equity and dignity for all in a safe and restored living space on Earth.	The Aichi Biodiversity Targets 2010; Sustainable management of resources and equitable distribution through institutions. Poverty eradication is considered the greatest global challenge. Effective solutions at the local and global levels, and converging interests among stakeholders. Mad cow disease outbreak 2003. Strong development of digital technology, mapping of terrestrial and marine ecosystems.	September 11, 2001 attacks; Digital bubble and stock market crash (2001-02); Third oil shock (2003-05). War in Afghanistan (2001) and Iraq (2003). Subprime mortgage crisis and other crashes from 2007 to 2020, the Great Recession. Pandemics (2003, 2009). Strong economic growth in China and India. Global GDP doubled between 1998 and 2010.
2011-2020³ A profound crisis of capitalism. The increase in material consumption is due to population growth, consumption trends mainly observed in developed economies, and the transformation of developing economies.	UNEP reports 2015, 2017, 2019; Planetary Boundaries and Planetary Health concepts 2015. WikiLeaks and Panama Papers 2011. Critical infrastructure (energy, buildings, transport, water supply, sanitation, and waste management) and the food supply sector contribute significantly to resource-related pollution worldwide and have negative impacts on the environment and human health.	Fukushima 2011. The Arab Spring. The COVID-19 pandemic 2020 and profound economic and migratory crises. Major social-economic effects. Extremism is gaining political ground (polarization). The crisis in Ukraine (> 2013). Social-economic crises in Latin America (> 2019). Democratic backsliding on the rise.
2021-2025^{4,5} Transfer through trade etc. of all types of environmental and health impacts from high-income consumer countries to middle- and low-income countries.	NASEM 2021a, 2021b, 2023; EU Beyond Growth (the Green Plan) 2023. Emphasis on primary resources: land, water, and biomass. Long-term, science-based decision-making is recommended. Multi-indicator dashboards and remote sensing data are currently available to policymakers, contributing to the development of early warning, transparency, and accountability policies.	Wars in the Middle East and surrounding areas, in Ukraine, Sudan, and the Suez Canal region, are spilling over. A new form of imperialism is emerging, eroding morality, international law, and the social-ecological progress of previous decades. The rise of AI, with the release of ChatGPT and DeepSeek, is also a factor. Social networks and platforms have addictive and harmful effects: capturing and maximizing screen time, spreading propaganda, etc. The world population reached 8 billion in November 2022.

Abbreviations (in order of appearance)

WHO - World Health Organization; ILO - International Labor Organization; IMF - International Monetary Fund; WB - World Bank; WTO ex-GATT - World Trade Organization; IPCC - Intergovernmental Panel on Climate Change; EMS - European Monetary System and a more general awareness of the limits of capital movements; RMA - Resource Management Act; MDG - Millennium Development Goals; SDG - Sustainable Development Goals; UNEP - United Nations Environment Programme; NASEM - National Academies of Sciences, Engineering, and Medicine of the US - Nobel Prize appeal and report on sustainability science (synthesis inspired by Pincemin & Negrutiu, 2024 and <https://en.wikipedia.org/wiki/2020s>).

Second, the failure to amortize depleted natural and social capital - unlike conventional economic capital - has generated a dual debt: ecological and social. This debt transcends both planetary and societal boundaries (Moore, 2015; Dixon-Declève et al., 2022; Rockström et al., 2023; Gupta et al., 2023). Jason Moore's formulation of "cheap nature and cheap labour" captures the dynamic of short-term gains at the expense of long-term sustainability. Uniquely in evolutionary history, one species has effectively breached the implicit ecological "contract" governing energy flows and trophic chains (Williams et al., 2015; Jørgensen et al., 2023a). By appropriating disproportionate shares of terrestrial biomass and evading the biophysical "taxation" that makes the biosphere a condominium, humanity - embedded within its technosphere - has destabilized the regenerative cycles of the planet. The framework of Planetary Boundaries (Rockström et al., 2023) suggests that several critical thresholds have already been crossed. Disruptions now affect water, nitrogen, and carbon cycles, as well as terrestrial thermodynamics¹ (Gorshkov et al., 2004; Schramski et al., 2015), thereby compromising ecosystem functions, biodiversity, and derived ecosystem services.

Third, these excesses erode systemic resilience and regenerative capacity. State shifts-non-linear transitions between system configurations (Meadows et al., 2005; Barnosky et al., 2012) - are gaining momentum. Systems once considered robust are increasingly vulnerable (Hamant, 2023).

Fourth, resource scarcity, finitude, and substitutability dominate economic and geopolitical discourse. Yet demographic dynamics - deeply intertwined with resource pressures - remain politically sensitive and often marginalized (Mann, 2018). Over-extraction/-production/-consumption of resources translates into contemporary forms of predatory imperialism that unfold within a context of acute social-ecological tension. Resource conflicts both drive and are intensified by these tensions (Gabriel-Oyhamburu, 2011; Acemoglu et al.,

2012; Jørgensen et al., 2023a; Farrell and Newman, 2024). This mutually reinforcing dynamic disproportionately affects countries already confronting poverty and structural vulnerability.

Fifth, although the relationship between pollution and health is widely acknowledged, global pollution levels continue to increase (Arguello Velazquez & Negrutiu, 2019; IPES-Food, 2017; Fuller et al., 2022). Expanding definitions of pollution - including ultra-processed foods, chemical additives, and performance-enhancing supplements - further aggravate the health burden. Industrial production now encompasses approximately 500,000 chemical substances (Brooks et al., 2020), many interacting in complex mixtures (the cocktail effect) whose cumulative impacts remain scientifically out of reach (Negrutiu, 2025a).

Within this context, the narrative of "systemic health" (Table 1) emerged comparatively late, gaining institutional prominence around 2015 and consolidating between 2021 and 2023 through work undertaken by the United States National Academies of Sciences in collaboration with the Chinese Academy of Sciences (NASEM, 2021a and b, 2023).

2. THE UNITED STATES NATIONAL ACADEMIES OF SCIENCES, ENGINEERING, AND MEDICINE (NASEM): LAST-MINUTE WHISTLEBLOWERS

The reports of the United States National Academies of Sciences, Engineering, and Medicine (NASEM) on the progress and limitations of sustainability research represent a markedly transformative position during what has been described as a decisive decade (2020-2030) in the face of unprecedented global challenges. Their conclusions are noteworthy for both their diagnostic clarity and their normative ambition. The principal arguments may be summarized as follows:

1. The global crisis constitutes a vast, uncontrolled experiment on the Earth's life-

¹ The energy dynamics of the biosphere makes the Earth System operate as a space battery (Schramski et al., 2015). Human activities and energy extracted in the last few centuries have made it possible to build today's intensive economy, a "debt" economy on living and fossilized biomass. The former is the most critical because there is no substitute or replacement energy for the direct conversion of diffuse low-quality solar energy to high quality organic compound chemical energy stored in living biomass.

support systems. Human activities have altered biophysical processes at planetary scale without adequate understanding of systemic feedbacks.

2. Guiding the technological revolution is imperative. In aggregate, technological progress has accelerated planetary destabilization. Future innovation must be explicitly oriented towards social and ecological objectives, including infrastructural transformations (see also Sverdrup & Ragnarsdóttir, 2014).

3. Power structures remain insufficiently integrated into social-ecological science. The latter remains methodologically underdeveloped in analysing power dynamics across time-space scales.

4. A persistent disconnect exists between empirical urgency and electoral politics. Scientific evidence indicates accelerating risks, yet political responses remain constrained by short-term electoral cycles (see also Carney, 2015).

5. Measurement systems must move beyond GDP. Indicators reflecting the genuine well-being of both people and nature are required, and should be adopted by financial and business sectors. Economic, environmental, and social externalities must be appropriately internalized.

6. Planetary Health has reached a societal inflection point. The recognition of intimate interdependencies between human health, animal health, and ecological integrity is evolving from conceptual framework to actionable commons-based narrative.

7. Justice-focused equity is central. Structural economic, social, and political drivers of systemic lock-in must be dismantled.

8. Adaptation governance and transformative change require institutional redesign. Effective linkages between knowledge and action must operate across scales, while confronting barriers rooted in power asymmetries and vested interests. Taken together, these observations suggest that shifting from a pathological diagnosis to a health-oriented framework reshapes the conceptual landscape and architecture underpinning sustainability discourses. It compels us to ask

three foundational questions: What narrative? What vocabulary? What grammar²?

The framework of Planetary Boundaries, which has received sustained scientific and political attention in recent years (Dao & Friot, 2025), offers a scientifically robust system for defining biophysical limits. When considered in an integrated manner, planetary boundaries can be analytically regrouped around two systemic drivers: food systems and global pollution (Arguello Velazquez & Negrutiu, 2019). This reframing constitutes a conceptual shift. It clarifies both the sources of destabilization and the loci of intervention (Fenner & Scheringer, 2021; Negrutiu, 2025a). These two components operate synergistically and generate what may be termed a dual “health bubble”: a set of slow, cumulative, and systemic risks that are often underestimated because of their insidious character. For example, the postwar massive proliferation of chemicals has become global and inescapable: humans and non-humans have become chemically transformed beings³ (Thomas, 2014 and references therein). The health-bubble perspective also sheds new light on climate change and biodiversity erosion (Weathers et al., 2025). It follows that planetary boundaries alone⁴ may not suffice to sustain a unifying grand narrative.

3. SYSTEMIC HEALTH: TOWARDS A GRAND NARRATIVE

These analyses converge in placing the concept of health at the centre of early twenty-first-century challenges. Health, in this sense, ranges from societal “care” (Serres, 2016), to persistent toxic contamination crises such as chlordecone pollution in the French West Indies (Santé Publique France, 2010; ANSES, 2024), to the COVID-19 pandemic, and the growing fragility of public health systems across the world.

The Lancet-University of Oslo Commission concluded that in a society grounded in social justice and ecological responsibility, health becomes simultaneously a precondition, an outcome, and an indicator of sustainability

² This is all the more important and urgent as the unravelling of political language taking place via ultraliberal-imperialist spheres is changing the grammar of recent decades (Miranda, 2026).

³ “We are what we eat” joins hands with “We are our chemically altered environment” (see Section 6).

⁴ Boundary values remain controversial for land use change or fresh water variables, as does the quantification of biodiversity loss, functional biosphere integrity, and global pollution (https://en.wikipedia.org/wiki/Planetary_boundaries; Dao & Friot, 2025). Furthermore, concerns have been raised about using boundaries in isolation. Also, there is the issue of whether economic and political control and management through thresholds would be meaningful.

This perspective is articulated in the “Manifesto for a Common Health” (Collart Dutilleul et al.,

The Manifesto raises a series of foundational questions (Figure 2):

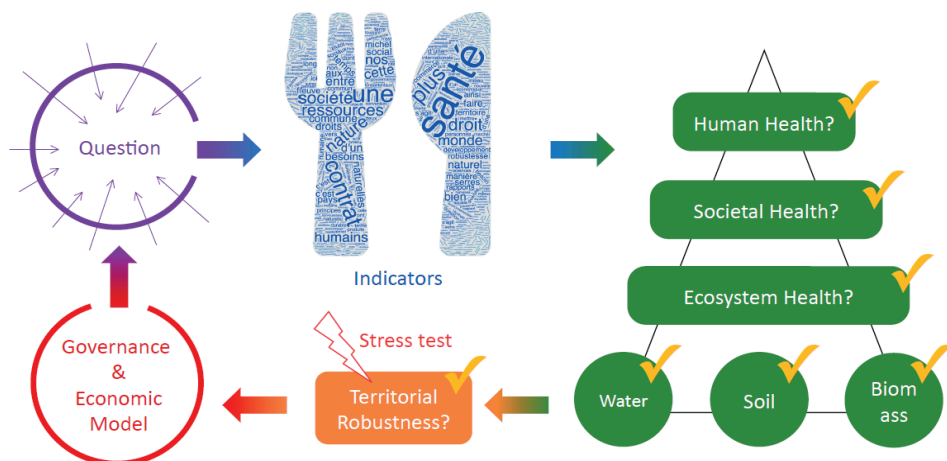


Figure 2. Systemic health is a way and a guide for inhabiting the Earth and its territories. It represents a profound paradigm shift. Outlined as a pyramid, the health of natural environments (water-soil-biomass) encompasses social and human health. At each stage, decisions must absolutely “check” the human, environmental, and social components of health. It is not a matter of imposing new rules, such as those advocated within the framework of Corporate Social and Environmental Responsibility, onto an existing economic model that has become obsolete, both socially and ecologically. On the contrary, it is the project itself, having undergone several cycles of health assessments, that builds a new, situated, and sustainable economic model. Nature and culture are prerequisites; the economic model is no longer a dogmatic input constraint, but an output rooted in our relationship with the world
(adapted from Collart Dutilleul et al., 2023)

Can systemic health integrate society into ecological cycles, functions, and interactions?

If “we are what we eat”, should the political narrative shift from “farm-to-fork” towards “fork-to-farm”, thereby aligning agri-food systems with public health (i.e., food democracy; see section 6.2)?

More fundamentally: what if health, rather than the market, were to guide the global economy (Laurent, 2020)?

4. SYSTEMIC HEALTH OVERTURNS THE LOGIC OF BUSINESS AS USUAL

Systemic health reconfigures the relationships between planetary boundaries, economic systems, and societal limits (Negrutiu, 2024). Rather than advocating degrowth *per se* (Jackson, 2009), it repositions the economy as a subsystem of the biosphere, tasked with balancing accessible resources against fundamental human needs (Collart Dutilleul et al., 2023). These needs correspond to those protected by human rights.

The appropriate equilibrium, therefore, is not between supply and demand, but between essential needs and available natural resources. This reframing clarifies the societal mission of markets: they must function within ecological constraints while ensuring equitable access to vital goods (to keep private profit and associated social-ecological debts in check).

Systemic health thus implies a renewed contractual framework. It seeks to balance what nature provides to human societies with what societies must return to nature. As the Manifesto observes, while the social contract historically aimed to pacify social relations by excluding violence and domination, it has failed to guarantee equitable pooling of vital resources. Yet ensuring effective access to resources necessary for collective well-being (*Supplementary Panel 2*) is the primary purpose of any legitimate contract. The challenge, therefore, is twofold: to utilize nature's resources prudently in satisfying human needs, and to mobilize human reason and institutional design to safeguard the needs of ecosystems. Human rights become effective only when they entail corresponding duties.

Health, initially mobilized as a corrective to economic logic, now extends to institutional and commercial governance. It informs climate policy, biodiversity protection, and economic reform. As Elizabeth Maruma Mrema (Deputy Executive Director of UNEP, UN Under-Secretary-General, and former Executive

Secretary of the Convention on Biological Diversity) observed in 2023 (McKinsey, 2023): *you can do everything under the sun as far as climate actions and solutions are concerned, but if nature is not part of the equation, global warming will continue to increase to unimaginable levels.*

For systemic health to become operational:

- Indicators and protocols must be grounded in robust scientific methodologies; accurate measurement conditions prudent judgement.

- Scientific communities must organize their research questions and instruments in genuinely transdisciplinary ways, while integrating law and governance structures.

- Regulatory capacity must expand to integrate economic activity within social and ecological constraints.

Health constitutes therefore both a normative compass and a regulatory principle. In a finite physical world, systematic measurement of human activities and full accounting of their social and environmental costs become indispensable.

5. WHAT AND HOW TO MEASURE? INTEGRATED SOCIAL-ECOLOGICAL INDICATORS MAKE UP SYSTEMIC HEALTH

What societies choose to measure reflects what they value. A health-based approach enables the construction of assessment systems that are preventive, collective, and democratic, thereby safeguarding public and common goods while incorporating the interests of future generations. Systemic health must demonstrate tangible relevance for present generations if it is to sustain long-term legitimacy.

Renewed interest in social-ecological planning⁵ (Ministère de la Transition écologique, 2023; Fofana & Hallosserie, 2024; Viennot, 2025; see also Thompson & Steiner, 1997) represents a good starting point for thinking about systemic health. Such planning strengthens long-term orientation, institutional legitimacy, and cross-sectoral coherence in science-policy interactions. Experimental sciences can provide calibrated datasets tailored to legal and regulatory needs. However, significant limitations persist. Many large-scale data systems fail to meet standards of

⁵ i.e., the integration of nature and humanity to create a healthy living environment through sustainable use of resources by adopting friendly materials, energy-saving technologies, recycling design, etc. It should be noted that the social dimension has not yet found its place in these planning mechanisms.

findability, accessibility, interoperability, and reusability - the FAIR principles (Wilkinson et al., 2016; Fairbrass et al., 2020; Argüello et al., 2022; Ramírez-Márquez et al., 2024). Political commitment to restructuring public data infrastructures is therefore urgent.

A couple of social-ecological evaluation frameworks illustrate operational feasibility. Together they embody the spirit and the letter of systemic health.

The Limits to Growth report to the Club of Rome (Meadows et al., 1972) pioneered integrated modelling of political, economic, social, and environmental systems based on resource finitude. Its recent update, *Earth for All* (Dixson-Declève et al., 2022), employs eleven synthetic parameters incorporating over 100 variables and feedback mechanisms. Its principal conclusion is unequivocal: reducing inequality is central to improving human well-being within planetary limits.

At national scale, New Zealand's *Resource Management Act* (1991) offers a pioneering example of integrated natural resource governance (Gow, 1997). Positioned at the apex of legislative hierarchy, it coordinates land, water, environmental, and economic law within a unified framework structured around the triptych "Avoid, Remedy, Mitigate". Compensation mechanisms were excluded. National environmental standards define long-term limits concerning water, estuaries, air, soil, biodiversity, and development.

At local scale, the Michel Serres Institute (Lyon) and the Lascaux Centre for Transition Studies (Bayonne) are developing integrated systemic health indicators applied, for example, at river basin level. Three interconnected dimensions are measured:

Public health. Starting from the definition that health is a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity (WHO, 1948), we can directly use sets of indicators deployed at the international (WHO) or national (Health Agencies) level. **Societal health** is addressed through the adjustment of basic needs to available resources (LASCAUX and CELT programs; Collart Dutilleul et al., 2023) and aims for social cohesion (see United Nations, 1994).

Ecosystem health refers to ecosystems' state or condition and involves maintaining their

organization, functions, diversity, productivity, resilience to stress, and autonomy over time (Argüello et al., 2022).

The objective is to integrate these three dimensions into national accounting frameworks currently dominated by GDP. Approximately half of global GDP depends on healthy ecosystems, and 20 per cent of countries face risks of ecosystem collapse (Swiss Re, 2020).

To test the feasibility of this approach, the health component of ecosystems was experimentally assessed in the Rhône River basin using an ecosystem capital accounting method (Argüello et al., 2022). This research indicates that amortizing the consumed ecological capital in the Rhône River basin would negatively impact the region's current GDP by at least 30 per cent. This work is accompanied by the development of mathematical models establishing dependencies and causal relationships between the three categories of health indicators.

These models provide a better understanding of interacting drivers and constraints and can support multidimensional political and economic trade-offs. Thus, cost-benefit analyses between the three levels of health - and in particular between ecosystem health and societal health - can accurately inform decision-making. For example, environmental risks alone have been estimated to account for 23 per cent of global deaths and 22 per cent of disability-adjusted life years (Prüss-Üstün et al., 2017).

One may therefore ask: what would society look like if the amortization of ecological, social, and public health capital were fully internalized? It is at this juncture that agriculture and food systems assume central importance. Positioned at the intersection of ecosystem integrity, social organization, and human nutrition, they constitute the operational core of systemic health.

6. WHAT EXACTLY IS AGRICULTURE? CERTAINLY NO LONGER THE ADJUSTMENT VARIABLE OF OUR SOCIETIES

If systemic health is to become operational, it must engage directly with the sector that lies at the intersection of ecosystems, societies, and human survival: agriculture.

The Industrial Revolution and subsequent technological and infrastructural transforma-

tions have reshaped agriculture into an activity increasingly dependent upon industrial inputs, mechanization, synthetic fertilisers, fossil energy, and globalized supply chains. In many regions, these processes have marginalized small-scale farms and weakened rural communities. Since 2022, the global population has exceeded eight billion, while more than half of the planet's ecosystems are now heavily anthropized (Barnosky et al., 2012; Ellis, 2019). Under such conditions, tensions surrounding agriculture have reached unprecedented levels. Recent conflicts within the European Union and debates surrounding the Mercosur trade negotiations (2025-2026) illustrate how agriculture has become a focal point of geopolitical, ecological, and social contestation (Jublin & de Ravignan, 2026). Historically, agriculture has functioned as the principal social, economic, and ecological adjustment variable of most human societies (Negrutiu, 2025b). This is paradoxical. Agriculture represents a paradigmatic case of social-ecological co-evolution and a defining feature of our species. Through domestication, systematic land-use transformation, and the construction of dedicated infrastructures, humans have continuously reshaped ecosystems (Jørgensen et al., 2023a). Cultural evolution initially conferred ecological advantage; over time, this advantage evolved into ecological dominance.

Today, agriculture (and therefore ecology) is inseparable from questions of national security and sovereignty. Yet it is simultaneously embedded in globalized markets that subordinate ecological and social considerations to short-term competitiveness and profits.

Agriculture thus embodies a dual character. It is a central driver of the "health bubble" described earlier - through land conversion, biodiversity loss, and resource depletion - but it also contains unique leverage points for systemic transformation (Schneider et al., 2023). For this reason, agriculture must constitute the starting point of any credible political and legal reorientation. In other words, translating into societal practice the principles "We are what we eat" and "Food is medicine" (here the microbiota comes in; see also Thomas, 2014) implies recognizing that food production is not merely an economic activity but a daily,

universal necessity. Every individual depends on agriculture and food, every day.

6.1. The Ecological Dimension of Agriculture - Unique Physical Resources

Within the systemic health framework, agriculture is structured around three primary and interdependent physical resources: *soil, water, and biomass* (Figure 2). These resources form the material foundation of food systems and possess distinctive characteristics: they are renewable yet exhaustible, non-substitutable, and non-relocatable (Negrutiu et al., 2020). Despite their fundamental importance, and contrary to basic economic logic, their large-scale degradation is not amortized within prevailing economic systems. Their management is therefore not merely technical but profoundly political and legal, given their centrality to food security, food sovereignty, and food democracy.

Biomass. All living matter is biomass. Human beings themselves are biomass, composed of approximately 70 per cent water. Food is biomass transformed. Current levels of human appropriation of biomass - estimated at approximately 40 per cent globally - constitute a major driver of habitat destruction, land-use change, biodiversity erosion, declining agro-ecosystem productivity, and climate change (Urban, 2015; Barnosky et al., 2012; Steffen et al., 2015). The scale of this appropriation fundamentally alters trophic structures and ecological balances.

Soil. Soils represent one of the most complex and fragile outcomes of evolutionary processes. Soil ecosystem multifunctionality - mediating interactions among water, air, microorganisms, and plant life - renders soils indispensable yet vulnerable (Negrutiu et al., 2020). Agriculture is inseparable from soils. It entails the intentional production of biomass through alteration of vegetation cover and soil structure. Land conversion remains the principal direct driver of habitat transformation across more than 40 per cent of the Earth's terrestrial surface (Vanbergen et al., 2020). It has been described as the largest geoengineering operation ever undertaken by humanity (Verbarg et al., 2015).

Water. Water scarcity and degradation have now entered a phase that may reasonably be described as post-crisis. Recent United Nations

assessments (Madani, 2026) employ unusually stark language: references to exhaustion of water resources (a “bankruptcy”), liquidation of natural capital, and the vulnerability of entire regions. More than four billion people experience severe water scarcity for at least part of the year. Water withdrawals and pollution increasingly exceed renewable inputs. Groundwater depletion, wetland loss, soil degradation, river modification, and glacial retreat impose damages that are either irreversible or repairable only at prohibitive cost. Humanity has entered what may be termed the age of hydrological irreversibility.

Taken together, agro-ecosystems represent a simplified and diminished form of nature: they are human-managed configurations of soil, water, and biomass. The manner in which these three resources are governed determines whether agriculture undermines or restores systemic health.

6.2. The Social Dimension of Food Systems - Peasants and Smallholder Farmers as Mediators with Nature

Food is a universal and indispensable resource, a vital individual and collective need (Supplementary Panel 2): it sustains life, labour capacity, and social organization. Yet food is more than caloric intake: it shapes power relations, cultural identity, and social cohesion. The United Nations Special Rapporteur on the Right to Food, Olivier De Schutter (2017), observed that contemporary food systems simultaneously produce hunger, ill-health, and ecological degradation. One person in ten remains undernourished, while obesity and diet-related diseases escalate globally (Sundiangu et al., 2025). The agri-food crisis thus encompasses multiple dimensions:

Moral: declining trust in industrial systems and erosion of dietary diversity;

Social: proletarianization, inequality, and economic marginalization of farmers;

Environmental: biodiversity loss and ecosystem degradation;

Political: disengagement and weakening of public regulatory authority;

Economic: concentration of power in oligopolistic agri-food corporations and expansion of liberalized trade regimes.

Initiatives such as the “Campaign to Improve the Nutritional Condition of Americans”, supported

by academic institutions, foundations, regulatory agencies, and members of the United States Congress (Food is Medicine Institute, 2025), illustrate attempts to reframe food as a public health intervention rather than a commodity. The “Food is Medicine” approach promotes dietary transformation as a therapeutic and cost-effective strategy for reducing healthcare expenditure and chronic disease burdens (Westcott, 2025).

Nevertheless, a substantial proportion of undernourished populations are, and that is the last straw, smallholder farmers, as well as residents of rural areas that depend upon small-scale agriculture for their livelihoods (Vanbergen et al., 2020). This underscores the persistent tension between globalized agricultural systems and local food security. Therefore no systemic health transition is possible without confronting structural inequalities.

As a matter of fact, systemic health reframes agriculture not as a mere production system, but as a social-ecological function. The propositions “We are what we eat” and “Food is medicine” fundamentally reconfigure social-ecological and political understandings of food. Food, currently treated as a commodity among others (Vivero Pol, 2013; Collart Dutilleul, 2018), becomes a collective health concern. The conventional “farm-to-fork” narrative, often deployed mechanically in policy discourse, may require inversion: a “fork-to-farm” democratic practice (i.e., food democracy; Collart Dutilleul, 2021) in which citizens’ dietary choices shape agricultural and public health policies. Such a reorientation implies joint governance of the core resources underlying food production - land, water, and biomass (Negrutiu et al., 2019, 2020; Collart Dutilleul et al., 2023).

Ultimately, farm-to-fork precisely indicates how the distribution of power within food systems operates. It can be reduced to three interrelated controls and one challenge (see also IPES-Food, 2026): Control of physical resources; Control of information and data (BigTech and BigAg are tightening their grip on farming through Artificial Intelligence); Control of regulatory rules; Who is defining the problems and the solutions (with finance and public policies in the driving seat). In this configuration, the progressive privatization of nature becomes evident.

7. SYSTEMIC HEALTH, AN INSTRUMENT OF SOCIAL-ECOLOGICAL TRANSFORMATION TO PURGE THE SYSTEM

Transitioning from systemic pathology to systemic health requires dismantling the health bubble whose manifestations differ across societies. Systemic health operates simultaneously as narrative and instrument. It entails reconfiguring economic, political, and social organization, and relearning how to recognize ecological limits. The social dimension in a social-ecological perspective is paramount, because it enables coherently articulating social and political values. Ultimately it may decide the fate of a society (Morris, 2013; Thomas, 2014).

Health thus becomes a narrative of social cohesion, collective responsibility, and long-term orientation. It integrates economy within society, and society within ecology. The approach outlined here demonstrates how health can operationalize other social-ecological frameworks, including Planetary Boundaries and the Sustainable Development Goals. Health, resources, and robustness (Figure 2) thereby become foundational policy criteria, adaptable across territories and scales. In this perspective, local and global dimensions are not opposed; nor are short-term and long-term considerations. Instead, they form nested layers of responsibility (Collart Dutilleul et al., 2023; Negrutiu, 2025b). Health may also function as an antidote to ecological denial. Across societies, two enduring expectations persist: health and justice (Negrutiu, 2022). These aspirations are inseparable and demand collective framing of, to name just a few, poverty, climate change, and biodiversity loss within a reordered hierarchy of priorities.

Yet contemporary societies are marked by deep social-political polarization (Varoufakis, 2024; Oxfam, 2025). Current geopolitical tensions undermine social-ecological cooperation and work against the systemic health framework. Since humanity cannot exist outside nature, transformative questions must be posed within

social-ecological space: Is agriculture an economic activity like any other? Is food a commodity like any other? Is a world without smallholder farmers conceivable or acceptable? To whom does nature belong?

Systemic health remains, therefore, a wager. It requires substantial collective effort and institutional redesign. Law occupies a central position in this undertaking (Negrutiu et al., 2019, 2023). Public institutions themselves face crisis under declining confidence in progress and scientific authority. The question arises: are they reformable (Frérot, 2016)?

Two trajectories emerge. One anticipates, prevents, and reorganizes societal systems to live within ecological constraints. The other ignores the “boomerang effect” of nature - whereby humanity now depends upon the systems it has destabilized. As Michel Serres (1990) wisely observed, we now depend on the things that depend on us. The message is clear: whatever humans do to the natural world finds its way back to humans.

In simplified terms, the central issue becomes whether an open legal framework can be designed in which social-ecological costs are systematically internalized. Such an approach would likely align economic activity with biospherical limits and would allow avoiding unduly disrupting the laws of Earth’s thermodynamics. Systemic health resonates therefore with indigenous cosmologies, such as that of the Kogi people of Colombia, who conceive their mission as safeguarding the balance of the Earth for present and future generations (Julien, 2022; Pyhälä, 2024). Western science has developed tools to measure such balances; governance through law must now ensure that quantification serves justice rather than technocratic control (Supiot, 2015; Varoufakis, 2024).

The decisive constraint, however, is temporal. The present decade (2020-2030) has been identified as pivotal (NASEM, 2023; Negrutiu et al., 2023). Given existing planetary and societal boundaries⁶, is there still time for

⁶ Namely, a human population that has quadrupled since the 1950s, the institutional and organizational scale of human societies, the haphazard use of the technosphere, the burden of systemic health, the asymmetries and incongruities of social capacity and group behaviour (political and cultural values, short-termism/long-term view, cooperation/competition, unbridled consumption/self-limitation, collective learning, connectivity, and information overload, levels of inequity, deleterious geopolitical power games, etc.) (Jørgensen et al., 2023a, 2023b; Faerron Guzmán et al., 2025).

inaction, or even gradual adaptation? Or has humanity hit a demographic glass ceiling while engaging in an evolutionary trap - a trajectory characterized by unprecedented constraints of combined social organization, polarized cultural landscapes, and rates of change exceeding governance capacity (Hulme, 2020; Waring et al., 2023)?

This question remains open. Systemic health offers a framework for answering it - but only if it is translated from narrative into institutional practice.

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NOTES

(1) The ambiguity of the decade: Environmental protection and the precautionary principle must be an integral part of the development process, while avoiding penalties for international trade and investment.

(2) A conceptually poor decade, shaken by major crises.

(3) The decade of UNEP and social protests (Occupy Wall Street, demonstrations in Hong Kong, Algeria, Iran, the “yellow vests” in France, and school strikes for climate).

(4) The NASEM (National Academies of Sciences, Engineering, and Medicine) decade. Over the past fifty years, the world’s population has doubled, raw material extraction has tripled, and gross domestic product has quadrupled, contributing to over 90 per cent of biodiversity loss and water stress, and accounting for approximately half of the impacts of climate change. Furthermore, global progress on the 2030 Agenda for Sustainable Development indicates that this agenda is “severely threatened” (2022). Academic science is being politically targeted.

(5) The new imperialism draws advantages from the capabilities of digital systems (particularly in the large-scale production of fake news), where the quantity of views on social networks generates value, and this occurs in all spheres of activity, including the sciences (NASEM, 2025). Quantity, enormity, and excess transform clicks into social facts, into something akin to truth. This constitutes a “collective fabrication of value” (Liogier, 2026).

Supplementary Panel 1. Comparative One Health and Planetary Health

The definitions and action plans they propose evolve and change over time (Lerner & Berg, 2017; Morand et al., 2020; Ruiz de Castañeda et al., 2023).

One Health tends to be exhaustive essentially on the public health aspects (human and animal health priority area) and has recently integrated a broad range of environmental aspects. The program is carried out by a quadripartite cooperation between the global international organizations WHO, WOAHA (animal health), FAO, and UNEP. This is a political network operating in association with governmental, financial, economic partners, civil society organizations, joint program networks, education and training activities, with national targets and priorities across sectors. For example, the 200 research networks - concentrated so far in Europe - investigate emerging infections and novel pathogens, endemic infections and NTDs, antimicrobial resistance, and extreme weather, water, environmental degradation, food safety, and food and nutrition security.

Planetary Health has been initiated by the Lancet-Rockefeller Foundation commission and the Planetary

Health Alliance is built on a strong academic network (400 universities, including Harvard, Oxford Martin School, Western, London School of Hygiene and Tropical Medicine, Sydney, California, Sunway), US and Chinese Academies, but also world leaders and experts from business, government (60 countries), and civil society organizations. The program is focused on teaching and training activities, and targets several environmental and health challenges (e.g., food systems and water, global pollution, climate, human pathologies).

The comparison highlights the fact that the main dimension that is apparently lacking or at least is not directly addressed is social health per se.

Supplementary Panel 2. Basic needs, accessible resources, and Societal Boundaries

Allocation of resources has been driven by a narrow economic-growth logic of development, cost recovery, and reliance on the market (Gupta & Lebel, 2020). Denying fair access to vital resources violates fundamental human rights and raises concerns about resource stewardship governance, with impacts on both social health and ecosystem health. While the adjustment of resources and needs is at the heart of business and family or community functioning, that concern is more discreet in public policies.

The essence of adjusting basic needs and resources implies considering the triptych “fundamental goods” (land, water, and wealth produced by natural resources, all of which are limited), “basic needs” (both economic and social, with food and health in the first place), and “fundamental rights” (the normative obligations of human rights). The latter is expressed in particular in the Draft Optional International Protocol on Economic, Social and Cultural Rights: “civil, cultural, economic, political, and social rights are universal, interdependent, and indivisible” (United Nations, 1994; Mahon, 2008). Therefore, resource justice becomes a pluralistic process about collective and individual relationships encompassing the distribution of rights and responsibilities on Common Pool Resources (distributive justice) and the role and ability of stakeholders to contribute to decision-making (procedural justice) (Ostrom, 2009; Wyborn et al., 2020).

Earth system justice is meant to allow living within biophysical boundaries in ways that enable fair access to essential resources and interrelated needs and services (Gupta & Lebel, 2020; Millward-Hopkins et al., 2020; Blake and Smith, 2022; Rockström et al., 2024). A just access to material resources concentrates on universal needs, such as food, water, energy, and infrastructures (e.g., housing, transport). This is based on international human rights principles extending to procedural and substantive justice (i.e., distributive, corrective, and restorative justice; Gupta et al., 2023), consisting of principles of equal distribution, meeting minimal needs

for all, and limiting excess resource use. They are likely to alleviate the unequal impacts of pollution, epidemics, or access to land, but also to address responsibilities for environmental degradation and disparities between countries, communities, and social and racial groups (Hickel et al., 2022).

There is significant work on what is considered a decent standard of living (Quality of Life, well-being; Costanza et al., 2007) that relates to social equity and protection, and to carrying capacity considerations (Mote et al., 2020). Human-rights advocates have long highlighted the imperative of ensuring every person's claim to life's essentials (sufficiency in dignity) and avoiding deprivations.

Gaining institutional power by articulating social well-being and ecological robustness sets focus on *collective needs*. This can be a productive approach to resource reallocation by aligning equity in wealth (i.e., the adjustment of accessible resources and fundamental social and economic needs) with a responsible maintenance of the life-support capacities of the biosphere. Rights and responsibilities can be aligned (Rammelt et al., 2023).

The operationalization of the Earth justice framework targets access and allocation means, such as (1) access to information in decision-making, to civic space, legal

remedies, minimum resources, and services, as well as (2) allocation of risks and harms, along with corresponding responsibilities for each of them. For example, access indicators have been established to quantify key material needs for food, water, energy, and infrastructure at two levels: escape from poverty and enabling a dignified life (Rammelt et al., 2023). Once evaluated, the minimum needs are integrated with the current levels of consumption figures and converted to planetary boundaries.

In summary, societal boundaries can become instrumental in promoting societal self-limitation with specifications debated according to sociocultural determinants implying institutional reframing of how goods, services, etc., are produced, distributed, and consumed (Brand et al., 2021). The difficulty with self-limitation at individual level lies in the fact that it has direct consequences: social marginalization or even (self)exclusion. Only a collective decision / option makes societal sense.

It is worth noting that societal boundaries have additional dimensions that embrace contrasting behavioural aspects, such as trust, immoderation, prestige. Of note, human needs expand with knowledge, technology, and material richness.

GENETIC AND BIOCHEMICAL REGULATION OF CAPSAICIN BIOSYNTHESIS IN *CAPSICUM* SPP.

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Abstract

Peppers (*Capsicum* spp.) are widely cultivated and consumed worldwide, with uses ranging from fresh vegetables in human diets to condiments, natural colorants, and medicinal products. Their sensory appeal is largely defined by pungency, a trait perceived differently among individuals but highly valued for both culinary and health-related properties. Pungency in peppers is primarily determined by the accumulation of capsaicin and related capsaicinoids in the fruit. Understanding the biochemical pathways responsible for capsaicinoid biosynthesis and the genetic mechanisms regulating this process has therefore been a major focus of *Capsicum* research. Advances in this area have provided critical insights into key biosynthetic enzymes, regulatory genes, and transcription factors controlling capsaicin production. Such knowledge is essential for pepper breeding programs, as it enables the selection of parental lines with targeted pungency levels. This review synthesizes recent progress in capsaicin biosynthesis, including pathway regulation, associated genes and transcriptional networks, and the development of molecular markers linked to pungency in pepper fruits.

Key words: bioactive compounds, hot pepper, pungency, spiciness.

INTRODUCTION

Pepper (*Capsicum* spp.) is a well-known vegetable, popular for its flavour, cultivated worldwide for fresh fruit and dried as a spice. Originally from tropical and humid regions of South and Central America, peppers are now grown and consumed worldwide. Studies indicate that pepper domestication began in central-eastern Mexico (Kraft et al., 2014). This region has numerous genetic varieties, including local populations with significant value for breeding and high potential as germplasm (Ramirez-Rodas et al., 2026).

After the Columbus expeditions, pepper cultivation spread to Europe and subsequently rapidly expanded worldwide (Koeda et al., 2019).

Hot or sweet, peppers belong to the Solanaceae family. Around the world, there are five domesticated species: *Capsicum annuum* L., *Capsicum bacatum* L., *Capsicum chinense* Jacq., *Capsicum frutescens* L., and *Capsicum pubescens* Ruiz & Pav.; all species are cultivated in numerous countries with variations in shape,

colour, and levels of spiciness (Kraft et al., 2014).

Pungent peppers have different degrees of pungency, and if they are very pungent, they cannot be used as fresh vegetables. Still, they can be used for pain relief, anti-inflammatory detumescence, appetite regulation, and rheumatism prevention, and they have also been proven to be effective as a repellent against pests and are very popular as a green protection pesticide (Zhang W. et al., 2022).

Important for humans' diet, bioactive compounds such as vitamins, anthocyanins, flavonoids, carotenoids, and capsaicinoids from peppers have been described as having protective effects on many aspects of human health. Peppers have been used for ages for their analgesic, anti-obesity, cardioprotective, memory-enhancing, and anti-metastatic activity; diabetic and gastroprotective, antimicrobial, anticancer, pharmacological, and neurological properties (Agarwal et al., 2017; Batiha et al., 2020; Clark & Lee, 2016; de Sá Mendes & Branco de Andrade Gonçalves, 2020;

Hayman & Kam, 2008; Sanati et al., 2018; Singh et al., 2022; Stoica et al., 2016).

Hot peppers are important for human health not only because of their capsaicinoid content and spiciness, but they are also important for other metabolism products like vitamin C, carotenoids, flavonoids, phenols, essential nutrients, and vitamins (Agapie et al., 2020). The high levels of vitamin C and carotene are enough to include peppers in the medicinal plant category (Pandia et al., 2012), and the production of foods that include hot pepper powder is a creative challenge for researchers. Sensory analyses of food products (jelly candy) that contain chili powder were conducted by Pop and colleagues, with good acceptance reviews from consumers (Pop et al., 2014).

Found exclusively in the *Capsicum* genus, capsaicin is an alkaloid responsible for pungency (Palma et al., 2020). Usually, pungency is graded on the Scoville scale, a tool invented by a pharmacologist. The Scoville scale measures the heat units based on the number of times the extracted compounds are diluted in sugar water until the pungency is undetectable. Pure capsaicin can go up to 16×10^6 on the Scoville scale (Hamed et al., 2019).

The most recent studies on capsaicin biosynthesis are compiled in this review, along with the transcriptional networks, pathway regulation, genes linked to pepper pungency, and molecular markers applicable for selecting *Capsicum* pungency levels.

CAPSAICINOID BIOSYNTHESIS

At every fruit development stage in the biochemistry and physiology of peppers, numerous changes occur. Volatile compounds that come with increased respiration and protein synthesis bring changes that include taste and flavour transformations in the later stages of maturation (Hamed et al., 2019).

Capsaicinoids such as capsaicin, dihydrocapsaicin, nordihydrocapsaicin, homodihydrocapsaicin, and homocapsaicin are responsible for pungency in chili peppers. The phenolic compounds responsible for 90% of the pungency are capsaicin and dihydrocapsaicin. Capsaicin (8-methyl-N-vanillyl-6-nonenamide) is a crystalline, lipophilic, colorless, and odorless alkaloid produced in various quantities

among pepper cultivars (Reyes-Escogido et al., 2011).

Capsaicin is found especially surrounding the seeds in placental tissue to protect them from animals. Birds don't feel the pungent taste, and it's easy to disseminate the seeds. Capsaicin is responsible for pungency, for the burning effect, and it produces pain, but capsaicin can also be used to relieve pain by boosting algesic pathways (Fattori et al., 2016). Initially, it was considered that the capsaicinoids were only synthesized in epidermal cells on the septum and stored in placental tissue. New findings reveal that the extremely pungent cultivars produce vesicles containing capsaicinoids on the inner surface of the pericarp (Jang et al., 2021; Park et al., 2019; Zhu et al., 2019).

Two different pathways produce capsaicinoids: the synthesis of vanillylamine by the phenylpropanoid pathway, giving the phenolic structure, and also through fatty acid metabolism derived from L-valine, leading to 8-methyl-6-nonenoyl-CoA (Kaiser et al., 2017; Srebcheva & Kostova, 2022). Their concentrations are related to fruit development (Reyes-Escogido et al., 2011).

Although the Scoville scale is widely known for measuring pungency, the main procedure to determine capsaicin and analogues is high-Performance Liquid Chromatography (HPLC), which allows quantification of capsaicin at under 100 Scoville units. HPLC can be combined with gas chromatography (GC) to determine pungency in raw spices, extracts, and pharmaceutical products (Reyes-Escogido et al., 2011).

The localization of capsaicin is mainly in vesicles of the epidermal cells of the placenta, but some pepper varieties also accumulate capsaicinoids in white ribs or other tissues (Milde et al., 2022). Because some species of pepper are pungent while others are not, environmental conditions are among the main factors influencing the levels of capsaicinoids inside fruits (Arce-Rodríguez & Ochoa-Alejo, 2019; Lagunovski-Luchian et al., 2016). It is known that hydric stress reduces the fruit yield and increases capsaicinoid levels; the fruits are smaller with a higher content of pungent alkaloids (Naves et al., 2019). Water deficit affects the phenylpropanoid pathway and increases capsaicin levels by raising the activity

of enzymes involved in the capsaicinoid biosynthesis pathway (Reyes-Escogido et al., 2011). A high quantity of moisture has the same effect, increasing the pungency levels to protect the seeds from fungi, which are known for thriving under high moisture conditions (Naves et al., 2019).

GENES RESPONSIBLE FOR CONTROLLING THE CAPSAICINOID BIOSYNTHESIS PROCESS

The identification of genes responsible for controlling the capsaicinoid biosynthesis process was performed using genetic fine mapping and biochemistry methods (Sun et al., 2020). Both pathways of synthesizing capsaicinoids were the subject of many studies trying to find the genes responsible for pungency in *Capsicum* spp., mostly by comparing pungent with non-pungent cultivars (Koeda et al., 2019).

Pun1, *PAMT*, *KAS*, and *BCAT* genes were associated with capsaicinoid production and are strongly linked with the level of spiciness in pepper fruits (Tanaka et al., 2017).

The main candidate for pungency seemed to be *Pun1* (Stewart et al., 2007; Stewart Jr et al., 2005), but the latest experiments demonstrated that there are many other genes responsible for the hot pepper's pungency. Understanding the pathways of capsaicin biosynthesis was the key to finding the genes involved in pungency. The reference genome analysis, RNA sequencing, and genome-wide association studies showed that there are many genes involved in capsaicin biosynthesis pathways, but only *Pun1* and *pAMT* have been proven to majorly affect the quantity of capsaicin (Kabita et al., 2019).

Pun1 encodes a BAHD-type acyltransferase, AT3 (capsaicin synthase), and is considered to be capable of catalyzing the amide bond between trans-8-methyl-6-nonenoyl-CoA (derived from branched-chain amino acid biosynthesis) and vanillylamine (derived from the phenylpropanoid pathway), resulting in the formation of capsaicin (Milde et al., 2022). Although it is not the only gene involved, *Pun1* is directly linked to capsaicinoid biosynthesis in its final step. Most non-pungent peppers have non-functional *pun1* alleles, and *Pun1* is also related to the biosynthesis of capsinoids, non-

pungent analogs of capsaicinoids (Han et al., 2019).

In a previous study, it was determined that crossing a non-pungent cultivar with several pungent cultivars will reveal that the loss of pungency is based on a single recessive gene, *CaKRI* (ketoacyl-ACP reductase) involved in the biosynthesis of an intermediate in the branched-chain fatty acid pathway that limits the entire capsaicinoid biosynthesis (Koeda et al., 2019). The regulatory genes responsible for capsaicin metabolism are still being studied, but so far it has been proven that silencing *Pun1* leads to a decreased level of capsaicin in *C. annuum*, *C. Chinense*, and *C. frutescens*, and silencing *pAMT* results in a decreased level of capsaicin in *C. frutescens* and *C. Chinense* (Zhang W. et al., 2022).

The opposite is also available, and crossing the pungency trait from *C. chinense* to a donor of *C. annuum* is possible. The presence of the *Pun1* and *Pun3* alleles from *C. chinense* is shown to significantly increase capsaicin and dihydrocapsaicin in the progeny. Interspecific hybridization between these species can increase pungency in *C. annuum* through dominance and interaction effects (Ahn et al., 2025).

An interesting study was conducted by Rathnayaka et al. (2021), in which two Japanese chilli pepper cultivars were subjected to drought stress. It is known that the levels of capsaicinoids in pepper fruits are related to fruit development and environmental conditions. The study analyzed the levels of capsaicin using the PCR technique for 18 genes involved in the capsaicinoid biosynthesis pathway, grouping the genes into two categories (Table 1). The first group of genes was related to the branched-chain fatty acid pathway, and the second group was related to the phenylpropanoid pathway in capsaicinoid biosynthesis. The results showed that drought stress increased the levels of capsaicinoids only in genes related to the first group. The genes from the second group are related to the biosynthesis of other secondary metabolites, such as lignin, flavonoids, or phenolic acids, and this could be the reason for no significant difference in gene expression (Rathnayaka et al., 2021).

Sixteen of these genes were also studied with two pungent cultivars, relating eight of them to

capsaicinoid variation within the cultivars. All of the genes related to capsaicinoid variation are part of the branched-chain fatty acid pathway (*Pun 1*, *PAMT*, *KAS*, *CaMYB31*, *BCAT*, *CaKR1*, *ACL*, *FAT*) (Kondo et al., 2021)

Numerous genes correlated with capsaicinoid levels were identified by Zhang (2022), who analysed 59 capsaicin synthesis-related genes. Distributed on 11 chromosomes, the genes showed different expression levels depending on the stage of fruit maturity (Zhang W. et al., 2022).

Table 1. Genes encoding proteins involved in capsaicinoid biosynthesis - Rathnayaka et al. (2021)

Pathway	Gene name
Branched-chain fatty acid pathway	<i>ACL</i> - acyl carrier protein
	<i>pAMT</i> - putative aminotransferase
	<i>Pun 1</i> - acyltransferase
	<i>WRKY 9</i>
	<i>CaKR1</i> - ketoacyl-ACP reductase
	<i>CaMYB31</i>
	<i>FAT</i> - ketoacyl-ACP reductase
	<i>KAS I</i> - ketoacyl-ACP synthase
	<i>KAS III</i> - ketoacyl-ACP synthase III
	<i>BCKDH</i> - branched-chain α -ketoacid dehydrogenase
	<i>ACS</i> - acyl-CoA synthase
	<i>BCAT</i> - branched-chain amino acid transferase
Phenylpropanoid synthesis pathway	<i>4CL</i> - α -coumaroyl-CoA ligase
	<i>PAL</i> - phenylalanine ammonia-lyase
	<i>C3H</i> - coumaroyl shikimate/quinic 3-hydroxylase
	<i>HCT</i> - hydroxycinnamoyl transferase
	<i>C4H</i> - cinnamate 4-hydroxylase
	<i>COMT</i> - caffeic acid O-methyltransferase

It was thought that capsaicinoids were exclusively biosynthesized only in the septum and stored in the placental tissue, but lately it was revealed that capsaicinoid accumulation is a quantitative trait regulated by the expression of *BCAT*, *KAS*, *CS*, *KR*, *C4H*, and *FatA* genes, and the same structural genes might control capsaicinoid biosynthesis both in the pericarp and placenta (Park et al., 2019).

New findings suggest that the capsaicinoid accumulation must be evaluated not only by studying the genes within the metabolic pathway, but also by studying the ones involved in cellular processes associated with capsaicinoid accumulation, such as transport and capsaicin storage. Their mechanisms could potentially influence indirect pathways (Burgos-Valencia et al., 2024; Fei et al., 2024).

TRANSCRIPTION FACTORS INVOLVED IN THE BIOSYNTHESIS PROCESS

The transcription factors (TFs) associated with regulatory genes were studied to determine which of them control pungency. Several *MYB* transcription factors and *AP2/ERF* are involved in the capsaicinoid biosynthesis pathway (Díaz et al., 2023).

Analysing the genes involved in regulating capsaicinoid biosynthesis, a pattern emerged, linking genes with higher expression to transcription factors. *KAS*, *PAMT*, and *AT3* genes showed a similar expression profile to *CaMYB31*, suggesting a strong link between them, even though *CaMYB31* was also found in vegetative tissue, indicating additional roles in other biological processes derived from the phenylpropanoid pathway (Arce-Rodríguez & Ochoa-Alejo, 2017; Burgos-Valencia et al., 2020).

Studying genes involved in the ethylene biosynthesis pathway and discovering that they exhibited a similar expression pattern to the genes associated with capsaicinoid biosynthesis, *COMT*, *pAMT*, and *KAS I* (the genes that enhance capsaicinoid biosynthesis) are activated by the *CaMYB108* transcription factor. Also, *CaMYB48* is possible to participate in capsaicinoid biosynthesis, but it has not been fully studied. The gene that induces endogenous ethylene production, *AP2/ERF* with the *CcERF2* transcription factor, is confirmed to be involved and associated with capsaicinoid biosynthesis (Wen et al., 2022).

Belonging to *Ap2/ERF* family, *ERF* transcription factors have an important role in plant growth and development processes. Related to responses to environmental states, the TFs are highly involved in all fruit developmental processes, stress responses, and defence against diseases, and are closely associated with capsaicinoid biosynthesis. Because capsaicinoid biosynthesis is affected by environmental stimuli, 142 members of the *ERF* family identified in pepper were studied, and four of them (*CaERF102*, *CaERF53*, *CaERF111*, and *CaERF92*) were strongly linked to the regulation of capsaicinoid biosynthesis (Song et al., 2020).

MYB is a large and important transcription factor family, including four groups: *4R-MYB*, *3R-MYB*, *R2R3-MYB*, and *MYB-related* subfamilies. *R2R3-MYB* is a dominant group, highly conserved, with 16 duplicated genes involved in the development processes of pepper fruit and ripening, and that makes a valuable contribution to capsaicinoid biosynthesis (Wang et al., 2020). *R2-R3-MYB* regulates multiple processes such as tissue development, abiotic stress response, and its metabolism, and also controls the phenylpropanoid pathway responsible for secondary metabolism products like anthocyanins (Han et al., 2019).

Altering the environmental conditions also alters the expression of key genes involved in capsaicinoid biosynthesis, which affect the levels of spiciness, and this represents important information for breeders. *MYB* transcription factors, especially *MYB31*, are related to Solanaceae-specific *MYB*, which is involved in capsaicinoid biosynthesis. *CaMYB48* plays a crucial role in regulating capsaicinoids by encoding a protein with a transcriptional activator function and can directly activate the transcription of capsaicinoid biosynthesis genes (Sun et al., 2020).

Previous studies have confirmed that *MYB31* and *MYB48* play key roles in capsaicin regulation, one of the precursors for capsaicinoids (Arce-Rodríguez & Ochoa-Alejo, 2017). Silencing *MYB48* reduces the expression of capsaicin biosynthesis pathway genes such as *AT3* and *KAS*, which affect the entire capsaicin biosynthesis. *AT3* gene, called *Pun1* (Srebcheva & Kostova, 2022), is an important point in the synthesis of capsaicin from vanillylamine and 8-methyl-6-nonenoyl-CoA, and *CaMYB37* is confirmed to bind to the *AT3* gene, activating its transcription, and both are linked in capsaicinoid biosynthesis. Liu proved that silencing *CaMYB37* changes the expression of capsaicin biosynthesis genes, and the capsaicin content is reduced (Liu et al., 2022). Studying 14 TFs that control *AT3* gene expression, Zhang et al. determined an undeniable link between the *AT3* gene and pungency in pepper fruit (Zhang B. et al., 2022). Silencing *CaMYB31* showed a significant reduction of capsaicinoid levels for the genes involved in capsaicinoid biosynthesis, such as

Kas, *BCAT*, *C4H*, *Comt*, and *pAMT*. Silencing *Pun1* has the same effect as silencing *CaMYB31*, reducing capsaicinoid production (Han et al., 2019).

Having an important role in response to environmental factors, a study based on drought stress applied to pepper plants determined a significantly higher expression for TFs *CaMYB31* and *WRKY9*, both involved in the branched-chain fatty acid biosynthesis pathway (Rathnayaka et al., 2021).

Recent studies confirm the important role of *R2R3-MYB* transcription factors in the regulatory role in the biosynthesis of secondary metabolites, and analyzing the promoter of the *CcMYB330* gene suggests that hormones and light may have an impact on capsaicin levels in peppers. Silencing *CcMYB330* impacted expression levels of *CcFatA*, *CcHCT*, *CcKAS*, *CcPAL*, and *CcBCAT*, suggesting that *CcMYB330* positively regulates the capsaicin biosynthesis (Cheng et al., 2025).

MYB TFs are related to the regulation of capsaicinoid accumulation, but are also important for the regulation of glucosinolates, carotenoids, and the phenylpropanoid metabolic pathway. Related to capsaicinoid biosynthesis, *CcMYB330* binds to the *CcPAL* structural gene with a positive impact, but *CcMYB4* TFs negatively regulate the expression of structural genes in the capsaicinoid biosynthesis pathway by binding with the *CcPAL2* promoter in *Capsicum chinense* fruits (Yu et al., 2024; Zhang M. et al., 2025).

The bHLH (basic helix-loop-helix) TFs constitute the second largest family of transcription factors and play crucial roles in several growth and development stages. Extensive research shows that bHLHs are highly involved in the regulation of plants' secondary metabolite biosynthesis, linked with salt tolerance in peppers and cold resistance. The *CcbHLH68* is a TF that has been demonstrated to influence the production of capsaicinoids (Cheng et al., 2026).

MOLECULAR MARKERS

Pungency is not fully elucidated at the genetic and molecular levels, but it is important for breeding programs that require the targeted use of compounds found only in *Capsicum*. The

Pun1 gene is highly related to pungency, although it is not the only gene that controls it, and marker-assisted selection helps to develop new lines of peppers and better understand the mechanisms behind pungency. Molecular markers allow the detection of pungency at the early stages of development, but identifying and describing a single, universal marker linked with capsaicinoid compounds is still a complex challenge. Using the PCR technique, specific markers based on detected polymorphism were developed, and *MAP1* was identified as a marker linked with pungency in accessions from *C. annuum*, *C. bacatum*, *C. chinense*, *C. frutescens* and *C. pubescens*, and also in wild species of *Capsicum* (Rodríguez-Maza et al., 2012).

A mutation in a ketoacyl-ACP reductase (*CaKRI* gene) was related to the loss of pungency in *Capsicum*, and using molecular markers to target the gene region with additional markers, also used to narrow down the specific DNA region, helped determine where the gene is located (Koeda et al., 2019).

Trying to find the genes responsible for pungency, a study from Bulgaria used 3 allele-specific primers (*Pun 1-1 fwd1*, *Pun 1-1 fwd2*, and *Pun 1-1 rev*) to identify the allelic state of the *Pun1* gene in parental peppers. Allele-specific markers were used to analyse the influence of the *Pun1* gene on capsaicin synthesis to determine if this could be the only gene responsible for pungency (Srebcheva & Kostova, 2022).

Breeding peppers with different levels of capsaicinoids remains a challenge for researchers, and using genomic selection and molecular markers could improve selection. A study using genome-wide markers and markers from GWAS (genome-wide association studies) was conducted. Using markers associated with QTLs for genes known to influence capsaicinoid biosynthesis, it was determined that they are located within the same QTL block as the capsaicinoid biosynthesis genes (Kim et al., 2022).

QTL mapping and GWAS studies were able to identify loci related to capsaicinoid content in peppers, mostly located on chromosomes 3, 6 and 10. The same locations were used for identifying the genes responsible for capsaicinoid biosynthesis. QTL analysis also searched chromosomes 4, 6, and 11, and genes

related to pungency were identified on chromosome 6. *Erf* (*Ethylene responsive factor*), *Jerf* (*Jasmonic acid and ethylene responsive factor*), *CaMYB31*, *Ankyrin*, and other genes encoding transcription factors are known to be responsible for capsaicinoid levels in the placenta and pericarp. SNPs, insertions, or deletions in exons of the *pAMT* gene generated mutations, and these mutations have been used for marker-assisted selection to control capsinoid contents in peppers, developing new varieties with high capsinoid contents (Jang et al., 2021).

Marker-assisted selection (MAS) is an important technique to select the desired traits from germplasm collections and to reduce breeding times. SSR (Simple Sequence Repeat) markers are highly polymorphic and are used mostly in genome mapping, variety identification, and genetic diversity estimation. SCAR (Sequence-Characterized Amplified Region) markers are used to detect traits of interest in genotypes for breeding programs. For instance, the SCAR marker *BF6-BR8* proved to be linked to capsaicinoid synthesis and pungency (Başak et al., 2022).

GWAS analysis suggests that structural genes in the capsaicinoid biosynthetic pathway determine capsaicinoid content. Next-generation sequencing tools that include whole genome resequencing, genotype-by-sequencing, and RNA-Seq are easier to use if a reference genome is available. Studying two accessions, pungent and non-pungent, it was demonstrated that other loci named *Pun3* are also related to capsaicinoid contents and encode the *CaMYB31* transcription factor, known to regulate *Pun1* expression and other fatty-acid-related genes. *CaMYB31* may be a candidate gene for *Pun3*, with a very important role in regulating the capsaicinoid biosynthetic genes (Han et al., 2019).

The quantitative trait locus *Capsaicinoid1* (*Cap1*), which is identical to *Pun3*, makes a major contribution to pungency accumulation. *Cap1/Pun3* encodes a specific transcription factor, MYB31, with an important role in regulating capsaicinoid levels in peppers, and it is related to *WRKY9*, both contributing to the evolution of highly pungent peppers (Zhu et al., 2019).

The aminotransferase (*pAMT*) encodes a protein in the phenylpropanoid pathway, and a mutation of *pAMT* was associated with the loss of pungency by producing capsinoids instead of capsaicinoids. In a QTL mapping study, the *pAMT* gene is associated with *Pun2*, a locus on chromosome 3 that contains the *pAMT* gene (Yi et al., 2022).

CONCLUSIONS

Capsaicinoid levels are highly dynamic during fruit development, being affected by many factors such as environmental conditions, biotic stress, and nutrient availability.

An important crop, cultivated worldwide for many beneficial traits, especially for human health, peppers are intensely studied for breeding purposes. Understanding pungency, the genes involved in pungency, the genes that encode the transcription factors, and the molecular markers that can be used for selecting the pepper pungency level are hot subjects on researchers' tables. The ultimate step in trying to completely understand and control spiciness has not yet been made, and only time and hard work will reveal the secrets of pungency mechanisms.

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ASSESSING THE CONSERVATION STATUS OF *CRAMBE TATARIA* SEBEÓK IN ROMANIA: DISTRIBUTION PATTERNS AND POPULATION EVALUATION METHODS

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Abstract

Crambe tataria Sebeók (Brassicaceae family) is a rare and vulnerable perennial steppe species with a fragmented distribution in Central and Eastern Europe. It is listed as vulnerable on the IUCN Red List, protected under national legislation, and included in Annex II of the Habitats Directive (92/43/EEC). Our field studies and literature review indicate that the species occurs mainly in steppe and forest-steppe regions in southern and eastern Romania. Population assessments in Romania show a favorable conservation status over three reporting periods to the European Commission. However, our field observations and monitoring reveal considerable variability in monitoring methods among experts, underscoring the need for standardized protocols to ensure accurate population evaluation and more effective conservation of the species. We can specify that, due to the fragility of habitats integrating Crambe and their multiple threats, the over-dimensioned population assessment does not take into account the growing strategies of the species. Therefore, the species remains at risk at the national level, and its conservation status requires urgent clarification to guide effective management measures.

Key words: *Crambe tataria, Brassicaceae, conservation status, distribution pattern, population evaluation methods.*

INTRODUCTION

The overall objective of sustainable development includes maintaining biodiversity while continuing or even encouraging human activities, taking into account economic, social, cultural, and regional aspects. The conservation of wild plant species (as part of the natural heritage of the European Community) is an essential and specific Community objective of preserving, protecting, and improving the quality of the environment, as more and more wild species are endangered. At European level, many species are threatened with extinction (species of Community importance) in the environment described by specific abiotic and biotic factors in which those species are found at any stage of their biological cycle (the habitat of a species) (H.D. 1992). Plant species are influenced by a set of factors that can affect the natural long-term expansion of the species and the abundance of their populations (Crawley, 2009; Silvertown & Charlesworth, 2009). According to the Habitats Directive

(1992), the conservation status of the plant species is good if: the species remains a viable element of its natural habitats based on population dynamics data for the species concerned species; the area of natural distribution of the species is not reduced or threatens to diminish in the near future; the species has and is highly likely to continue to have a habitat sufficiently extensive to maintain its population in the long term.

The conservation status of a species it is revealed by species monitoring (IUCN SSC, 2026) that requires the systematic collection and analysis of recurring observations or measurements to assess variations in condition and progress toward achieving the management goals. Clearly formulated objectives are fundamental to effective monitoring, as they determine what is measured, how accurately, and at what frequency (Elzinga et al., 2001).

Monitoring can be conducted directly, through counts or measures of species performance (cover or reproductive output), or indirectly, by observing habitat conditions, associated species,

or potential threats to the focal species (Marsh & Trenham, 2008).

Monitoring programs are crucial for identifying threatened species, assessing management interventions, and tracking invasive/pest species. Nevertheless, their decentralized nature often limits researchers' ability to obtain a comprehensive understanding of existing monitoring practices (Marsh & Trenham, 2008). Recent research highlights that there is a wide range of methodologies, indicators, and databases currently available to address diverse monitoring requirements. However, there are still significant challenges in both data collection and its application to monitoring and decision-making. These include inconsistent or insufficient methods, tools, and technologies; limited institutional capacity and financial resources; poorly defined indicators and monitoring frameworks; data gaps in existing databases; and restricted access to or exchange of information (IUCN SSC, 2026).

In Romania, there are 56 plant species listed in Annexes II and V of the Habitats Directive (1992) requiring monitoring for establishing the best conservation strategies, and *Crambe tataria* Sebeók (Brassicaceae family) is one of them.

Based on the historical use of *C. tataria* species (and other species from the Brassicaceae family) (source of biofuel applied in agriculture, the food and technical industry, soil bioremediation, etc.), *research* aimed at: the oil composition and some morphological characters (Comlekcioglu et al., 2008), chemical composition and morphological properties, as well as pulping potential (Tutus et al., 2010), seed and fruit fatty acid compositions (Barut et al., 2022) of *C. tataria* stalks growing in Turkey; mass consumption of the underground parts by Hungarian ethnic groups living in the Carpathian Basin (Dénes et al., 2012) and the traditional utilization of the species in the Carpathians from Eastern Europe (including its ecological and conservation status) (Paniagua-Zambrana & Bussmann, 2024).

The resistance of the *C. tataria* species under extreme environmental conditions has been studied by investigating the biological features of the species: the anatomical structure of the vegetative organs (root, stem, leaf) revealing its anatomical adaptations to arid climatic conditions from Western Kazakhstan (Aktobe

region) (presence of a thick cuticle, a developed sclerenchymatic structure, and a robust root system that allows the plant to withstand water scarcity and extreme temperatures) (Turalin et al., 2019); morpho-physiological adaptations (deep roots, reserve organs, vegetative rest) increasing the species' resistance to low temperatures, thus allowing it to survive cold winters from the Ukrainian steppes (Gritsenko, 2025).

Studies developed in the western Kazakhstan/Aktobe region have suggested an active reproductive capacity of the *C. tataria* species but high vulnerability to ecological disruption. Analyses have been conducted on the characteristics of the age stages (juvenile, immature, generative mature, etc.) in the populations, describing the demographic structure and showing the predominance of generatively mature individuals (Kupriyanov et al., 2020a). The individuals of the species are mosaically distributed, and both their natural density and regeneration are influenced by edaphic factors; thus, the researchers emphasise the importance of preserving natural habitats for maintaining their viability (Kupriyanov et al., 2020b).

Other studies applied to *C. tataria* have made important contributions in the field of genetics and biotechnology, highlighting essential aspects of regeneration, genetic variability, and the genetic structure of natural populations. Pushkarova et al. (2018) analysed the regeneration capacity and genetic variability of *C. tataria* in *in vitro* cultures, highlighting that the species can be effectively regenerated through tissue cultures using different types of explant; micropropagation processes do not significantly affect genetic stability, although minor somaclonal variations may occur; *in vitro* techniques may support *ex situ* conservation and controlled multiplication of this rare species. Chokheli et al. (2022) assessed genetic diversity and produced DNA fingerprinting showing moderate genetic variability in the populations of *C. tataria*, indicating a sufficient genetic basis for adaptation but a vulnerability to geographical isolation; the potential use of molecular markers (RAPD, ISSR, SSR) to identify and monitor genotypes; and the importance of maintaining genetic diversity for long-term preservation. Thus, for conserving

natural populations of *C. tataria*: the researchers developed *in vitro* propagation methods for creating more plant material, providing genetically homogeneous regenerated plants, and reintroducing them into its natural habitats. Studies for identifying potential habitats for the restoration of populations of *C. tataria*, and characterizing areas with favourable ecological conditions for reintroduction (steppe, piedmont soils, sunny slopes) have been developed in the Republic of Moldova (Izverscaia & Ghendov, 2020). Here, the first controlled reintroduction of the species nationwide documenting the steps of reintroduction (collection of biological material, cultivation, transplantation, and monitoring of the establishment success) (Izverscaia et al., 2021).

In Crimea, the phytocoenological characteristics of *Crambe* populations, and the structure of plant communities, the composition of accompanying species, and edaphic conditions (Mykhailova, 2013).

Recently reports about the presence of new populations of the species in the Ulytau region (Kazakhstan) (Kubentayev et al., 2024) and the North Dnieper Steppe (Ukraine) (Kyiak, 2025) have determined extending the knowledge regarding the natural distribution area of the species, signalling the need to protect it in the Asian-central steppe ecosystems.

In Romania, *C. tataria* had been included in an assessment and monitoring programme at the national level (Mihăilescu et al., 2015; M.O. 3351, 2023), describing standard monitoring methods, population status assessment criteria, and habitat parameters. At the local level, concrete data on the local distribution and conservation status of the species have been highlighted through the inventory and mapping of *C. tataria* in small protected areas (Bădărău, 2019). Over the years, on Romanian *C. tataria* populations had been realised through a more comprehensive study regarding detailed ecological and chorological research, analysing morphological variation, demographic structure, geographical dispersion, and the influence of environmental factors on density and regeneration (Chirilă, 2021, 2022); the habitat preferences of the species, identifying the optimal conditions for development (southern slopes, limestone soils, xerophilic steppe vegetation) and the causes of population decline

(Chirilă et al., 2022). Farther more, Chirilă et al. (2025) developed the first detailed study on the genetic structure of the populations of *C. tataria* in Romania, using ISSR markers. The results showed: moderate levels of genetic diversity among the analysed populations; significant genetic differentiation between geographic groups, suggesting habitat fragmentation and a possible restriction of genetic flow; and the need for *in situ* and *ex situ* conservation to maintain genetic diversity at the species level.

Our paper aims to develop population assessments in Romania over three reporting periods for the European Commission; the standardization in monitoring methods among experts to ensure accurate population evaluation and more effective conservation of the species.

MATERIALS AND METHODS

This Pontic-Pannonian relict species was first published in *Tataria Hung.*: 7 (1799) is primarily distributed across steppes and meso-xerophilic grasslands in Central and Eastern Europe, extending into Western Asia (Marhold, 2011). The Eur-Asian distribution comprises: Austria, Bulgaria, Central European Russia, Czechia-Slovakia, East European Russia, Hungary, Italy, Kazakhstan, Krym, North Caucasus, NW Balkan Peninsula, Romania, South European Russia, Transcaucasus, Türkiye, Ukraine, West Siberia (RBGK, 2026) (Figure 1).



Figure 1: Distribution of *Crambe tataria* in Eur-Asia (after Euro+Med PlantBase and RBGK, 2026)

In Romania, the species is treated as vulnerable (Dihoru & Dihoru, 1994; Oltean et al., 1994; Witkowski et al., 2003) and rare (Oltean et al., 1994). From a legal perspective, it is present in Appendix I of the Bern Convention (1982), Annex IIb of the Habitat Directives (1992) and Romanian Law 49 (2011) in the Continental (Figure 2a) and Steppe bioregions. Nyárády (1955) publishes one of the first morphological and taxonomic descriptions of the genus *Crambe* in the flora of Romania, establishing the basis of the botanical classification of the *C. tataria* species. He describes *C. tataria* as honey/nectar source plant (very large inflorescence) (Figure 2b), with a very long root (about 20 cm), “thick as an arm”, black-brown, with white, fleshy and sweet (containing starch and sugar), with a multicapitate root collar (Figure 2c) consumed alone as salad and together with leaves as vegetables.

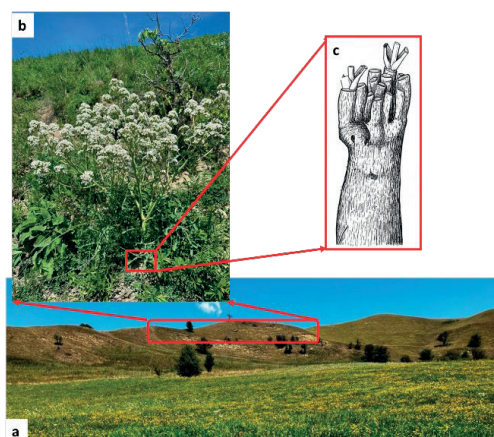


Figure 2: Transylvanian *Crambe tataria* habitat (a) (photo: Onete M.), habitus (b) (photo: Mehedin B.) and morphology of the upper part of the root (c) (after Nyárády, 1955)

For better establishment of the monitoring program, we needed to know more about plant species biology (life history and expectancy, flowering period, annual variability in flowering, etc.). The ontogeny of *Crambe tataria* Sebeók was studied in western Kazakhstan, revealing that the species is a slow-developing perennial (12-30 years to maturity), with age-related stages defined by shifts in root structure and flowering capacity: juvenile (a rosette of 3-4 large leaves, lacking the hard

pubescence typical of adults; often die with the early May heat); immature (prior to the development of the shoot, the thin root system reaching 40 cm deep); virginal (the root system reaches depths of 100-150 cm); generative (young - forming vegetative buds on the shortened shoot (Figure 2c), habits - shortened rosettes produce 1-2 terminal buds from which generative shoots develop; middle/old - vegetative buds are not formed; the underground rhizome often branches into two, rarely three). In arid conditions, the root system might reach 4 m in depth, with branching densities decreasing, and individuals may never bloom (remaining in virginal/non-flowering state indefinitely). In less severe areas, individuals matured (became generative) in 5-7 years, showing high plasticity to resist extreme arid environments. The „tumbleweed” (running) dry former inflorescence bearing seeds is instrumental in ensuring dissemination by wind (anemochory) of the seeds over long distances, a vital adaptation for survival in open steppe habitats (Kupriianov et al., 2020a).

We analysed the publications concerning the *C. tataria* species from Romania (scientific articles, reports, standard data forms, and management plans) to obtain a good estimation of the species distribution and for the evaluation of the methods used by different authors for the species.

According to the Habitats Directive (92/43/EEC), Articles 11 and 17 stipulate that species listed in Annexes II and IV must be regularly monitored, with their conservation status reported to the European Commission every six years. The field assessment and monitoring of the conservation status of the *C. tataria* species of community interest (2007-2023) involved the estimation of the conservation status of the species for three reporting periods (every six years) to the European Commission (2007-2012, 2013-2018 and 2019-2023). We collected the data from the field using data forms that comprised: the location of the individuals (registered with GPS Garmin eTrex Summit), population parameters, habitat characteristics, pressures and threats (P.O.S. project, 2011; Mihăilescu et al., 2015a; 2015b; P.O.I.M., 2023; Annex 1 from M.O. 3351, 2023; Mihăilescu et al., 2024).

RESULTS AND DISCUSSIONS

Our assessments of *Crambe tataria* populations at national level started during the first reporting period (2007-2012) and continue even in the present. Knowledge about the biology, ecology, distribution, population density, and zoology has been highlighted in comprehensive publications over the years (Mihăilescu et al., 2015a, 2015b).

C. tataria found in the ecological (mesoxerophilic-mesophilic species on sunny, dry, and eroded or grassy hills and bushes) (Gafta & Mountford, 2008; Bădărău, 2019; Chirilă, 2021; Chirilă et al., 2022) and developmental optimum in steppe and forest-steppe regions from Romania (Chirilă, 2022; 2023; Chirilă et al., 2022; 2025) (Figure 3).

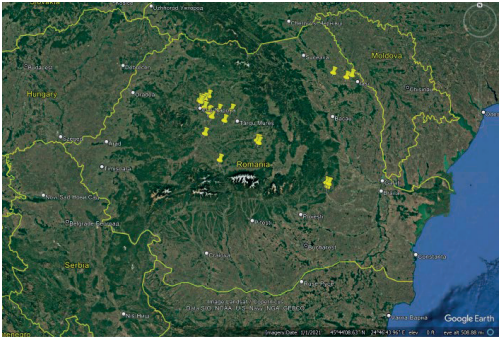


Figure 3: The distribution of *Crambe tataria* in Romania

Chirilă (2021) and our assessment at the national level highlighted that the distribution sites of *C. tataria* populations mentioned in the old Romanian literature are far from being confirmed. The species is presented as being hosted in many Natura 2000 protected areas in Romania (F.C., 2011) (Table 1), showing small populations integrated in different conservation categories (sozology) thus being rare (R), vulnerable (V) or endangered (EN) in all of the protected areas due mainly to anthropic impact (overgrazing, burning the vegetation, etc.). If the factors affecting narrowly distributed populations continue to operate, the species might become: endangered/threatened (its survival is improbable); vulnerable (it almost passes into endangered/threatened); rare (the population of the species is very small and under risk) (Oltean et al., 1994).

The integrative habitat 6240* Sub-pannonic steppic grasslands is largely distributed in Transylvania (Cluj, Mureș, Alba, Sibiu, Brașov) and 62C0* Ponto-Sarmatic steppes is mainly distributed in N-W Romania (Iași, Vaslui, Suceava, Botoșani) since toward Central-South (Galați, Buzău) the habitats change (Table 1).

Table 1. The presence and conservation category (sozology) of *Crambe tataria* in protected area from Romania (according to S.F., 2011)

Protected areas		County	Sozology
Natura 2000 Code	Name		
6240* Sub-pannonic steppic grasslands			
ROSCI0333	Pajiștile Sărmășel - Milaș - Urmeniș	Bistrița Năsăud, Cluj, Mureș	P
ROSCI0238	Suatu -Cojocna - Crairăt	Cluj	R
ROSCI0099	Lacul Știucilor - Sic - Puini - Bonșida	Cluj	R
ROSCI0295	Dealurile Clujului Est	Cluj	P
ROSCI0040	Coasta Lunii	Cluj, Mureș	R
ROSCI0331	Pajiștile Balda - Frata - Miheșu de Câmpie	Cluj, Mureș	R
ROSCI0408	Zau de Câmpie	Mureș	R
ROSCI0079	Fânețele de pe Dealul Corhan - Săbed	Mureș	R
ROSCI0187	Pajiștile lui Suci	Alba	R
ROSCI0211	Podișul Secașelor	Alba, Sibiu	V
ROSCI0093	Insulele Stepice Șura Mică - Slimnic	Sibiu	R
ROSCI0227	Sighișoara - Târnava Mare	Mureș, Sibiu, Brașov	R
62C0* Ponto-Sarmatic steppes			
ROSCI0171	Pădurea și pajiștile de la Mărzești	Iași	EN
ROSCI0080	Fânăturile de la Glodeni	Vaslui	R
ROSCI0286	Colinele Elanului	Vaslui	R
ROSCI0081	Fânețele seculare Frumoasa	Suceava	R
ROSCI0082	Fânețele seculare Ponoare	Suceava	R
ROSCI0399	Suharau - Darabani	Botoșani	R
40C0* Ponto-Sarmatic deciduous thickets			
ROSCI0151	Pădurea Gârboavele	Galati	V
1530* Pannonic salt-steppes and salt-marshes			
ROSCI0272	Vulcanii Noroiși de la Păcelele Mari și Păcelele Mici	Buzau	R

Legend: R – rare; V -Vulnerable, EN – endangered/ threatened.

The distribution of *C. tataria* in these habitats shows its preferences for dry, oligotrophic, and alkaline or saline substrates, sunny slopes. There are major ecological differences between locations, ranging from the xerophilous habitat (Buzău) to the mesophilous conditions (Sibiu), which directly affect plant vigor. Population survival is threatened by the impact of human activities in the surrounding areas, such as the use of pesticides in adjacent crops or habitat fragmentation caused by land-use changes.

Factors such as fires (which destroyed over 90% of the Insulele Stepice Șura Mică - Slimnic site in 2011 in Sibiu County), grazing, and mowing have an immediate and severe impact on population viability.

Repeated and standardized surveys of plant (and animal) populations are essential for evaluating changes in abundance, distribution, and environmental factors, thus supporting conservation and management strategies. Methods such as transect and plot sampling are typically used to quantify population dynamics and overall health (Gibbs et al., 1998). The protocol and methodology for monitoring the conservation status of plant species of community interest have been published as guides (Mihăilescu, et al., 2015 a, b, c; M.O. 3351 - Annex 1, 2023). The description of the field methods is essential for detecting the two key population parameters: population size and the size of the one or more habitats of the species (Higgins et al., 1996). Regarding population size, the following information should be provided: the minimum proportion of the population that must be monitored, the number and size of monitoring plots, as well as the criteria for selecting their locations, the specific parameters (counted or estimated), such as the number of individuals (genets and/or ramets – terms that are not always easy to distinguish). In the case of populations with a limited distribution range and a small number of individuals, it is recommended to count all individuals and measure the entire distribution area (Davy & Jefferies, 1981; Gauthier et al., 2017; Bernardo et al., 2019; McDonald & Thompson, 2004). For populations with a large number of individuals, methodologies usually involve surveying a representative number of plots, so that a significant portion of the population (at least 10%) can be estimated in total (Gibbs et al., 1998; Elzinga et al., 2001; IUCN SSC, 2026). The species fact sheet also includes several practical details for planning field activities: the monitoring frequency within one year or over a six-year reporting cycle; the optimal period for inventorying (months of flowering, fruiting, etc.); the minimum number of people required for conducting the field survey; the number of days per year necessary for the inventory work. Following CSM (2014), we grouped the species in groups accordingly

with their integrative habitats (where they have their ecological optimum for survival and reproduction). The direct and indirect attributes (quantitative features or characteristics) are mandatory. If the attributes are not observed, it indicates unfavourable environmental conditions for the species' existence (Table 2). This group of species grows in habitats with relatively large areas, often grazed or at least occasionally trampled by sheep.

The optimal monitoring period depends on each species' phenology, ideally during the flowering period. The number and placement of monitoring plots depend on the continuity or fragmentation of the habitat within the studied area. The fixed-point photographic method may also be used as a complementary monitoring tool.

Table 2: The attributes, objectives, and evaluation method for *C. tataria* populations (after M.O. 3351, 2023)

Attribute	Objective	Evaluation method
Direct attributes		
Presence / absence (P)	The species must be present	Identification / recording of the species
Population size – number of individuals	At least the minimum size of a viable population; no decline >10% of the total number	Counting of functional individuals
Population size – distribution area	No more than 10% loss from the total distribution area of the population/ species	Mapping
Regeneration success	The number of fruits should not decrease compared to the normal number; at least 10% of the total population should produce fruits, spores, or seeds	Counting fruits or estimating fruit/spore/seed production
Indirect attributes		
Availability of suitable niche	Sufficient area of suitable habitats for maintaining populations; no loss of habitat area or linear extent (rock surfaces, slopes, etc.)	Mapping (area or length)
Negative indicator: physical disturbance / grazing	Grazing or trampling only by wild animals, occasionally	Visual assessment
Negative indicator: shading	Tree / shrub cover should be <10%	Visual assessment
Negative indicator: competition	Accompanying species should not be numerous or overlapping with the target species	Visual assessment
Negative indicator: alien species (plants or animals)	Alien plants and animals have minimal or no presence	Visual assessment (e.g. Braun-Blanquet, DAFOR, etc.)

Our data (location of the individuals, population parameters/attributes, habitat characteristics, pressures and threats) for characterizing the conservation status of *C. tataria* at the national level have been reported to the European Comision at the end of every reporting period (2007-2012, 2013-2018 and 2019-2023). Thus, the European database (EIONET, 2025) allows the comparative analysis of the three reporting cycles (2001-2006, 2007-2013, and 2013-2018) for *Crambe tataria* Sebeók (Brassicaceae) under the Habitats Directive (92/43/EEC, Art. 17) and provides a coherent picture of the species' conservation status evolution in Central and Eastern Europe, highlighting both ecological trends and the effectiveness of the implemented protection measures (Table 2) (EIONET, 2026). The 2001-2006 period represented a baseline stage, characterized by insufficient information and limited geographic coverage. Only small populations were reported in a few countries (Italy, Czech Republic, Hungary, and Slovakia), showing declining trends and degraded habitats. The overall species status was assessed as "U1 – Unfavourable-Inadequate" or "Poor."

During 2007-2013, the reporting range expanded through the inclusion of Eastern European countries (Romania, Bulgaria), where viable populations still existed. More accurate data were collected on population structure and habitat quality, based on standardized monitoring. The species' condition was transitioning between "U1" and "FV", showing initial signs of improvement in the East.

In 2013-2018, the reporting range remained extensive. Populations were stable or increasing in Romania, Bulgaria, and Slovakia, being evaluated as "FV-Favourable" for Romania and Bulgaria. Steppe habitats were well preserved in Eastern Europe, largely due to reintroduction measures (e.g., in the Republic of Moldova) (Izverscaia & Ghendov, 2020; Izverscaia et al., 2021) and improved management of protected areas, contributed significantly to the recovery of the species' conservation status.

The database created by our research team comprises all the necessary characteristics (attributes) for characterizing the conservation status of a species as stipulated in Table 3 (EIONET, 2026a).

Table 3: Evaluation of the conservation status of *C. tataria* at European level (After EIONET, 2026)

ETC/BD treated member states' data																				Period 2001-2006									
MS	Reg	Range (km ²)			Population			Habitat for the species (km ²)				Future prosp.	Overall Assess.	Areas from gridded maps(km ²)			Quality												
		Area	% MS	Trend	Ref.	Size&Unit	% MS	Trend	Ref.	Area	% MS			Trend	Suitable	Range	% MS	Distrib.	% MS	Range	Population	Habitat							
IT	CON	110	100	=	>110	7-7 loc.	100	=	>7	N/A	N/A	N/A	N/A	Good	U1	600	100	300	100	G (12/2006)	M (12/2006)	()							
CZ	PAN	873	64.4	=	873	10000-15000 indiv.	77.2	=	15000	0.94	62.3	X	0.94	Poor	U1	2400	55.10	600	23.50	G (12/2006)	M (2005-2006)	M (12/2006)							
HU	PAN	478	35.3	=	478	2500-3000 indiv.	17	=	3000	0.40	26.4	=	0.50	Poor	U1	1753	40.30	1753	68.70	G (2006)	G (2006)	G (2006)							
SK	PAN	5	0.4	=	5	500-1400 indiv.	5.9	=	1400	0.17	11.2	=	N/A	Good	FV	200	4.60	200	7.80	G (12/2006)	G (12/2006)	G (12/2006)							

Treated data from Member States reports																				Period 2007-2012									
MS	Reg	Range (km ²)			Population			Habitat for the species (km ²)				Future prosp.	Overall assessment				Areas from gridded maps(km ²)												
		Area	% MS	Trend	Ref.	Size&Unit	% MS	Trend	Ref.	Area	% MS		Qual.	Trend	Suitable	Cur. CS	Qualifier	Prev. CS	Nat. of ch.	Range	% MS	Distrib.	% MS						
BG	BLS	800	100	x	800	10-10 indiv.	100	x	>10	0.90	100	m	x	0.90	N/A	U2	=	N/A	800	100	700	100							
AT	CON	100	1.7	0	=100	725-725 indiv.	0.4	=	>725	0.03	N/A	m	=	0.12	N/A	U1	=	N/A	nc	100	8.30	100	10						
BG	CON	600	10	x	600	8-8 indiv.	N/A	x	>8	0.20	N/A	g	x	0.20	N/A	U1	=	N/A	100	50	400	40							
IT	CON	500	8.3	0	=500	3000-3500 indiv.	1.8	=	=3000	N/A	N/A	m	=	N/A	N/A	U1	=	U1	d	500	41.70	500	50						
RO	CON	4800	85	0	=4800	50000-300000 indiv.	97.8	0	=50000	1000	100	m	0	1000	N/A	FV	=	N/A	N/A	N/A	N/A	N/A	N/A						
CZ	PAN	1200	64.9	0	=1200	3708-6788 indiv.	62.7	0	=3708	0.94	64.5	m	0	N/A	N/A	U1	=	U1	nc	1200	70.60	800	53.30						
HU	PAN	500	27.1	0	=500	2500-2700 indiv.	31.1	0	=2500	0.40	27.3	m	0	0.40	N/A	U1	=	U1	nc	500	29.40	500	33.30						
SK	PAN	148.25	8	0	=148.25	200-850 indiv.	6.3	0	=200	0.12	8.2	m	0	N/A	N/A	FV	=	FV	N/A	N/A	200	13.30							
RO	STE	100	100	0	=100	10-100 indiv.	100	0	=10	100	100	g	0	100	N/A	FV	=	N/A	N/A	100	N/A	100	100						

Member States reports																				Period 2013-2018													
MS	Region	Range (km ²)			Population			Habitat for the species			Future prospects			Overall assessment				Distribution area (km ²)															
		Surface	Status (% MS)	Trend	Min	Max	Best value	Unit	Type est.	Method	Status (% MS)	Trend	FRP	Unit	Occupied suff.	Unoccupied suff.	Status	Trend	Range prosp.	Population prosp.	Hab. for sp. prosp.	Status	Cur. CS	Prev. CS	Prev. CS trend	Status Nat. of ch.	CS trend Nat. of ch.	Distrib.	Method	% MS			
BG	BLS	1900	100	=	1900	3	3	N/A	i	minimum	b	100	=	7	I	N	Y	FV	=	unk	poor	poor	unk	U1	U1	=	U2	=	noChange	noChange	600	b	100
AT	CON	100	3.57	=	N/A	N/A	725	i	estimate	a	3.02	x	=	N	N	Y	U1	=	good	poor	poor	unk	U1	U1	=	U1	=	noChange	genuine	100	a	5.56	
BG	CON	1200	62.96	=	1200	4	4	N/A	i	minimum	b	3.02	=	5	I	Y	Y	U1	=	unk	unk	unk	unk	U1	U1	=	U1	=	noChange	noChange	600	b	33.33
IT	CON	500	19.94	=	5000	6000	N/A	i	estimate	a	23.48	=	=	Y	Y	Y	U1	=	poor	poor	good	unk	U1	U1	=	U1	=	knowledge	noChange	500	a	27.78	
RO	CON	1000	35.71	=	8000	30000	N/A	i	estimate	a	72.46	=	35000	I	Y	Y	Y	FV	=	good	good	good	FV	FV	=	FV	=	noChange	noChange	600	a	33.33	
CZ	PAN	1200	91.59	=	1200	3900	5000	N/A	i	interval	a	67.47	=	6000	I	Y	Y	U1	=	good	poor	poor	unk	U1	U1	=	U1	=	noChange	noChange	800	a	50
HU	PAN	600	36.86	=	1150	1800	N/A	i	estimate	a	22.33	=	=	N	Y	Y	U1	=	poor	bad	poor	unk	U1	U1	=	U1	=	genuine	genuine	600	a	37.50	
SK	PAN	148.25	7.61	=	159	1085	N/A	i	estimate	a	9.50	=	=	Y	Y	Y	U1	=	good	poor	poor	unk	U1	U1	=	FV	=	knowledge	knowledge	200	b	12.50	
RO	STE	300	100	=	100	1000	N/A	i	estimate	a	100	=	1000	I	Y	Y	Y	FV	=	good	good	good	FV	FV	=	FV	=	noChange	noChange	200	a	100	

Legend: FV Favourable XX Unknown U1 Unfavourable-Inadequate U2 Unfavourable-Bad

The EU-level biogeographical conservation status of species within each region was determined through an integration of information provided by Member States, or by applying a weighting system to their national assessments. When comprehensive data (i.e. population attributes) were available, they were combined and evaluated using the same criteria employed at the national level. In cases where data were incomplete or inconsistent, the assessment relied on a weighted aggregation of national evaluations, reflecting both the condition and proportion of the species or habitat represented in each country. As with Member State evaluations, the biogeographical status at the EU scale was categorized as favourable, unfavourable–inadequate, or unfavourable–bad (EIONET, 2026b).

The data recorded for the period 2007–2012 (following Romania's accession to the European Union) were scarce and more descriptive; observations regarding the species' ecology, phenology, and growth strategies were made (P.O.S. project, 2011; Mihăilescu et al., 2015a; 2015b). For the reporting period 2007–2013, and 2013–2018 the database improved mainly to the standard database required by the European Commission (P.O.I.M., 2023; Annex 1 from M.O. 3351, 2023; Mihăilescu et al., 2024). The Commission realized that there are inconsistencies in the database due to the monitoring methodology adopted by every reporting country and decided to uniformize the national databases for a better integration in the European database. For the upcoming report (due by 31 July 2025), the European Commission approved in 2023 a standardized reporting format for species types (DG Environment, 2023), that we used for *C. tataria* standard conservation evaluation.

Together with numerical population trends, researchers need to integrate abiotic factors (soil, microclimate) and biotic interactions (pollination or competition with invasive species like for instance black locust shoots). Analysing the structure and dynamics of its habitat determine a better understanding of the persistence of the species (Higgins et al., 1996; Gauthier et al., 2017).

According to the Habitats Directive (1992), Favourable Conservation Status (FCS) implies long-term population viability and a stable

natural range. In Table 3, the European evaluation for the species is marked as Inadequate (U1), reflecting the failure to meet these criteria due to combined anthropogenic and natural pressures. In Romania, the viability of the species' in both bioregions where it is present (Continental and Steppe) is Favourable (FV) due to management measures taken for the protected areas comprising *C. tataria* populations, especially because the integrative habitat are priority (*) meaning that there are natural habitat types in danger of disappearance; thus,, the Community has particular responsibility for their conservation.

CONCLUSIONS

Crambe tataria is a multifunctional species valued for its agricultural, industrial, and ecological applications, including biofuel production and soil restoration. Studies reveal significant biochemical and morphological diversity, as well as strong anatomical and physiological adaptations that enable survival in arid steppe environments. Despite its reproductive potential, populations remain vulnerable to habitat disturbance and soil degradation. Moderate genetic variability and successful in vitro regeneration support both in situ and ex situ conservation approaches. Overall, integrating ecological, anatomical, and genetic insights is essential for effective management and long-term preservation of this rare steppe relict.

This perennial species has a slow developmental cycle, requiring between 12 and 30 years to reach maturity. Its longevity, coupled with the fact that, in arid conditions, plants may remain vegetative and never flower, makes populations extremely vulnerable to ecological disturbances. In Romania, calcareous steppe habitats remain essential yet fragile, requiring continued protection from agricultural intensification and land-use pressures. There are major ecological differences between sites that directly affect plant vigour. Due to its rarity, fragmented, relict distribution, and habitat destruction, *C. tataria* is protected by national and international laws applied in Romania.

The habitats supporting *Crambe tataria* are priority habitats: fragile and facing multiple threats, mainly anthropogenic (overgrazing,

vegetation burning, pesticide use, and habitat fragmentation) that affect *C. tataria*'s survival. Although official reports to the European Commission indicate a favourable conservation status for this species, field observations suggest that these evaluations may be overstated, as they often overlook the species' specific growth strategies. The favourable status reported in Romania is largely due to management measures implemented in protected areas (Natura 2000 sites), where the species' habitats are considered priorities. However, many sites cited in older literature have not been recently reconfirmed. A proper conservation assessment must integrate not only numerical data but also elements such as soil structure, microclimate, and biotic interactions (e.g. pollination or competition with invasive species like *Robinia pseudoacacia*). Although the situation appears stable on paper, the species' biological fragility and the lack of a unified data collection methodology call for an urgent reassessment of its conservation status to guide truly effective management actions. The species remains at risk at the national level.

There is considerable variation in the methods used by different experts, highlighting the need for standardized protocols to ensure accurate population assessments and effective conservation measures.

At European level, between 2001 and 2018, *C. tataria* transitioned from being a "vulnerable and poorly known species" to one with locally favourable status, stable populations, and partially restored habitats in Eastern Europe, while still remaining threatened in the western part of its natural range. This evolution reflects the effectiveness of regional coordination in conservation policies and the continuous monitoring framework established by the Habitats Directive.

The number of reporting countries increased over time. The overall population trend shifted from decline in the first period to growth in Eastern Europe later on. The conservation status evolved from "U1" (Unfavourable) through a transitional phase to "FV" (Favourable) at both local and regional levels.

The general trend indicates slow but steady improvement, supported by greater awareness and better integration of conservation measures into European biodiversity policies.

Monitoring programs play an essential role in identifying and assessing the conservation status of species, as well as in evaluating the effectiveness of management actions. But their different structure makes it difficult for researchers to gain a comprehensive overview of current monitoring efforts. There are varied methods, indicators, and databases to support most species and ecosystem monitoring needs, and the collection and use of data in conservation planning are still constrained by major obstacles such as inconsistent methodologies, insufficient institutional and financial resources, weak or poorly harmonized indicators, incomplete datasets, and restricted access to or exchange of information. To overcome these barriers, it is vital to strengthen institutional and technical capacity within national agencies/institutions, expand data acquisition through coordinated research and conservation projects, align indicators and methodologies, and promote open data sharing in formats accessible to scientists, practitioners, and decision-makers.

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USE OF GENERATIVE ARTIFICIAL INTELLIGENCE (GenAI)

The authors declare the use of Generative Artificial Intelligence (GenAI) tools in the preparation of this manuscript. GenAI was used exclusively for language editing and text refinement and for image clearance (Figure 2c). All scientific content, data analysis, and

interpretations were developed and verified by the authors.

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INFLUENCE OF PHENOLOGICAL STAGE ON BIOACTIVE COMPOSITION IN *MENTHA* × *PIPERITA* L. CULTIVARS 'CORDIAL' AND 'CRISTAL'

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Abstract

Mentha × *piperita* L. is a valuable aromatic species rich in bioactive volatile compounds. This study evaluated the influence of phenological stage and genotype on biomass, essential oil composition, total phenolic content, total flavonoid content, and antioxidant capacity in two cultivars, 'Cordial' and 'Cristal'. Plants were harvested at vegetative growth (BBCH 31-39), bud stage (BBCH 51-59), and full flowering (BBCH 65). Essential oils were analyzed by GC-MS, and antioxidant capacity was related to phenolic content. Results showed that both factors significantly influenced all parameters. 'Cristal' exhibited higher biomass production, while 'Cordial' showed greater accumulation of secondary metabolites and higher antioxidant capacity. Antioxidant activity strongly correlated with total phenolics. These findings highlight the importance of cultivar selection and harvest stage depending on whether biomass yield or phytochemical quality is targeted.

Key words: essential oil, antioxidant capacity, phenolic content, *Mentha* × *piperita*, phenological stage, GS-MS.

INTRODUCTION

The genus *Mentha* (Lamiaceae) is among the most taxonomically complex groups of aromatic plants, comprising approximately 18–19 species and over 11 natural hybrids, characterized by high morphological plasticity and extensive interspecific hybridization (Božović et al., 2015; Mogosan et al., 2017). This genetic and morphological diversity underlies the remarkable variability observed in agronomic performance and chemical composition among mint species and cultivars.

In Romania, peppermint cultivation has a long-standing tradition dating back to 1908, encompassing economically important species such as *Mentha piperita*, *M. spicata*, and *M. suaveolens* (Moldovan & Oprean, 2014). Breeding programs conducted at the Fundulea station have resulted in the development of high-performance cultivars including 'Cordial', 'Columna', 'Cristal', 'Coral', 'Mencris', and 'Record', characterized by high menthol content

and productivity. Among these, 'Cordial' and 'Cristal' are widely cultivated due to their stable oil quality, elevated menthol levels, and resistance to fungal diseases. 'Cordial', registered in 1989, is a medium-tall plant with robust purple stems and elongated leaves, producing 98-150 q ha⁻¹ fresh herbage, with an essential oil content of 2.0-4.3% and menthol concentrations ranging from 49-70%. 'Cristal', registered in 2000 and derived from 'Cordial' × *Mentha arvensis* hybrids, exhibits a compact growth habit, strong regenerative capacity, and resistance to rust and powdery mildew, with productivity of 95-170 q ha⁻¹ fresh herbage, essential oil content of 4.4-4.9%, and menthol levels of 49-75%, making it suitable for industrial steam distillation and mechanized harvesting systems (Păun et al., 1988). The chemical diversity and high menthol content of these cultivars directly influence both yield and pharmacological properties, highlighting the link between breeding, cultivation, and industrial and therapeutic applications.

The diversity of the genus is also reflected in the pronounced chemical polymorphism of its essential oils, typically classified into menthol-, menthone-, carvone-, or linalool-type chemotypes, each derived from distinct biosynthetic pathways (Božović et al., 2015). Peppermint essential oil is a complex mixture of volatile compounds, predominantly monoterpenes and phenolic derivatives, with menthol and menthone as the principal bioactive constituents. Variability in volatile composition, including compounds such as α -terpinolene and terpinen-4-ol, further expands its functional profile and industrial applicability (Ali et al., 2019; Pintilia et al., 2025).

In addition to volatile fractions, *Mentha* species accumulate significant amounts of non-volatile phenolic compounds, particularly phenolic acids and flavonoids, which substantially contribute to biological activity. The antioxidant mechanisms of *Mentha* polyphenols involve free radical scavenging through hydrogen atom or electron donation, inhibition of lipid peroxidation, and chelation of pro-oxidant metal ions (Dorman et al., 2003; Shan et al., 2005). Rosmarinic acid and flavonoid derivatives have also been shown to modulate endogenous antioxidant defense systems and reduce oxidative stress markers in biological models (Kapp et al., 2013). Consistently, a positive correlation has been reported between total phenolic and flavonoid content and the overall antioxidant capacity of mint extracts (Mimica-Dukić et al., 2003).

The accumulation of both volatile and non-volatile metabolites in *Mentha* is strongly influenced by genotype, environmental conditions, cultivation practices, and developmental stage. Variations in light intensity, temperature, and water availability can significantly affect phenylpropanoid and terpenoid metabolism, thereby altering antioxidant potential and essential oil composition (Shan et al., 2005). Biomass production is generally higher under conventional systems, whereas organically cultivated peppermint often exhibits increased essential oil content, likely due to moderate stress stimulating secondary metabolism (Oroian et al., 2017). Climatic variability, particularly elevated temperatures, may enhance oil biosynthesis but also induce qualitative changes, such as reduced menthol content and increased pulegone or menthyl acetate levels, alongside

significant shifts in menthone and menthofuran concentrations (Heydari et al., 2018).

Phenological stage represents another critical factor influencing both yield and chemical quality. Essential oil composition and content can vary substantially depending on harvest timing, with the flowering stage frequently associated with maximal oil accumulation and modifications in the relative proportions of key monoterpenes such as menthol and menthone (Sourestani, 2016). Moreover, combined hydric and thermal stress may impair shoot regrowth and canopy development, preventing plants from reaching technological maturity for subsequent harvests and ultimately reducing productivity.

Due to their flavoring properties and a wide spectrum of biological activities, *Mentha* species are extensively used in the food, pharmaceutical, and cosmetic industries. Peppermint essential oil exhibits antimicrobial, antioxidant, neuroprotective, anti-inflammatory, and therapeutic effects (Mogosan et al., 2017; Hudz et al., 2023). Pharmacological studies have demonstrated antispasmodic, carminative, and sedative properties, smooth muscle relaxation relevant to irritable bowel syndrome, as well as cardioprotective, hypotensive, and hypolipidemic effects associated with antioxidant mechanisms (McKay & Blumberg, 2006; Hudz et al., 2023; Malcheva et al., 2023). Despite the recognized industrial and medicinal importance of peppermint, relatively few studies have systematically evaluated how phenological development influences essential oil composition, alongside the accumulation of other bioactive compounds such as polyphenols and flavonoids. A comprehensive understanding of these variations is essential for optimizing harvest timing, improving oil quality, and maximizing therapeutic potential.

Therefore, the present study aimed to evaluate the influence of developmental stage on essential oil composition, biomass production, and the concentration of key constituents such as menthol and menthone in the peppermint cultivars 'Cordial' and 'Cristal'. Additionally, total polyphenol and flavonoid contents were assessed in relation to antioxidant capacity, in order to clarify how phenological development modulates the industrial and therapeutic value of these cultivars.

MATERIALS AND METHODS

Plant material

The plant material consisted of two Romanian peppermint (*Mentha piperita* L.) cultivars, 'Cordial' and 'Cristal', both developed through national breeding programs. The plants were grown at the National Agricultural Research and Development Institute Fundulea (NARDI Fundulea), Romania, in the experimental field dedicated to the maintenance and renewal of the medicinal and aromatic plant germplasm collection.

The experimental plots were arranged in a randomized complete block design with three replicates per cultivar, each plot having a harvestable area of 5 m². Planting followed standard agronomic practices recommended for peppermint cultivation to ensure uniform plant density and optimal soil moisture conditions. Two planting dates were included to evaluate

potential interactions between planting time and phenological development.

Harvesting was conducted four times during the 2025 growing season, corresponding to key phenological stages: *intensive vegetative growth* (BBCH 31-39), *floral bud stage* (BBCH 51-59), and *full flowering*, and full flowering stage (25 June). Fresh herbage yield was recorded for each plot at each harvest, and cumulative yield was subsequently calculated.

The characterization of fresh mint leaves was carried out at the Research Center for Studies of Food Quality and Agricultural Products, from the University of Agronomic Sciences and Veterinary Medicine of Bucharest.

The methods, summarized in Table 1, included the determination of shoot density, fresh mass, dry matter content, total phenolic and flavonoid contents, essential oil yield, essential oil composition, and antioxidant capacity.

Table 1. Analytical methods used for the determination of biometric, phytochemical, and antioxidant parameters

No.	Property	Symbol	Unit	Determination Technique / Equipment	Reference
1	Shoot density	S	Shoots m ⁻²	Counting shoots within a single 1 m ² quadrat;	Kent, 2012
2	Fresh mass	FM	g m ⁻²	Aerial biomass harvested from a single 1 m ² ; weighed immediately using an analytical balance	FAO, 2006
3	Dry mass	DM	%	Oven-drying at 105°C until constant mass	AOAC 934.01, 2016
4	Total phenolic content	TPC	mg gallic acid equivalent (GAE) g ⁻¹	Folin–Ciocalteu method; UV–Vis spectrophotometer (760 nm)	Perez et al., 2023
5	Total flavonoid content	TFC	mg rutin equivalents (RE) g ⁻¹	AlCl ₃ colorimetric assay; UV–Vis spectrophotometer (510 nm)	Chang et al., 2002
6	Essential oil yield	EO	% (mL/100 g)	Hydrodistillation (3 h) using a Clevenger apparatus	Adams, 2007
7	Essential oil composition	EOC	%	Gaz chromatography (GC-MS)	Ion et al., 2020
8	Antioxidant capacity	AC	mg trolox equivalents (TE) g ⁻¹	DPPH assay; UV–Vis spectrophotometer (515 nm)	Bujor et al., 2016

All replicated measurements, namely total phenolic content, total flavonoid content, and antioxidant capacity, were performed in triplicate, and the results are presented as mean ± standard deviation (SD). In contrast, shoot density, fresh mass, dry mass, essential oil yield, and essential oil composition were each determined from a single measurement. Accordingly, these latter data are reported descriptively, and no inferential statistical analyses were applied; therefore, they should be interpreted as indicative.

RESULTS AND DISCUSSIONS

Growth and biomass of peppermint cultivars

During the intensive vegetative growth stage, the 'Cristal' cultivar exhibited a higher shoot density (393 shoots m⁻²) compared with 'Cordial' (235 shoots m⁻²), whereas 'Cordial' showed slightly higher fresh and dry biomass per plant (Table 2). This suggests a genotype-specific allocation strategy, with 'Cristal' investing more in shoot proliferation and 'Cordial' allocating resources toward greater

biomass accumulation per shoot. Such variability among mint genotypes is consistent with the high phenotypic plasticity reported for *Mentha* species (Telci et al., 2004). At the bud formation stage, both cultivars demonstrated a marked increase in fresh biomass, particularly ‘Cristal’, which reached 3,923 g m⁻² fresh mass compared with 2,221 g m⁻² in ‘Cordial’. This stage corresponds to an optimal period for vegetative growth under favorable climatic conditions, characterized by longer daylength and adequate water availability (Telci et al., 2004). However, dry mass values were lower than those observed at flowering, reflecting the higher water content of plant tissues during this phase. These results agree with Zheljazkov et al. (2010), who reported that the first harvest at bud formation typically provides the highest fresh herbage and essential oil yields due to optimal regrowth conditions and sufficient physiological time for biomass accumulation.

Table 2. Peppermint growth and biomass at different growth stages

Cultivar	Shoots (m ⁻²)	Fresh mass (g m ⁻²)	Dry mass (%)
Intensive growth (BBCH 31-39)			
‘Cristal’	393	500	22.92
‘Cordial’	235	560	24.92
Bud formation (BBCH 51-59)			
‘Cristal’	308	3,923	15.52
‘Cordial’	194	2,221	17.54
Flowering (BBCH 65)			
‘Cristal’	280	1,685	38.87
‘Cordial’	235	2,313	33.33

During the flowering stage, both cultivars showed a reduction in shoot density, accompanied by decreased fresh mass but a pronounced increase in dry mass, particularly in ‘Cristal’ (38.87%). This pattern reflects progressive tissue lignification and a higher proportion of structural dry matter, a common phenomenon in advanced phenological stages. Similar trends were reported by Nazem et al. (2019), who observed lower fresh herbage yields at later growth stages despite increased dry matter concentration. Additionally,

Zheljazkov et al. (2010) demonstrated that harvesting at flowering generally results in lower essential oil yields compared with bud formation, largely due to reduced regrowth capacity and shorter recovery periods.

Bioactive compound variation

During the intensive vegetative growth stage, the ‘Cordial’ genotype exhibited a markedly higher total phenolic content (15.17 ± 0.13 mg GAE g⁻¹) compared with ‘Cristal’ (6.64 ± 0.42 mg GAE g⁻¹), suggesting an early activation of secondary metabolism in ‘Cordial’, likely associated with antioxidant protection of rapidly developing tissues (Figure 1). Phenolic compounds play a key role in protecting young plant organs against oxidative stress and environmental constraints (Farnad et al., 2014; Dirar et al., 2019).

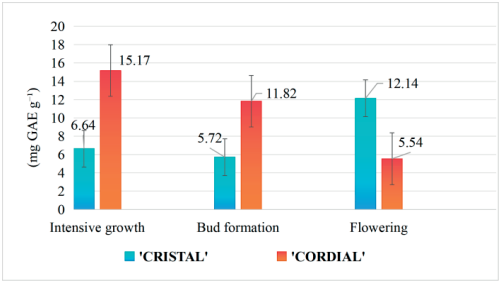


Figure 1. Total phenolic content of fresh mint leaves. Columns represent mean values (N = 3); bars indicate $\pm$ standard deviation (SD)

At the bud formation stage, TPC increased in ‘Cristal’ (5.72 ± 0.58 mg GAE g⁻¹), while ‘Cordial’ showed a decrease (11.82 ± 0.10 mg GAE g⁻¹). This shift reflects a genotype-specific metabolic response during the transition from vegetative to reproductive growth. Phenolic biosynthesis is strongly influenced by developmental stage, and the onset of reproductive development may stimulate phenolic accumulation as an adaptive response to increased physiological stress (Kaddour et al., 2022; Abidi, 2025).

During flowering, ‘Cristal’ reached its highest TPC value (12.14 ± 0.46 mg GAE g⁻¹), whereas ‘Cordial’ recorded its lowest level (5.54 ± 0.38 mg GAE g⁻¹). In ‘Cristal’, phenolic synthesis appears enhanced toward plant maturity, likely as a protective mechanism against elevated oxidative stress associated with higher

temperatures and increased metabolic demand. Similar responses have been observed in *Mentha spicata* subjected to thermal and water stress, where polyphenol accumulation increased under adverse conditions (Katar et al. 2017).

In southeastern Romania, a maximum air temperature of 30°C recorded in May, combined with total monthly precipitation of 89 mm (INCDA Fundulea weather station), can create conditions conducive to moderate thermal and transient water stress, depending on rainfall distribution and soil water-holding capacity. Although 89 mm may appear sufficient at the monthly scale, uneven rainfall distribution or short dry intervals between precipitation events can limit effective soil moisture availability during active vegetative growth.

Considering total flavonoid content, the ‘Cordial’ cultivar exhibited substantially higher levels ($164.27 \pm 4.79 \text{ mg RE g}^{-1}$) than ‘Cristal’ ($51.80 \pm 2.17 \text{ mg RE g}^{-1}$) during the intensive vegetative growth stage, indicating an early activation of flavonoid biosynthesis likely associated with the protection of rapidly expanding tissues against oxidative stress (Figure 2). Comparable trends have been reported in other species (*Morus alba*, *Cyperus rotundus*, *Blepharis linariifolia*), where young organs accumulate elevated flavonoid concentrations as a defensive strategy against environmental and oxidative challenges (Dirar et al., 2019).

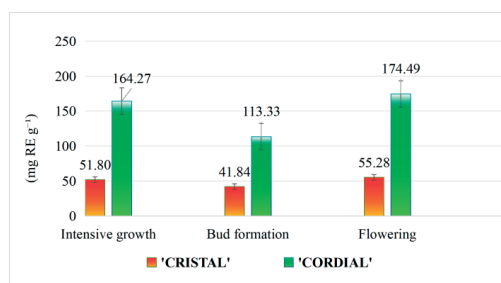


Figure 2. Total flavonoid content of fresh mint leaves. Columns represent mean values (N = 3); bars indicate $\pm$ standard deviation (SD)

At the bud formation stage, TFC decreased in both cultivars; however, ‘Cordial’ maintained higher levels ($113.33 \pm 2.26 \text{ mg RE g}^{-1}$) than ‘Cristal’ ($41.84 \pm 3.38 \text{ mg RE g}^{-1}$). This pattern reflects genotype-dependent modulation of secondary metabolism during the transition

from vegetative to reproductive growth. Flavonoid biosynthesis is highly sensitive to developmental metabolic reprogramming and the plant’s capacity to synthesize phenolic derivatives under changing physiological demands (Farnad et al., 2014).

During the flowering stage, TFC increased again, reaching maximal values in ‘Cordial’ ($174.49 \pm 6.38 \text{ mg RE g}^{-1}$), whereas ‘Cristal’ showed a moderate increase ($55.28 \pm 4.29 \text{ mg RE g}^{-1}$). The late-stage accumulation in ‘Cordial’ may confer enhanced tolerance to oxidative stress associated with reproductive development. Such trends are consistent with previous studies linking elevated flavonoid production at advanced developmental stages to stress-defense mechanisms and intensified secondary metabolism (Dirar et al., 2019).

Antioxidant capacity

Antioxidant capacity (Figure 3) was influenced by both phenological stage and genotype. In the ‘Cristal’ cultivar, antioxidant capacity increased markedly from $34.50 \pm 1.27 \text{ mg TE g}^{-1}$ during intensive growth to $101.89 \pm 4.83 \text{ mg TE g}^{-1}$ at bud formation, followed by a substantial decline to $54.52 \pm 1.98 \text{ mg TE g}^{-1}$ at flowering. In contrast, ‘Cordial’ consistently exhibited considerably higher values across all developmental stages, reaching $186.57 \pm 1.04 \text{ mg TE g}^{-1}$ during intensive growth, $183.64 \pm 4.15 \text{ mg TE g}^{-1}$ at bud formation, and $162.46 \pm 1.12 \text{ mg TE g}^{-1}$ at flowering.

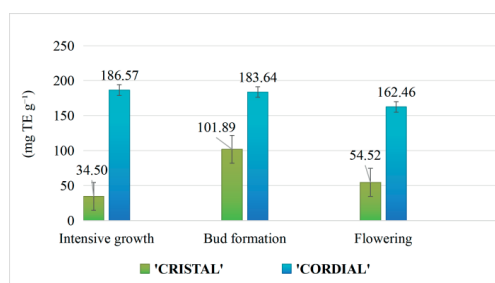


Figure 3. Antioxidant capacity of fresh mint leaf extracts. Columns represent mean values (N = 3); bars indicate $\pm$ standard deviation (SD)

Antioxidant capacity reached its maximum at the bud formation stage in ‘Cristal’, whereas in ‘Cordial’ the highest values were recorded during intensive vegetative growth and remained comparably high at bud formation, followed by a decline at flowering. Throughout

all phenological stages, 'Cordial' maintained substantially greater antioxidant capacity than 'Cristal'. This stage-dependent pattern likely reflects enhanced accumulation of phenolic compounds in young, metabolically active tissues, followed by metabolic reallocation toward reproductive development. Comparable developmental trends have been reported in *Mentha* species (Božović et al., 2015; Jianu et al., 2021).

Total phenolic content, total flavonoid content, and antioxidant capacity exhibited clear genotype- and stage-dependent variation. During intensive vegetative growth, 'Cordial' showed substantially higher phenolic and flavonoid levels than 'Cristal', corresponding to markedly greater antioxidant capacity. At bud formation, antioxidant capacity peaked in 'Cristal' despite comparatively lower phenolic concentrations, suggesting the contribution of additional antioxidant constituents, while 'Cordial' maintained consistently high activity. During flowering, 'Cordial' reached maximal flavonoid levels and retained superior antioxidant capacity, whereas 'Cristal' showed a weaker correspondence between phenolic content and AA. Overall, antioxidant capacity was more closely associated with flavonoid accumulation in 'Cordial', while in 'Cristal' the relationship appeared less direct and likely influenced by other bioactive compounds.

Environmental stress during early vegetative stages may further stimulate phenolic biosynthesis, enhancing antioxidant potential, as observed in *Mentha spicata* grown under arid conditions (Kaddour et al., 2022). The consistently higher antioxidant capacity in 'Cordial' suggests genotype-dependent differences in secondary metabolism, consistent with previous findings in *Mentha* cultivars (Jianu et al., 2021). The lower values recorded in 'Cristal', particularly at flowering, likely reflect reduced phenolic accumulation at this stage.

Phenological stage also strongly affected biomass production and essential oil characteristics. Vegetative stages were associated with higher shoot density and fresh biomass, especially in 'Cristal', whereas the transition to reproductive development corresponded with intensified secondary metabolite synthesis. Such shifts are consistent

with reports indicating that metabolic flux is redirected from primary growth to essential oil biosynthesis during reproductive phases (Zheljazkov et al., 2010; Oroian et al., 2017).

Essential oil yield and composition

The data show that essential oil yield varies both with cultivar and phenological stage. In the early vegetative stage, 'Cordial' exhibits slightly higher EO content (0.62%) than 'Cristal' (0.54%), suggesting that 'Cordial' initiates oil biosynthesis earlier during leaf expansion, likely reflecting a genotype-dependent activation of monoterpene pathways.

At the bud formation stage, EO yield decreases in 'Cristal' (0.41%) while remaining relatively stable in 'Cordial' (0.60%). This divergence may indicate a temporary metabolic shift in 'Cristal', where assimilates are preferentially allocated toward biomass and reproductive organ development, temporarily reducing oil accumulation. In contrast, 'Cordial' maintains a more stable oil production during this stage, reflecting a consistent investment in secondary metabolism.

During flowering, EO yield increases markedly in 'Cristal' (1.52%), surpassing 'Cordial' (1.10%). This trend highlights that flowering is a critical phenophase for essential oil accumulation, particularly in 'Cristal', when metabolic flux is redirected toward the production of volatile compounds. The higher oil content at flowering may be associated with increased monoterpene synthase activity and enhanced metabolic demand linked to reproductive growth (Rasooli et al., 2002; Singh et al., 2009; Verma et al., 2010).

The analysis of volatile oil composition revealed pronounced qualitative and quantitative differences between the two cultivars, indicating distinct chemotypic orientations (Figure 4). In 'Cordial', oxygenated monoterpenes dominated throughout all phenological stages, with menthol and menthone as the principal constituents (Figure 4a). Menthol content increased progressively from the intensive growth stage (33.77%) to flowering (46.19%), whereas menthone remained relatively stable (28.54-31.03%). This shift toward higher menthol levels at flowering is consistent with reports showing that menthol accumulation intensifies from early to late bloom, while

menthone, its biosynthetic precursor, tends to stabilize or decline as enzymatic conversion progresses (Grulova et al., 2015). A moderate increase in menthofuran at the bud formation stage (2.88%) suggests a temporary diversion of metabolic flux toward secondary side-pathways, a phenomenon associated with developmental stage and environmental factors such as temperature and water availability (Taherpour et al., 2017). Sesquiterpenes, including caryophyllene and germacrene D, were present only in low proportions (1.91% and 0.97%, respectively), confirming that ‘Cordial’ retains a classical “menthol-type” peppermint oil profile comparable to those described by Mahboubi & Kazempour (2014). The presence of D-limonene, isomenthone, neo-menthol, ($\pm$)-menthol, isomenthol, neoisomenthol (*tentative identification*), piperitone, and menthyl and isomenthyl acetates further supports a strong metabolic flux toward menthone reduction and menthol ester formation, characteristic of high-quality peppermint oil.

In contrast, ‘Cristal’ exhibited a markedly different chemical profile (Figure 4b), dominated by linalool across all phenological

stages (50.55-65.25%), indicating a chemotype distinct from ‘Cordial’. Genotype-driven variation in essential oil composition is widely reported in *Mentha* species and is attributed to genetic regulation of terpene synthase activity (Nazem et al., 2019). ‘Cristal’ was characterized by compounds associated with the carvone biosynthetic pathway, including 1,8-cineole, γ -terpinene, (-)-borneol, terpinen-4-ol, cis-dihydrocarvone, citronellol, pulegone, carvone, geranial, neodihydrocarveol (*tentative identification*), and dihydrocarvyl acetate. Carvone content increased substantially from the intensive growth stage (5.81%) to bud formation and flowering (~16.5%), suggesting activation of the limonene–carvone metabolic route. Such developmental shifts in monoterpene composition are consistent with previous studies demonstrating that essential oil profiles vary according to phenological stage and metabolic demand (Grulova et al., 2015). The relatively low menthone and menthol levels in ‘Cristal’ further emphasize its divergent biosynthetic orientation compared with traditional menthol-type peppermint oils (Taherpour et al., 2017).

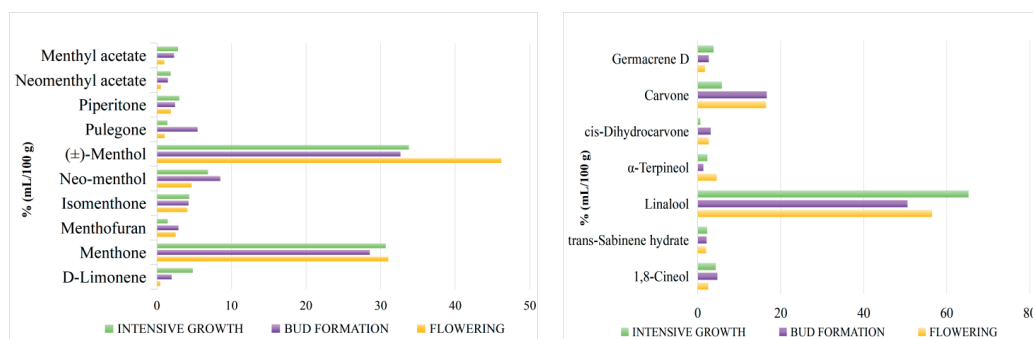


Figure 4. Main volatile compounds of a) ‘Cordial’ and b) ‘Cristal’

Across both cultivars, early growth stages were characterized by a greater diversity of minor monoterpenes, including α -pinene, β -pinene, myrcene, and limonene, which declined toward flowering. This pattern reflects the preferential accumulation of monoterpenes in young, actively expanding leaves, consistent with observations in peppermint showing that leaves older than three weeks contribute less to essential oil biosynthesis (Grulova et al., 2015). A gradual reduction in certain sesquiterpenes, such as germacrene D and β -caryophyllene, at

later stages aligns with evidence that environmental conditions and light quality can modulate farnesyl pyrophosphate FPP-derived biosynthetic pathways (Maffei et al., 1999). Additional differences were evident in the sesquiterpene fraction, with ‘Cristal’ containing aromadendrene, alloaromadendrene, and γ -cadinene, whereas ‘Cordial’ was characterized by (E)- β -farnesene, cubenol, globulol, viridiflorol, and T-murolol. Although these compounds occurred in lower proportions than monoterpenes, their distinct distribution further

supports genotype-dependent regulation of terpenoid biosynthesis.

CONCLUSIONS

The present study demonstrates that both phenological stage and genotype significantly influence biomass production, essential oil composition, and antioxidant capacity in peppermint cultivars. Vegetative stages favored biomass accumulation, particularly in 'Cristal', whereas secondary metabolite synthesis was more pronounced during early growth and bud formation, especially in 'Cordial'. Antioxidant capacity closely followed phenolic content dynamics, confirming the strong relationship between total polyphenols and radical scavenging capacity.

Clear genotypic differences were observed: 'Cordial' exhibited superior essential oil quality and antioxidant potential, while 'Cristal' showed greater vegetative vigor and biomass productivity during intensive growth and bud formation. Environmental conditions further modulated these responses, enhancing essential oil accumulation but limiting regrowth capacity. These findings highlight the importance of selecting appropriate cultivars and harvest timing according to the intended production objective (biomass vs. phytochemical quality).

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QUANTITATIVE ANALYSIS OF HUMAN PRESSURE AND CLIMATE ARIDITY IN THE PESCEANA RIVER BASIN, SOUTHWEST OLTENIA, ROMANIA

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Abstract

Quantitative analysis of aridity and drought at the local and regional levels has gained significance in recent years due to the impacts of global warming and variable precipitation, as well as their impact on the quality of life. In this study, a quantitative analysis of the temporal evolution of aridity conditions was performed in the Pesceana River Basin, Southwest Oltenia, Romania, using the De Martonne aridity index (AI_{DM}). Statistical data on the number of inhabitants in 2024 and the area occupied by forest in 2014 are taken from the Tempo online database, and data on temperature and precipitation for the period 2015-2024 are taken from the National Meteorological Administration (ANM) and other sources. According to the results of the AI_{DM} investigation, the Pesceana River Basin exhibits very humid and extremely humid climatic types, characterizing this location as having high humidity, abundant vegetation, and, often, high temperatures, which create a suffocating environment. At the same time, the I_{nat} results show that the basin's rural area is mostly characterized by landscapes with a moderate balance affected by anthropogenic intervention over time. Specifically, I_{nat} values between 20% and 40% were obtained for the six administrative-territorial units in this area.

Key words: AI_{DM} , I_{nat} , precipitation, temperature, human pressure on the environment.

INTRODUCTION

Human pressure on the environment, commonly referred to as a threat to biodiversity, is an action taken by individuals with the potential to harm nature (Martins et al., 2012).

Furthermore, according to Ionuș et al. (2011), anthropogenically induced transformations can have a significant influence on the environment and, especially, on the relief; the increasing vulnerability of land to dangerous geomorphological phenomena represents one of the most pressing problems that local communities currently face.

According to the literature, arid areas cover approximately 41% of the Earth's land surface (Yao et al., 2020; Coleine et al., 2024), and, according to estimates, by 2100, their area could further increase by 12% to 23% (Schlaepfer et al., 2017; Yao et al., 2020; Krivoguz, 2025).

Aridity is notable in the extra-Carpathian regions of Romania, mainly in the plains and

plateaus of the country. Increases in aridity in these regions are triggered by two factors: (1) the constant increase in temperature and (2) potential evapotranspiration (Vlăduț & Licurici, 2020). In Romania, aridity has been highlighted and investigated by Croitoru et al., (2013) in the extra-Carpathian regions (Croitoru et al., 2013); by Paltineanu et al., (2007) and Vlăduț et al. (2017) in the south-eastern and southern regions of Romania (Paltineanu et al., 2007; Vlăduț et al., 2017); by Dragotă et al. (2011), Peptenatu et al. (2013), Prăvălie (2013), and Vlăduț (2010) in the Oltenia region (Vlăduț, 2010; Dragotă et al., 2011; Peptenatu et al., 2013; Prăvălie, 2013); by Lungu et al. (2011), Prăvălie & Bandoc (2015), and Tiscovschi et al. (2013) in the Dobrogea region (Lungu et al., 2011; Tiscovschi et al., 2013; Prăvălie & Bandoc, 2015); by Pelin (2016) in Moldova (Pelin, 2016); and by Lungu et al. (2012) for the Danube Delta.

AI_{DM} is most typically used in studies that fall under the fields of climatology/bioclimatology,

agricultural and land resource management, and water resource management. This index is also extremely useful in environmental assessments (Nistor & Mîndrescu, 2019; Cutini et al., 2021; Dimkić et al., 2022; Jafarpour et al., 2023; Gur et al., 2024).

The present research focuses on the temporal evolution of aridity in the Pesceana River Basin, Southwest Oltenia, Romania, using AIDM. The main motivation for choosing to investigate this aridity index is its ability to simultaneously evaluate two research variables, namely temperature and precipitation. This ability is supported by recent research that confirms its value for investigating aridification not only at the national and global levels but also regionally and locally (Dikareva et al., 2021; Heidarizadi & Jamali, 2022).

Previous studies on the Pesceana River Basin have focused on the use of GIS techniques for evaluating land use (Măceşeanu et al., 2026) and investigating forest habitats and plant associations (Măceşeanu & Făgăraş, 2023), while others have quantified invasive plant species in European habitats (Măceşeanu & Făgăraş, 2024). Thus, this study represents the first comprehensive quantitative analysis of human pressures and climatic aridity in the Pesceana River Basin in southwestern Oltenia, Romania.

The main objectives of this study were (1) to calculate the AIDM for two meteorological stations, RM Vâlcea and Drăgăşani, and (2) to assess human pressure on the environment in the Pesceana River Basin's rural area.

MATERIALS AND METHODS

Study Area

The Pesceana River Basin is located in the southwestern region of Oltenia (Figure 1), and more precisely in the southeastern part of Vâlcea County, Romania. Also, on the map in Figure 1 are mapped the eight administrative-territorial units (Amărăşti, Creţeni, Glăvile, Guşoeni, Lădeşti, Mitrofani, Pesceana, and Suteşti), localities, national roads, the hypsometry of the relief steps, and the hydrographic network. The climate of the investigated area is temperate-continental, being also influenced by the location of the Pesceana River Basin in the Subcarpathians (Marinică & Marinică, 2016).

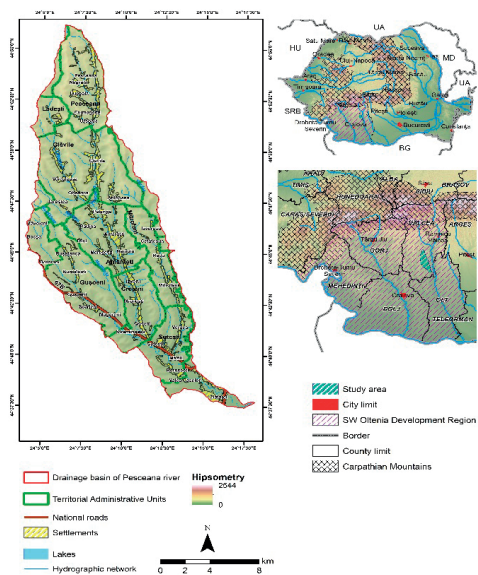


Figure 1. Location of the Pesceana River Basin in regional and national context
Source: Authors' own image created in ArcGIS 10.7.2. software

Data Sources and Methodology

In this study, the authors used a wide range of methods, including descriptive statistical methods (Creţan et al., 2023; Rotaru et al., 2023), statistical-mathematical (Popescu, 2014), and cartographic methods (Desculţu et al., 2024; Drăguleasa et al., 2024), as well as one of the newest methods of the 21st century: “desk research” (Creţu et al., 2021; Niţă, 2021; Mazilu et al., 2023; Vilcea et al., 2023; Mazilu et al., 2024). The authors used several statistical data sets, including those from the National Institute of Statistics (NIS) and the National Meteorological Administration (ANM), and, based on these data sets, various tables were created (Popescu et al., 2019; Popescu et al., 2022; Popescu et al., 2023; Popescu et al., 2025).

For the study area, the authors chose to use the digital elevation model (DEM) at a resolution of 30 x 30 (Niţă et al., 2025). The topographic map was georeferenced with longitude and latitude using ArcGIS software, version 10.7.2, with the boundary of the study area demarcated. The Geographic Information System (GIS) is an integrated hardware and software system capable of capturing, collecting, storing, and analyzing (Drăguleasa et al., 2023; Gordan et al., 2024; Călina et al., 2025a; Călina et al., 2025b; Călina et al., 2025c) as well as displaying

geographically referenced (spatio-temporal) information in order to support decision-making for the development of an area or territory, which, in this case, is the Pesceana River Basin. The De Martonne aridity index (AI_{DM}) was used to quantitatively assess the degree of humidity in the geographical space of the Pesceana River Basin. This index was calculated using the following formula (Martonne, 1920; Martonne, 1925; Martonne, 1926; Coscarelli et al., 2004):

$$AI_{DM} = \frac{P}{(T+10)}$$

where:

AI_{DM} - De Martonne aridity index;

P - annual precipitation (in mm);

T - average annual air temperature (in °C);

10 - coefficient added to obtain positive values.

The AI_{DM} was calculated for two meteorological stations that frame the basin—the RM Vâlcea meteorological station in the north and the Drăgășani meteorological station in the south.

Human (anthropic) activities exert pressure on the environment that differs between types of spaces and areas. The values of human pressure “highlight the directions through which man intervenes in the structure of the biotic envelope” (Pătroescu, 1996).

The formula applied by the Food and Agriculture Organization of the United Nations (FAO) to calculate this indicator is (Vijulie, 2010):

$$P = \frac{S(ha)}{N(loc)}$$

where:

P - human pressure;

S - analyzed area (hectares);

N - number of inhabitants in the analyzed area.

In order to assess the natural state of the landscape, a synthetic index was calculated—namely, the *naturalness index*—for the rural landscape of the Pesceana River Basin (Dumitrașcu, 2006), its value represents the share of the area covered by forest in the analyzed territorial unit. This index was calculated using the following formula (Niculae, 2012):

$$I_{nat} = \frac{S_{pad}}{S_{tot}} \times 100$$

where:

I_{nat} - naturalness index;

S_{pad} - area occupied by forest (hectares);

S_{tot} - total area.

RESULTS AND DISCUSSIONS

AI_{DM} - De Martonne aridity index

The AI_{DM} is used to assess the degree of dryness of a given environment by classifying it into one of seven classes - which range from “dry” to “extremely humid” - based on climatic parameters, i.e., the two research variables taken into account in this study: air temperature and precipitation (Charalampopoulos et al., 2023; Claro et al., 2023; Charalampopoulos et al., 2025).

In order to identify trends, in this study, the index’s formula was applied to each year or shorter periods to obtain data sets for calculating trends. According to the values obtained by calculating the AI_{DM} , the climatic type of a region can be classified as shown in Table 1.

Table 1. Climate type according to AI_{DM}

Climate type	AI_{DM} value
Arid	< 10.0
Semi-arid	$10.0 \leq I_{DM} < 20$
Mediterranean	$20.0 \leq I_{DM} < 24.0$
Semi-humid	$24.0 \leq I_{DM} < 28.0$
Humid	$28.0 \leq I_{DM} < 35.0$
Very humid	$35.0 \leq I_{DM} \leq 55.0$
Extremely humid	$I_{DM} > 55.0$

Source: Adapted by the authors from Baltas (2007) and Croitoru et al., 2013 (Baltas, 2007; Croitoru et al., 2013)

Based on the climatic parameters (air temperature and precipitation), the AI_{DM} was calculated for two meteorological stations (RM Vâlcea and Drăgășani) for the period 2015-2024 (Table 2 and Table 6). The highest AI_{DM} index was recorded at the RM Vâlcea station in 2018 (81.51), and, at the opposite pole, a value of 40.04, at the Drăgășani station in 2024.

Table 2. AI_{DM} values for the period 2015-2024

Weather station	Annual precipitation (mm)		Average annual temperature (°C)		AI_{DM}	
	2015	2024	2015	2024	2015	2024
RM Vâlcea	704.9	532.5	13.3	14.3	63	47.23
Drăgășani	701.4	441.6	13.1	14.7	63.54	40.04

Source: Authors’ own calculations based on data from the National Administration of Meteorology database 2025, ANM (ANM, 2024)

Table 3. AI_{DM} values for the period 2016-2023

Weather station	Annual precipitation (mm)		Average annual temperature (°C)		AI_{DM}	
	2016	2023	2016	2023	2016	2023
RM Vâlcea	750.5	745.2	12.6	13.6	69.56	64.79
Drăgășani	683.7	690.1	12.6	14.1	64.26	58.94

Source: Authors’ own calculations based on data from the National Administration of Meteorology database 2025, ANM (ANM, 2024)

Table 4. AI_{DM} values for the period 2017-2022

Weather station	Annual precipitation (mm)		Average annual temperature (°C)		AI _{DM}	
	2017	2022	2017	2022	2017	2022
RM Vâlcea	820.7	684.3	12.6	13	75.13	62.63
Drăgășani	663	664.6	12.8	13.5	61.79	59.22

Source: Authors' own calculations based on data from the National Administration of Meteorology database 2025, ANM (ANM, 2024)

Table 5. AI_{DM} values for the period 2018-2021

Weather station	Annual precipitation (mm)		Average annual temperature (°C)		AI _{DM}	
	2018	2021	2018	2021	2018	2021
RM Vâlcea	929.7	695.6	13	12.3	81.51	66.55
Drăgășani	891.8	693.8	12.8	12.7	79.67	64.62

Source: Authors' own calculations based on data from the National Administration of Meteorology database 2025, ANM (ANM, 2024)

Table 6. AI_{DM} values for the period 2019-2020

Weather stations	Annual precipitation (mm)		Average annual temperature (°C)		AI _{DM}	
	2019	2020	2019	2020	2019	2020
RM Vâlcea	706	612.7	13.5	13.3	62.29	56.06
Drăgășani	616.8	603.8	13.8	13.6	54.69	54.39

Source: Authors' own calculations based on data from the National Administration of Meteorology database 2025, ANM (ANM, 2024)

Human pressure on the environment

The human pressure on the environment - a synthetic indicator of the landscape's quality and degree of artificialization (Dumitrașcu, 2006; Vijulie, 2010) - was evaluated in the study area.

Table 7. Human pressure in the Pesceana River Basin, Romania

Administrative-territorial unit	Surface (hectares)	Number of inhabitants (number of people)	Human pressure
Amărăști	2.843	1608	1.76
Crețeni	2.680	2116	1.26
Glăvile	5.005	1824	2.74
Gușoeni	3.665	1233	2.97
Lădești	5.013	1808	2.77
Mitrofani	1.724	920	1.87
Pesceana	2.876	1427	2.01
Sutești	2.491	2113	1.17

Source: Authors' own calculations based on data from the National Institute of Statistics. TEMPO Online database 2025, NIS [42] and Ciobotea et al., 2012 (Ciobotea & Avram, 2012; Ciobotea & Avram, 2012)

Analyzing the statistical data for the eight administrative-territorial units in the Pesceana River Basin, it is observed that the highest human pressure (2.97) was recorded for Gușoeni and the lowest (1.17) was recorded at the opposite pole, in Sutești (Table 7).

The dynamics of the rural landscape in the Pesceana River Basin are expressed as the change in naturalness (Table 8) as well as diversification as a result of intensified anthropogenic activity.

Table 8. I_{nat} values for 2014

Administrative-territorial unit	Surface (hectares)	Spad	I _{nat} 2014
Amărăști	2.843	531	18.67%
Crețeni	2.680	842	31.41%
Glăvile	5.005	1941	38.78%
Gușoeni	3.665	1385	37.78%
Lădești	5.013	1547	30.85%
Mitrofani	1.724	758	43.96%
Pesceana	2.876	735	25.55%
Sutești	2.491	582	23.36%

Source: Authors' own calculations based on data from the National Institute of Statistics (TEMPO Online database 2025, NIS) (NIS, 2025) and Ciobotea & Avram, 2012 (Ciobotea & Avram, 2012; Ciobotea & Avram, 2012)

Thus, only one administrative-territorial unit is characterized by landscapes with a strongly affected balance (index values lower than 20%): Amărăști (18.67%).

From the analysis of the values obtained, most administrative-territorial units (six) are characterized by landscapes with balances that are moderately affected by anthropogenic intervention over time (index values between 20% and 40%).

Relatively few of the communes (Mitrofani 43.96%) are administrative-territorial units with a weakly affected ecological balance of landscapes (index values between 40% and 50%).

CONCLUSIONS

According to the calculated AI_{DM} values, the Pesceana River Basin is characterized as exhibiting the very and extremely humid climatic types. Thus, we can conclude that the Pesceana River Basin is characterized by high humidity, abundant vegetation, and, often, high

temperatures, which creates a suffocating environment.

When analyzing the degree of naturalness of the basin's rural area, it was found to be mostly characterized by landscapes with a balance moderately affected by anthropogenic intervention over time, with I_{nat} values between 20% and 40% obtained for the six administrative-territorial units considered here. In order to capture the full complexity of climatic aridity and human pressure on the environment, future studies would benefit from integrating these objective findings with qualitative and sociodemographic information in the form of a questionnaire. A second limitation of this research is the statistical data employed; future research should also include field statistical data, such as sampling coordinates for plant association types.

ABBREVIATIONS

The following abbreviations are used in this manuscript:

AI _{DM}	De Martonne aridity index
NAM	National Administration of Meteorology
GIS	Geographic Information System
DEM	Digital Elevation Model
NIS	National Institute of Statistics
FAO	Food and Agriculture Organization of the United Nations

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EFFICACY OF THE NATIVE ENTOMOPATHOGENIC NEMATODES (EPN) ON THE SURVIVAL OF *CERATITIS CAPITATA*

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Abstract

Ceratitis capitata (Wiedemann, 1824) (Mediterranean fruit fly) is one of the most economically damaging pests of fruits globally, causing significant yield losses and trade restrictions. Despite the presence of both native and non-native entomopathogenic nematodes (EPNs) in South African soils, indigenous species remain underexplored for use in pest management programs. This study objective was to evaluate the efficacy of two indigenous isolates; *Steinernema jeffreyense* and *Heterorhabditis bacteriophora* against the larval and pupal stages of *C. capitata* under laboratory conditions. To achieve the objective, a 2×5 factorial experiment with five replications in a completely randomised design was set up. Variables measured were larval and pupal mortality, mortality speed, EPN infectivity, and the number of pupae after larval exposure. There were no significant interactions between EPN and concentration for the larval trial, while all variables in the pupal trial had highly significant interactions. Generally, both species caused larval and pupal mortality across treatments, with *H. bacteriophora* performing better than *S. jeffreyense*. Larval and pupal mortalities were concentration dependent, with the highest larval mortality at 25 IJs and pupal mortality significant only at 250 IJs. These findings suggest potential for biological control of *C. capitata*.

Key words: biological control, *Ceratitis capitata*, entomopathogenic nematodes, *Heterorhabditis bacteriophora*, *Steinernema jeffreyense*

INTRODUCTION

Phytopathogenic pests, such as *Ceratitis capitata*, are widely distributed polyphagous pests of commercial fruits in South and North America, Southeast Asia, Europe, and Africa (Authority et al., 2019, Dong et al., 2022, Jaffar and Lu, 2022, Papadogiorgou et al., 2024, Authority et al., 2025). *Ceratitis capitata* attacks over 300 plant species, particularly in tropical and subtropical regions, including South Africa (Abd-Elgawad, 2021; Sookar et al., 2021; Dong et al., 2022). The infestation by *Bactrocera dorsalis* (Hendel, 1912) and *C. capitata* is associated with estimated yearly financial losses of US\$1 billion worldwide and US\$42 million in Africa (Motswagole et al., 2019, López et al., 2024). Management of *C. capitata* in orchards includes behavioural, chemical, biological, cultural, and mechanical tactics, with synthetic chemicals being the most used (Candia, 2019, Reddy et al., 2020, Ganie et al., 2022, Rodriguez et al., 2024, Wang et al., 2024). However, the use of synthetic chemicals poses ecotoxicological risks, which may include water and soil pollution, insect resistance, and chemical

residues on fruits, affecting the export quality of the crop (Mokrini et al., 2020, Cruz et al., 2022, Lengai et al., 2022, Ali et al., 2023). Therefore, there is a clear demand for eco-friendly alternatives that are also highly effective. Biological control has attracted research and developmental attention as a reliable alternative to reducing reliance on synthetic chemicals, because it is very effective, with minimal environmental contamination and insect resistance (Baker et al., 2020, Lengai et al., 2022, Maurya et al., 2022, Costa et al., 2023, Kasana, 2024).

Entomopathogenic nematodes (EPNs) are one biological agent that has demonstrated effectiveness in the management of many insect pests, including *Thaumetotibia leucotreta* and many tephritid fruit flies (Belien, 2019; Coleman, 2020; Mkiga, 2020). The use of entomopathogenic nematodes could help alleviate fruit fly densities in many cropping systems (Fouad et al., 2020, Sallam et al., 2024). Unlike chemicals, entomopathogenic nematodes actively seek out their hosts by detecting chemical cues released by the larvae or pupae in the soil (Zhang et al., 2021, Kallali et

al., 2024). Currently, there is limited information on the use of locally isolated EPNs in the management of fruit flies. Therefore, the current study objective is to evaluate the efficacy of two local EPN isolates: *Steinernema jeffreyense* and *Heterorhabditis bacteriophora*, in the management of *C. capitata*.

MATERIALS AND METHODS

Study sites

The study was conducted at the University of Mpumalanga, Mbombela Campus, in South Africa under laboratory conditions.

Sampling and rearing of insects

Sourcing and rearing of mealworm (Tenebrio molitor) (Linnaeus, 1758) larvae

Mealworm larvae were sourced from Feeders Africa and reared in containers filled with wheat bran, which served as the primary substrate and food source (Figure 1). Freshly grated carrots were provided as a food source for the adults and a moisture source for the larvae. The containers had holes to ensure adequate ventilation, yet small enough not to allow the insect to escape (Figure 1). Egg cardboard crates were placed inside the containers to provide shelter and ovipositing surfaces for the adult beetles (Figure 2).



Figure 1. Rearing container of mealworms with wheat bran, ventilation holes, and grated carrot provided as a food and moisture source. Photo by NC Shabalala (2025)

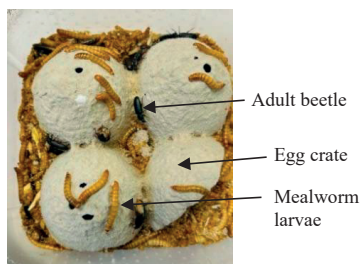


Figure 2. Rearing container of mealworms showing egg cardboard crate used as shelter and ovipositing surfaces for adult beetles. Photo by NC Shabalala (2025)

Sourcing and rearing of Ceratitis capitata

Five hundred eggs of *Ceratitis capitata* were sourced from Citrus Research International, Mbombela, South Africa. The eggs were placed in a circular container filled with a carrot-based medium (Tsitsipis, 1989) prepared by mixing carrot powder (BioNutricia extract) with warm water to achieve a moist consistency (Figure 3). The mixture served as the primary food source for the larval stage. The container was then placed inside a 27 L clear plastic container (Figure 4). Distilled water was sprayed regularly on the carrot mixture to maintain a high-humidity environment. The eggs were allowed to hatch and larvae to develop through the first three instar stages before being moved out, mimicking their natural behavior of exiting fruit to pupate. Unhatched eggs were left in the original circular container until they hatched; those that did not hatch were disposed of. The third instar larvae were collected using forceps and used for the larval bioassay. Some of the third instar larvae were left in the circular container inside the 27 L container until they reached the pupal stage. The pupae were then collected using forceps for use in the pupal bioassay; the rest were transferred into a Petri dish and subsequently placed back into a new 27 L container, which had a net sleeve through a side opening, to ensure adequate ventilation and prevent adult flies from moving out. Once adults emerged, they were provided with a sugar-yeast mixture (3:1) in a Petri dish, along with water supplied in a circular container lined with a paper towel that was lightly moistened (Figure 5). For egg collection, a custom-designed artificial oviposition device was used. This consisted of a plastic container with damp paper towel and rotten *Citrus sinensis* on either side of the device, sealed with perforated lids (Figure 6). The holes allow adult females to insert their ovipositors and lay eggs into the medium, ensuring a continuous supply of eggs.

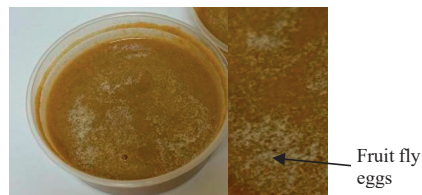
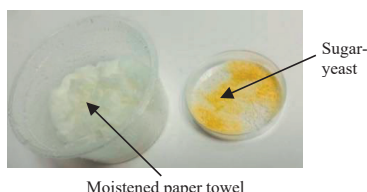


Figure 3. Carrot-based medium in circular containers with fruit fly eggs. Photo by NC Shabalala (2025)



Carrot-based medium

Figure 4. Circular container with carrot-based medium placed inside a 27L clear plastic container used for rearing larvae. Photo by NC Shabalala (2025)



Moistened paper towel

Figure 5. Sugar-yeast mixture (3:1) in a Petri dish, with water provided in a circular container lined with moistened paper towel. Photo by NC Shabalala (2025)

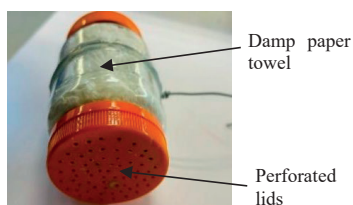


Figure 6. Custom-designed artificial oviposition device consisting of a plastic container with damp paper towel and rotten *Citrus sinensis*, sealed with perforated lids. Photo by NC Shabalala (2025)

Sourcing and rearing of entomopathogenic nematodes

Entomopathogenic nematodes (EPNs) were obtained from the University of Stellenbosch, South Africa. The *in vivo* approach was used for rearing the EPNs as previously described (Shapiro-Ilan et al., 2016). Briefly, four Petri dishes (9 cm in diameter) were filled with circular filter paper (Whatman No. 1). About 800 μ L suspension containing the EPNs were evenly distributed onto the filter paper in each Petri dish. Ten mealworm (*Tenebrio molitor*) last instar larvae were then introduced into each Petri dish. The Petri dishes were covered with their lids, labelled with the date and EPN species, and placed inside a 5L plastic container to maintain humidity. The containers were stored at room temperature (20–25°C). Petri

dishes were examined daily for signs of infection and larval death, as determined by the discoloration or decay of the mealworm larvae. After two days, cadavers were carefully removed and rinsed with distilled water on a 25 μ m sieve, then transferred to sterile 9 cm Petri dishes. Ten days later, the 9 cm Petri dish containing wet filter paper and cadavers was placed inside a 15 cm-wide glass Petri dish, leaving the small Petri dish open. The 15 cm Petri dish was filled with water to half the level of the 9 cm Petri dish. The large Petri dish was closed, labelled accordingly, and left undisturbed at room temperature until the infective juveniles began to emerge and move into the water contained in a 15 cm Petri dish. Once EPN concentrations were high enough to become visible in the water, the 9 cm Petri dish was removed. The water containing EPNs was carefully poured into a beaker and allowed to settle for approximately 30 minutes. The infective juveniles (IJs) suspension was then transferred into culture flasks. The flasks were stored horizontally to ensure even distribution of nematodes. The nematode suspension was kept at 12.8°C. The flasks were shaken weekly to improve oxygen levels in water and prevent EPNs from climbing out of water, the lid was slightly loosened to allow air exchange.

Experimental design and bioassays

Larval bioassay

A 5×2 factorial experiment in a completely randomised design was used to test effect of EPNs on *C. capitata*. The first factor consisted of five EPN concentrations of 0, 25, 50, 75 and 100 IJs per 50 μ L and the second factor were the two EPNs; *Steinernema jeffreyense* or *Heterorhabditis bacteriophora*. The EPN inocula were prepared from the culture flask by diluting the nematode suspensions with distilled water. Prior to inoculation, a 13 mm diameter filter paper disc was placed in each well of a 24-well bioassay plate. Each EPN was tested in 12 alternating wells of a 24-well plate to minimize potential IJ migration between wells. For each treatment, 50 μ L of the nematode suspension was pipetted into the well, and a single third-instar fruit fly larva of *C. capitata* was placed on the filter paper. Control wells received only 50 μ L of distilled water and fruit fly larvae. A completely randomized design with five

replicate plates per treatment was employed, resulting in 60 larvae per treatment per nematode. The plates were covered with a paper towel and a glass plate, secured with a rubber band. Each respective treatment plate was then placed inside a 2-L plastic container lined with damp paper towels to maintain humidity. All plates were incubated at 25 ± 2 °C. Larval survival was recorded daily for a period of three days. Mortality was determined at each observation by the difference between the total number of larvae introduced and those surviving. Dead larvae were dissected under a stereomicroscope to confirm the presence of nematode infection.

Pupal bioassays

To ascertain the survival of pupae, the same procedure described for larvae was followed, except that the EPN concentrations tested were 0, 100, 150, 200, and 250 IJs per 50 µL. Prior to inoculation, a 13 mm diameter filter paper disc was placed in each well of a 24-well bioassay plate. Each concentration was applied by pipetting 50 µL of the nematode suspension into the well. A single one-day-old pupa of *Ceratitis capitata* was then placed into each treated well. Control wells received only 50 µL of distilled water and pupa. Each species was tested in 12 alternating wells of a 24-well plate. A completely randomized design with five replicate plates per treatment was used. The plates were covered with a paper towel and a glass plate, secured with a rubber band, and placed inside 2-L plastic containers lined with damp paper towels to maintain humidity. All plates were incubated at 25 ± 2 °C. The number of adults emerging was recorded daily for both nematodes. The experiment was terminated on the 10th day. Pupae that failed to turn into adults or showed visible signs of infestation (e.g., discoloration, decay, or disintegration) were dissected, and if nematodes were found inside, then they were recorded as dead. Nematode infection was confirmed by the presence of EPNs inside a dissected pupa. Mortality was

calculated as the difference between the number of pupae introduced and the number of adults that successfully emerged.

Statistical analysis

Data were tested for normality using the Shapiro-Wilk test (Shapiro & Wilk, 1965). The data found to deviate from normality were transformed to meet the assumptions of ANOVA using $\log_{10}(x + 1)$ or arcsine, depending on the data type. The entire data set were subjected to analysis of variance (ANOVA) through Statistix 10 software (Gomez & Gomez, 1984). Where significant differences were detected, means were separated using the Least Significant Difference (LSD) test at the 5% probability level ($P \leq 0.05$).

RESULTS AND DISCUSSIONS

Shapiro Wilk test showed that all variables (mortality, mortality speed, pupation, and infected) were not normally distributed ($P \leq 0.05$) and were transformed accordingly.

Larval bioassay

There were no significant interactions between EPN and concentration on all measured variables. Concentrations had significant effects on all measured variables, whereas EPN were only significant for larval mortality speed. Concentration effects were concentration dependent, with concentrations of 25, 50, 75, and 100 IJs/larva not differing from each other for mortality, mortality speed, pupation, and infectivity, but all differed from the control (Table 1). The control was significantly different from all other concentrations, except 75 IJs for mortality (Table 1). The highest mean mortality, mortality speed, and infectivity of 27.4, 4.0, and 30.9% occurred at 25 IJs/50 µl, followed by 50 IJs/50 µl at 19.90, 2.8, and 26.6 %, respectively, while the control recorded the least at 1.6, 0.1, and 0%, respectively. The highest pupation of 91.8% occurred at the control.

Table 1. Effect of entomopathogenic nematode concentration on *Ceratitis capitata* larval mortality (M), mortality speed (MS), number of pupae (P), infectivity (I), and Relative Impact (RI)

Concentrations	M (%)	RI (%)	MS	RI	P (%)	RI (%)	I (%)
0	0.02 ^b (1.6)	-	0.03 ^b (0.1)	-	0.13 ^a (91.8)	-	0.00 ^b (0)
25	0.28 ^a (27.4)	1612	0.56 ^a (4.0)	3900	0.80 ^b (69.3)	-24	0.32 ^a (30.9)
50	0.20 ^a (19.9)	1143	0.48 ^a (2.8)	2700	0.94 ^b (77.6)	-15	0.27 ^a (26.6)
75	0.17 ^{ab} (15.7)	881	0.38 ^a (2.7)	2600	1.02 ^b (84.3)	-8	0.24 ^a (22.4)
100	0.19 ^a (19.2)	1100	0.57 ^a (3.2)	3100	0.87 ^b (75.8)	-17	0.17 ^a (16.6)
F value	3.07		5.78		4.74		4.84
P value	0.028*		0.001**		0.003**		0.003**
LSD _{0.05}	0.078		0.131		0.117		0.080

*Significant at $P \leq 0.05$ **Highly significant at $P \leq 0.001$; Values in brackets are untransformed means; All means followed by the same letter are not statistically different from each other. $RI (\%) = \left(\frac{Treatment}{Control} - 1 \right) \times 100$

Heterorhabditis bacteriophora (3.92) exhibited a higher mortality speed compared to *Steinernema jeffreyense* (1.20) (Table 2). Relative to *S. jeffreyense*, *H. bacteriophora* had 3.27 times faster mortality (Table 2).

Table 2: Effect of *Steinernema jeffreyense* and *Heterorhabditis bacteriophora* on *Ceratitis capitata* larvae mortality speed across all concentrations

Species	Mortality speed
<i>S. jeffreyense</i>	0.27 ^b (1.20)
<i>H. bacteriophora</i>	0.54 ^a (3.92)
F value	10.19
P value	0.002**
LSD _{0.05}	0.082

**Highly significant at $P \leq 0.001$; Values in brackets are untransformed means

Both entomopathogenic nematodes infected fruit fly larvae, with *H. bacteriophora* achieving the highest infectivity among treatments. A nonlinear relationship between concentration and infection was observed, where infectivity increased at lower concentrations but decreased at higher ones. This could be due to density-dependent interactions inside the host (Selvan, 1993). At lower concentrations, mortality increased, probably because the number of nematodes was adequate for effective searching, locating, and penetrating the host. However, when too many nematodes invade a single host, they begin to compete for limited nutrients, reducing their survival and successful development. This could explain the observed reduction in infectivity at higher concentrations relative to lower concentrations.

Mortality speed, defined as how quickly nematodes killed the host, was highest at 25 IJs/larva. This indicates that from the first day

after inoculation, larvae began dying more rapidly compared with other treatments. More larvae pupated in the control, as expected of the fly life cycle, which is approximately three days to pupate (Weldon, 2022). At higher concentrations, increased pupation rates were observed, particularly at 75 IJs/larva. This may suggest that larvae accelerated pupation to escape infection pressure. However, no direct studies have shown that *Ceratitis capitata* pupate faster when exposed to EPNs; however, a similar phenomenon has been reported in other insect–parasite systems. *Lobesia botrana* larvae exposed to parasitoids developed faster and reached maturity earlier, possibly as a strategy to reduce the duration of exposure to parasitism (Vogelweith et al., 2013). Infectivity values were generally higher than mortality at most concentrations, however, the opposite trend occurred at 100 IJs/larva, where mortality exceeded infectivity. This was likely because some larvae died early from physical damage caused by excessive nematode penetration attempts, before the symbiotic bacteria could establish and produce metabolites that suppress the host immune system or outcompete other microbes (Grewal et al., 1994). Since the nematodes rely on their symbiotic bacteria to create a suitable environment and provide nutrition the larvae need to survive and proliferate. As a result, when confirming the cause of death, no nematodes were found even though the larvae were dead.

Thus, the plateau in mortality above 25 IJs/larva in this study aligns with findings where further increases in nematode density result in diminishing returns.

Previous reports have indicated a linear concentration-dependent mortality, in which

mortality increases consistently with increasing concentration, as observed in *S. carpocapsae*, *H. bacteriophora*, and *S. feltiae* (Elhadidy, 2021; Yağcı, 2021; Sayed, 2023). This pattern often occurs when host-searching behaviors favor nematode movement and infection success and also where food supply is non-limiting. Both EPNs used in the current study are considered cruising foragers that actively search for hosts (Rakubu, 2024; Langford, 2014). However, in this study, *H. bacteriophora* showed faster killing ability, with mortality observed from the first day. It exhibited higher mortality relative to *S. jeffreyense*, likely due to key differences in reproductive strategy (Cassells, 2024). *Heterorhabditis bacteriophora* produces hermaphrodites in its first generation inside the host cadaver, allowing reproduction even when few infective juveniles are present, whereas *S. jeffreyense* requires male–female mating in the first generation, which may slow early propagation. Another contributing factor could be the size of infective juveniles, *H. bacteriophora* is smaller (512–671 µm) (Adams and Nguyen, 2002) compared to *S. jeffreyense* (784–1043 µm) (Malan, 2016), which gives *H. bacteriophora* an advantage in penetrating host openings. Additionally, the presence of a dorsal tooth in *Heterorhabditis* enables active cuticle penetration (Griffin et al., 2005). These traits, combined with efficient host searching and rapid early reproduction, likely contributed to the superior performance of *H. bacteriophora* in this study.

Pupal bioassay

There were significant interactions between EPN and concentration on all measured

variables. Concentration effects varied with changes in nematode concentration, with 100 IJs/pupa not differing significantly from 150 and 200 IJs/pupa in mortality, and 100 and 150 IJs/pupa not differing significantly in infectivity. However, all other concentrations (0, 100, 150, 200, and 250 IJs/pupa) differed significantly from each other (Table 3).

Table 3. Combined effect of *Steinernema jeffreyense* and *Heterorhabditis bacteriophora* concentrations on mortality and infectivity of *Ceratitis capitata*

Concentration	Mortality (%)	Infectivity (%)
0	0.07 ^a (6.6)	0.00 ^d (0)
100	0.55 ^{bc} (48.3)	0.05 ^c (4.9)
150	0.45 ^c (40.0)	0.07 ^c (6.6)
200	0.64 ^b (54.1)	0.16 ^b (15.8)
250	1.00 ^a (80.0)	0.27 ^a (26.6)
F value	62.52	48.18
P value	0.000**	0.000**
LSD _{0.05}	0.060	0.021

**Highly significant at P ≤ 0.001; Values in brackets are untransformed means

The highest mean mortality and infectivity of 80.0 % and 26.6 %, respectively, both occurred at 250 IJs/pupa. The next concentration was 200 IJs/pupa with mortality and infectivity at 54.1 % and 15.8 %, respectively, while the control (0 IJs/pupa) recorded the least mortality and infectivity of 6.6 % and 0 %, respectively. For *Steinernema jeffreyense*, concentrations of 0, 100, 150, and 200 IJs/pupa did not differ significantly in mortality, while 250 IJs/pupa caused a significantly higher mortality (68.3%) than all other concentrations (Table 4).

Table 4: Interactive effect of entomopathogenic nematode and concentration on mortality and infectivity of *Ceratitis capitata* pupae

Concentration	Mortality				Infectivity	
	<i>S. jeffreyense</i>		<i>H. bacteriophora</i>		<i>S. jeffreyense</i>	<i>H. bacteriophora</i>
	Means	RI	Means	RI	Means	Means
0	0.07 ^c (6.67)	-	0.07 ^c (6.67)	-	0.00 ^d (0)	0.00 ^d (0)
100	0.17 ^c (16.67)	150	0.93 ^{bc} (80.00)	1099	0.07 ^c (6.67)	0.03 ^{cd} (3.33)
150	0.08 ^c (8.33)	25	0.81 ^{cd} (71.67)	975	0.00 ^d (0)	0.13 ^b (13.33)
200	0.22 ^c (21.67)	225	1.06 ^b (86.67)	1199	0.08 ^b (8.33)	0.24 ^a (23.33)
250	0.75 ^d (68.33)	924	1.25 ^a (91.67)	1274	0.25 ^a (25.00)	0.29 ^a (28.33)
F value	15.56				7.10	
P value	0.000**				0.000**	

**Highly significant at P ≤ 0.001; Values in brackets are untransformed means; All means followed by the same letter are not statistically different from each other. $RI\ (\%) = \left(\frac{Treatment}{Control} - 1 \right) \times 100$

Infectivity for *S. jeffreyense* differed significantly across concentrations, except between 100 and 200 IJs/pupa. For *Heterorhabditis bacteriophora*, mortality differed significantly across all concentrations, except that 100 IJs/pupa did not differ from 150 and 200 IJs/pupa (Table 4). The 250 IJs/pupa treatment of *S. jeffreyense* did not differ significantly from the 150 IJs/pupa treatment of *H. bacteriophora* in mortality. Infectivity of *H. bacteriophora* differed significantly among concentrations; however, 200 and 250 IJs/pupa did not differ significantly, while 100 IJs/pupa did not differ from the control.

Heterorhabditis bacteriophora exhibited a higher mortality (67%) and infectivity (14%) when compared with *Steinernema jeffreyense* with 24 % and 8 %, respectively, (Table 5). Relative to *S. jeffreyense*, *H. bacteriophora* had 2.8 times faster mortality and 1.75 times faster infectivity (Table 5).

Table 5. Effect of *Steinernema jeffreyense* and *Heterorhabditis bacteriophora* on mortality and infectivity of *Ceratitis capitata* pupae

Species	Mortality (%)	Infectivity (%)
<i>Steinernema jeffreyense</i>	0.26 ^b (24.33)	0.08 ^b (8.00)
<i>Heterorhabditis bacteriophora</i>	0.82 ^a (67.33)	0.14 ^a (13.67)
F value	218.60	17.44
P value	0.000**	0.000**
LSD _{0.05}	0.038	0.013

**Highly significant at $P \leq 0.001$; Values in brackets are untransformed means

In the pupal bioassays, mortality increased with concentration. For example, *S. jeffreyense* caused 67% mortality at 250 IJs/pupa, which was higher than at other concentrations, while *H. bacteriophora* caused 92% mortality at 250 IJs/pupa. These observations are in full agreement with other reports that observed concentration-dependent mortality in pupal bioassays (Aatif, 2019; Rakubu, 2024). Such outcomes illustrate that while larval-stage responses may plateau due to density dependence at the currently used concentrations, the pupal stage continues to increase with increasing doses. This could be due to differences in anatomical and physiological constraints between larvae and pupae. Pupae, have a thicker cuticle that reduces mobility and

also pose a greater barrier to infection. Higher IJ concentrations increase the chances of penetration through sheer greater numbers or opportunities for infection. Mortality was higher than infectivity across all concentrations because not all observed deaths are due to nematode infestation. Infectivity, however, was confirmed only when nematodes were found inside pupae that failed to emerge. Some pupae did not emerge, but dissection revealed fully formed adults inside; these were recorded as alive. Pupae that failed to emerge and showed no signs of adult development were recorded as dead.

CONCLUSIONS

Both entomopathogenic nematodes caused significant mortality against *Ceratitis capitata* larvae and pupae, with *Heterorhabditis bacteriophora* performing better across all concentrations. The larval stage exhibited density-dependent mortality effects, whereas the pupal stage showed concentration-dependent mortality.

Based on these findings, both nematodes are promising candidates for the biological control of *C. capitata*, but *H. Bacteriophora* appears to be particularly more efficient at higher concentrations when targeting less mobile pupal stages, while lower concentrations were still effective for the larval stage.

Further field experiments are needed to validate these laboratory results under variable environmental conditions. Future studies should also consider factors such as soil type, nematode persistence, and formulation improvements to enhance field performance and ensure the effective integration of EPNs into integrated pest management (IPM) programs.

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THE IMPACT OF DIGITALIZATION IN THE SUSTAINABLE DEVELOPMENT OF VEGETABLE GROWING AND ATTRACTING YOUNG FARMERS. REVIEW

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Abstract

The vegetable production sector is facing increasing pressures generated by climate change, market volatility, and the growing demand for fresh, safe, and sustainable products. This paper examines the role of digitalization in the transformation of modern vegetable production through the integration of technologies such as unmanned aerial vehicles (UAVs), Internet of Things (IoT) sensors, artificial intelligence (AI), digital farm management platforms, and Agricultural Knowledge and Innovation Systems (AKIS). The findings indicate that digitalization contributes to reduced resource use, improved production quality, and enhanced operational efficiency. The study also highlights the strategic role of young farmers in the adoption of digital technologies, as well as the importance of AKIS in facilitating knowledge transfer and accelerating innovation processes. Digitalization is therefore analyzed not only as a form of technological advancement, but also as a catalyst for ecological, economic, and social sustainability within the vegetable production sector.

Key words: precision horticulture, young farmers, sustainable farming.

INTRODUCTION

The digitalization of agriculture has experienced significant acceleration over the past decade, driven by technological advances in the fields of smart sensors, Internet of Things (IoT) systems, drones, data analytics platforms, and applications based on artificial intelligence

(Canicatti & Vallone, 2024; Abobatta, 2024). These technologies enable the collection and processing of large volumes of data concerning environmental conditions, crop status, and resource utilization, thereby facilitating the development of agricultural systems based on predictive analytics and automated decision-making processes (Figure 1).

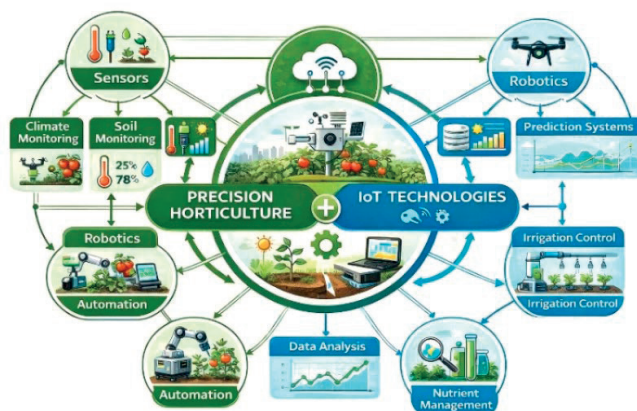


Figure 1. Innovative technological solutions in horticulture in 2025,
Source: Authors' own conception

Recent literature highlights that artificial intelligence and digital systems represent essential components of precision agriculture, contributing to crop monitoring, plant disease detection, the optimization of resource use, and improvements in agricultural productivity (Kumar et al., 2024; Padhiary et al., 2024; Aijaz et al., 2025).

In horticulture, and particularly in vegetable production, digital technologies are used to monitor crop development, optimize irrigation and fertilization practices, reduce post-harvest losses, and improve the traceability of agricultural products. The integration of data obtained from drones, sensors, and remote sensing systems enables farmers to observe spatial variability in environmental conditions and adopt management strategies tailored to the plot level, thereby enhancing the efficiency of horticultural production (Kumar & Bhatt, 2023; Getahun et al., 2024; Raj & Prahadeeswaran, 2025).

In this context, the concept of precision horticulture has become a central element of the digital transformation of the horticultural sector. Precision agriculture represents an approach based on the use of digital technologies for the continuous monitoring of crops and the variable-rate application of agricultural inputs, with the objective of optimizing production while reducing environmental impact. Recent studies emphasize that the integration of IoT sensors, drones, and artificial intelligence algorithms enables real-time crop monitoring and supports the adoption of management decisions grounded in data analysis (Nautiyal et al., 2025; Miller et al., 2025). However, the development of digital agriculture also has an important socio-economic dimension (Awais et al., 2025).

International organizations and academic studies underline that digital technologies can contribute to the modernization of the agricultural sector and increase the attractiveness of agriculture for younger generations by creating opportunities for the development of production models based on innovation and data-driven decision-making (Irawan et al., 2025). At the same time, there is a risk of the emergence of a “digital divide”

between farmers who have access to digital infrastructure and technological skills and those who do not benefit from these resources (FAO, 2022; Mihai et al., 2025).

Maintaining the economic viability of farms, particularly small- and medium-sized enterprises, represents a major challenge in the context of current technological transformations. Although digital technologies and artificial intelligence-based applications can contribute to improving agricultural management efficiency, the level of adoption of these technologies remains limited in many regions. Recent studies indicate that the adoption of digital technologies is influenced by factors such as farm size, farmers’ educational level, access to digital infrastructure, and investment capacity (Kramarz & Runowski, 2025). In this context, European agricultural policies are placing increasing emphasis on the development of digital competencies and the promotion of innovation in rural areas. Support programs targeting young farmers and initiatives for agricultural digitalization aim to facilitate access to technology, strengthen human capital, and accelerate the adoption of digital solutions in the agricultural sector (European Commission, 2023).

MATERIALS AND METHODS

The study analyzed the specialized literature (2015-2025) from the following sources:

- International scientific databases (ScienceDirect, Scopus, SpringerLink, MDPI, Web of Science);
- Reports and policy documents from organizations such as FAO, the European Commission, and the OECD.

Search strategy included the use of keywords such as “smart greenhouse”, “precision horticulture”, “IoT in agriculture”, “sensor-based irrigation”, “digital horticulture”, and “vegetable production systems”, as well as the use of Boolean operators (AND, OR) to combine them.

Articles published between 2015 and 2025, in English, were selected to reflect the most recent technological advances in the field.

Data analysis was carried out by compiling relevant information (authors, year, type of technology used, monitored parameters, and main results) into a tabular file created in Microsoft Excel. Subsequently, the data were compared and

synthesized to highlight the main research directions: digitalization of production processes (use of drones, sensors, and IoT in vegetable production); artificial intelligence in vegetable production; young farmers and the adoption of digital technologies; knowledge transfer and the role of AKIS in accelerating digitalization; and digitalization and sustainability in vegetable production.

The adopted methodology provides an overview of the current state of research on digitalization in horticulture and contributes to the identification of future research directions in digital vegetable production.

RESULTS AND DISCUSSIONS

The study's findings highlight a significant increase in academic and institutional interest in the digitalization of vegetable production and the role of young farmers in adopting digital technologies. Five major research directions were identified:

- (1) Digitalization of production processes: the use of drones, sensors, and IoT in vegetable production;
- (2) Artificial intelligence in vegetable production;
- (3) Young farmers and adoption of digital technologies;
- (4) Knowledge transfer and the role of AKIS in accelerating digitalization;
- (5) Digitalization and sustainability in vegetable production.

1. Digitalization of production processes: drones, sensors, and IoT in vegetable production

1.1. Drones (UAVs) as a central tool for monitoring and intervention

Recent literature emphasizes that drones (UAVs – Unmanned Aerial Vehicles) have become an important instrument in precision agriculture, being used for crop monitoring, assessment of plant health, and the application of phytosanitary treatments (Yogalakshmi et al., 2025). The use of multispectral and hyperspectral sensors mounted on UAVs enables the rapid collection of plot-level data and the early detection of plant stress, disease, and pest outbreaks. These technologies contribute to the optimization of crop

management and enhance farmers' decision-making processes (Guebsi et al., 2024). The integration of drones into agricultural management systems enables crop mapping and monitoring of spatial variability within agricultural fields, providing near real-time information on crop conditions and facilitating rapid interventions when phytosanitary issues arise. Recent literature considers drones an essential component of digital agricultural ecosystems, integrated with IoT sensors and data analytics platforms to support precision agriculture (Sharma et al., 2024). Moreover, images collected by drones can be analyzed using artificial intelligence algorithms. The use of convolutional neural networks (CNNs) for image analysis enables the automated detection of diseases and plant stress with high accuracy, contributing to the rapid diagnosis of phytosanitary problems (Ferentinos, 2018).

1.2. IoT sensors: monitoring soil, microclimate, and nutrition

Sensor-based systems are a fundamental element of precision horticulture, particularly in controlled environments such as greenhouses and tunnels. Multiparameter sensors can monitor real-time factors such as soil moisture, temperature, pH, light, and CO₂ concentration, providing essential data for optimizing plant growth conditions (Mansoor et al., 2025).

Integrating these sensors into IoT systems enables continuous data collection and transmission to digital farm management platforms. These systems facilitate the adoption of data-driven irrigation and fertilization strategies, contributing to reduced water consumption and improved efficiency in the use of agricultural inputs (Toselli et al., 2023; Jaiswal et al., 2025).

In modern horticulture, the concept of the “smart greenhouse” is becoming increasingly important. Systems based on sensor networks, digital platforms, and AI algorithms allow for the automatic control of temperature, ventilation, and irrigation, stabilizing production conditions and enhancing energy efficiency (Bicamumakuba et al., 2025; Kumar & Runowski, 2025).

1.3. System integration: from monitoring to automation

The digitalization of production processes in horticulture is evolving from simple monitoring systems toward integrated digital ecosystems,

where data from drones, IoT sensors, and AI-based analytics are used to automate agronomic decision-making. This approach is characteristic of Precision Agriculture 4.0, where data analysis and predictive models enable the automatic adjustment of irrigation, fertilization, or ventilation according to environmental conditions and crop status (Raj & Prahadeeswaran, 2025).

The integration of AI and IoT technologies contributes to the development of autonomous agricultural systems capable of analyzing large volumes of data and optimizing the use of agricultural resources (Awais et al., 2025). Recent studies indicate that these systems can improve crop productivity and foster the development of more efficient and sustainable agricultural systems (Zhang et al., 2024; Nautiyal et al., 2025).

2. Artificial intelligence in vegetable production

Artificial intelligence (AI) represents a central pillar in the digital transformation of modern agriculture. In vegetable production, AI applications are employed for automatic image analysis, early detection of plant diseases, yield estimation, irrigation optimization, and real-time decision support. The literature highlights a clear shift from mere data collection via drones and sensors toward advanced data interpretation using machine learning and deep learning algorithms, facilitating the development of predictive and automated agriculture (Kamilaris et al., 2017; Padhiary et al., 2024; Awais et al., 2025).

2.1. Aerial and ground image analysis using deep learning algorithms

Image analysis is one of the most important applications of AI in precision horticulture. Deep learning algorithms, particularly convolutional neural networks (CNNs), are used to automatically extract relevant features from multispectral, hyperspectral, or RGB images captured by drones, ground-based cameras, or mobile devices (Ferentinos, 2018; Awais et al., 2025).

Recent studies demonstrate the effectiveness of these methods for early disease detection. CNN systems can identify symptoms of foliar diseases and nutrient deficiencies with high

accuracy, often outperforming traditional diagnostic methods (Ferentinos, 2018; Wakchaure et al., 2023). More recent research shows that deep learning models can rapidly process large volumes of agricultural images and detect multiple types of plant diseases or stresses in a very short time (Awais et al., 2025).

Integrating UAV-acquired aerial images with AI algorithms allows the early detection of water stress or nutrient deficiencies before symptoms become visually apparent. Subtle variations in leaf color or vegetation indices can provide valuable information for fertilization adjustments and crop management optimization (Canicatti & Vallone, 2024; Padhiary et al., 2024).

2.2. AI for irrigation, fertilization, and resource management

Beyond diagnostic applications, AI is increasingly used to optimize the use of agricultural resources. Predictive models based on machine learning can analyze climate data, soil moisture, and crop status to estimate optimal water and fertilizer requirements at different plant growth stages (Kamilaris et al., 2017; Mana et al., 2024). When integrated with IoT sensor networks, these models enable the automatic, real-time adjustment of irrigation and fertilization according to environmental conditions and crop needs (Gupta et al., 2025; Mansoor et al., 2025). In intensive horticulture, such systems contribute to resource-use efficiency and reduce input consumption (Jaiswal et al., 2025).

Similarly, the integration of robotic systems with AI algorithms supports the automation of production processes. Robotic platforms can perform tasks such as crop monitoring, application of phytosanitary treatments, or selective harvesting, increasing operational efficiency in horticulture (Hamrani et al., 2025). AI algorithms can also automatically regulate ventilation, shading, and fertilization systems in greenhouses, maintaining a stable microclimate and improving uniformity in production (Zhang et al., 2024).

2.3. AI in post-harvest processes: sorting, quality, and traceability

In the post-harvest phase, AI is used for automated classification of horticultural products based on size, color, and visual quality. Vision-based systems reduce sorting errors and improve batch standardization, enhancing the quality of

market-ready products (Ferentinos, 2018; Wakchaure et al., 2023; Matache et al., 2025). Moreover, AI technologies in supply chain management facilitate product traceability and quality monitoring throughout transport and distribution. Analysis of logistics data and the use of predictive algorithms can reduce post-harvest losses and optimize commercial flows in the horticultural sector (Gamage et al., 2024; Raj & Prahadeeswaran, 2025).

2.4. Integrated AI-IoT Systems: the foundation of autonomous agriculture

Current trends in horticulture focus on integrating AI with IoT technologies to develop autonomous production systems. Systems based on multiparameter sensors connected to digital platforms and AI algorithms allow automatic control of irrigation, ventilation, lighting, and fertilization in greenhouses and tunnels (Zhang et al., 2023; Bicamumakuba et al., 2025).

The integration of these technologies enables the analysis of historical data alongside real-time information to continuously adjust production parameters. AI-IoT systems thus form the foundation of the “smart agriculture” concept, in which production processes are continuously monitored and optimized based on data generated by the digital infrastructure (Abobatta, 2024).

3. Young farmers and the adoption of digital technologies

Young farmers play a crucial role in the digitalization of agriculture, being more open to innovation and generally possessing higher levels of digital literacy compared to older generations (Mihai et al., 2025; Aranguri et al., 2025). Higher education levels, easier access to information, and familiarity with digital environments facilitate the adoption of modern technologies, such as agricultural sensors, drones, crop monitoring systems, and digital farm management applications (Klerkx et al., 2019; Wolfert et al., 2017). By using these technologies, young farmers contribute to the development of precision agriculture and the optimization of production processes through data-driven decision-making.

At the same time, the literature identifies barriers that may limit the adoption of digital

technologies, including high equipment costs, limited access to financing, and insufficient digital infrastructure in rural areas (Rotz et al., 2019). Additionally, the lack of advisory services and specialized training programs can slow down farm digitalization (Klerkx et al., 2019; FAO, 2019).

Nevertheless, the integration of digital technologies enhances resource-use efficiency, reduces production costs, and improves crop productivity, particularly in horticulture and vegetable production (Wolfert et al., 2017; Rotz et al., 2019). Digitalization also increases the attractiveness of agriculture for younger generations by reducing reliance on labor-intensive work and transforming agricultural activities into more innovative, technology-oriented processes (FAO, 2019).

Furthermore, the use of digital technologies and intelligent crop monitoring systems allows farmers to make more informed decisions and improve the economic performance of their farms (Markovits, 2023). Consequently, digitalization is considered a key factor in revitalizing the agricultural sector and attracting young people to farming, contributing to the development of more efficient and sustainable production models (Mihai et al., 2025).

4. Knowledge transfer and the role of AKIS in accelerating digitalization

Knowledge transfer is an essential factor in the adoption of digital technologies in agriculture. Agricultural Knowledge and Innovation Systems (AKIS) facilitate the flow of information between research, agricultural advisory services, farmers, and the private sector, supporting the rapid dissemination of innovations and the integration of digital technologies in farm management (European Commission, 2023; Knierim et al., 2022). Access to advisory services, training, and knowledge-sharing networks is considered critical for the adoption of technologies such as agricultural sensors, data platforms, IoT systems, and digital analytics tools used in precision agriculture (Klerkx & Rose, 2020; OECD, 2021). Under the 2023–2027 Common Agricultural Policy reform, AKIS are recognized as a strategic pillar for promoting innovation and digitalization in the agricultural sector. Their integration into European agricultural policies aims to strengthen collaboration among farmers, research

institutions, universities, agricultural advisors, and technology companies, improving knowledge transfer and accelerating the adoption of digital solutions in agriculture (European Commission, 2023).

Agricultural advisors play a central role within these systems, acting as intermediaries between research and practice. They support farmers in interpreting technical information, using digital tools, and integrating precision agriculture technologies into production processes (Ingram & Maye, 2020; Knierim et al., 2022). Studies show that farmers who have access to advisory and specialized technical support adopt digital technologies more rapidly and utilize agricultural data more effectively.

Farmers' digital skills are also a key determinant in the adoption of modern technologies. Professional training programs, technology demonstrations, and participatory innovation initiatives contribute to skill development and enhance decision-making in farm management (Klerkx & Rose, 2020; OECD, 2021). Simultaneously, digital platforms and agricultural innovation networks facilitate experience exchange among farmers and access to technical information, accelerating technology diffusion and the development of locally adapted solutions (European Commission, 2023; Kountios et al., 2024).

Strengthening AKIS, expanding advisory services, and developing digital training programs are therefore essential for accelerating agricultural digitalization and supporting farm modernization in the transition toward more innovative and sustainable agricultural systems (Del Puente et al., 2026).

5. Digitalization and sustainability in vegetable production

Digitalization is increasingly associated with sustainability goals, providing tools to optimize resource use and enhance the economic and social performance of farms. Digital technologies - including agricultural sensors, IoT systems, drones, data analytics platforms, and AI applications - enable real-time crop monitoring and support farmers' decision-making. The literature highlights that

integrating these technologies contributes to the development of more efficient and resilient agricultural systems, optimizing resource use and reducing environmental impact (Wolfert et al., 2017; Klerkx et al., 2019).

In vegetable production, characterized by high input intensity and the need for continuous crop monitoring, digital technologies significantly improve ecological sustainability. Precision agriculture, based on soil sensors, aerial imagery, and crop monitoring systems, enables variable-rate application of water, fertilizers, and pesticides, reducing resource consumption and environmental impact (Gupta et al., 2025). Sensor-based smart irrigation systems and digital control platforms contribute to more efficient water use, while drones and remote sensing technologies facilitate early disease and pest detection and targeted phytosanitary interventions (Hamrani et al., 2025). In controlled environments, digital climate-control systems allow for automatic adjustment of temperature, humidity, and ventilation, stabilizing production conditions and reducing energy consumption (Zhang et al., 2024).

Beyond ecological benefits, digitalization strengthens the economic sustainability of vegetable farms. Real-time crop monitoring and data analysis allow for optimized input use and reduced operational costs, while predictive models and data analytics support yield estimation and production planning (Kamilaris et al., 2017; Ferentinos, 2018). These technologies help reduce losses, improve production efficiency, and enhance the economic resilience of farms-particularly important for the vegetable sector.

Digitalization also impacts the social dimension of sustainability. Automating labor-intensive agricultural processes transforms farmers' activities into more monitoring-, analysis-, and data-management-oriented work (Rotz et al., 2019). At the same time, digital technologies generate new skills and professional roles in agriculture and can increase the sector's attractiveness to younger generations, contributing to professionalization and farm modernization (Klerkx et al., 2019).

Therefore, digitalization is a key factor in consolidating sustainability in vegetable production by integrating ecological, economic,

and social dimensions of agricultural development.

CONCLUSIONS

The literature analysis confirms that digitalization is a key factor in the modernization of vegetable production. Technologies such as drones, IoT sensors, and artificial intelligence algorithms enable real-time data collection and interpretation, facilitating predictive, automated, and efficiency-oriented agriculture.

From an ecological perspective, digitalization reduces the use of water, fertilizers, and pesticides, contributing to the protection of natural resources and enhancing the resilience of horticultural systems.

From an economic standpoint, smart technologies optimize costs, reduce losses, and improve production planning.

The social dimension is supported through the active involvement of young farmers, who adopt digital solutions more rapidly and contribute to the transformation of agricultural business models. Nevertheless, the success of the digital transition depends on the strengthening of AKIS, access to professional training, and the development of rural digital infrastructure.

Digitalization in vegetable production is not merely a technological innovation but a strategic process of structural transformation, integrating ecological sustainability, economic competitiveness, and generational renewal in the agricultural sector.

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MANAGEMENT OF BIODIVERSITY IN AGRICULTURAL SYSTEMS IN ROMANIA

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Abstract

Biodiversity plays a fundamental role in maintaining the stability, productivity, and resilience of agricultural systems. In Romania, agricultural landscapes are characterized by a combination of traditional farming practices and modern intensive systems, which generate both opportunities and pressures for agroecosystems. This paper aims to analyze the importance of biodiversity management in agricultural systems, with a focus on sustainable farming practices that support ecosystem functions and long-term agricultural viability. The study highlights the role of environmentally friendly agricultural practices, diversified cropping systems, and landscape elements in conserving biodiversity and enhancing ecosystem services. By promoting balanced management strategies that integrate agricultural production with environmental protection, biodiversity-friendly farming can contribute to food security, climate change adaptation, and sustainable rural development in Romania

Key words: agriculture, biodiversity, ecosystem, services, sustainable.

INTRODUCTION

Biodiversity is a fundamental component of agricultural systems, playing a crucial role in maintaining ecosystem stability, productivity, and resilience (FAO, 2019). Agricultural biodiversity encompasses the diversity of species, habitats, and ecological processes that support food production, including soil organisms, pollinators, natural enemies of pests, and functional landscape elements. These components contribute to essential ecosystem services such as nutrient cycling, soil fertility, pollination, and biological regulation, which are vital for sustainable agricultural production (IPBES, 2019).

Across Europe, agricultural intensification and land-use change have been identified as major drivers of biodiversity loss in farmland ecosystems (European Commission, 2020). In Romania, agricultural landscapes are characterized by a high degree of heterogeneity, shaped by the coexistence of traditional low-intensity farming systems and modern intensive agricultural practices. Traditional farming systems, particularly those associated with extensive grasslands and mixed crop–livestock systems, have historically supported high levels of biodiversity. However, recent trends toward

intensification, land abandonment, and the simplification of cropping systems have led to habitat fragmentation and a decline in farmland biodiversity, with significant implications for ecosystem functioning and rural sustainability (Eurostat, 2022).

The integration of biodiversity conservation into agricultural management has therefore become a priority at both national and European levels. Sustainable agricultural practices, such as crop diversification, reduced soil disturbance, and the conservation of semi-natural habitats, are increasingly recognized for their potential to enhance biodiversity and ecosystem services within agricultural landscapes (FAO, 2021). In this policy context, agri-environmental and agri-environment-climate measures represent key instruments for encouraging farmers to adopt environmentally responsible management practices and for mitigating the negative impacts of agricultural intensification on biodiversity (European Commission, 2021).

The aim of this paper is to analyze the management of biodiversity in agricultural systems in Romania, with particular emphasis on the role of sustainable farming practices and policy measures in conserving agroecosystems. By examining current challenges and opportunities, the study seeks to highlight the

importance of biodiversity-friendly management strategies for ensuring environmental protection, food security, and sustainable rural development.

MATERIALS AND METHODS

This study adopts a qualitative and descriptive research design focused on the analysis of biodiversity management in agricultural systems in Romania. The methodological approach is based on the review of scientific literature, the analysis of statistical data, and the examination of policy documents relevant to agricultural biodiversity and sustainable farming practices. This approach is appropriate for exploring general trends and relationships between agricultural management practices and biodiversity-related outcomes at the national level.

The research relies exclusively on secondary data sources and does not include field-based measurements or experimental analyses. The methodology is therefore aligned with the descriptive nature of the results presented in this study.

Data Sources

The study is based on scientific literature addressing agricultural biodiversity, ecosystem services, and sustainable agriculture. Peer-reviewed articles and institutional reports were used to establish the theoretical background of the research.

In addition, official documents and reports published by the Food and Agriculture Organization of the United Nations (FAO), the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES), and the European Commission were consulted to contextualize biodiversity management within European and national agricultural policies.

Statistical data related to land use and agricultural management were obtained primarily from Eurostat, the Statistical Office of the European Union, and from publicly available datasets. These data were used to illustrate trends relevant to biodiversity management in Romanian agriculture.

Analytical Approach

The analysis is based on a descriptive interpretation of trends derived from the selected statistical data and literature sources. Particular attention was given to the evolution of agricultural areas managed under low environmental impact systems, as this indicator reflects the increasing integration of environmentally friendly practices into agricultural management.

In addition, the Harmonised Risk Indicator 1 (HRI1) was considered as a complementary indicator to illustrate changes in pressure related to pesticide use at the national level. The interpretation of this indicator was limited to trend analysis and did not involve quantitative modeling or causal assessment.

The results are presented in the form of a table and figures, which support the discussion of observed trends and their potential implications for biodiversity management in agricultural systems.

Limitations of the Study

The study is based on secondary data and aggregated indicators, which may limit the ability to capture local-scale biodiversity responses. Furthermore, the descriptive nature of the analysis does not allow for the establishment of causal relationships between agricultural practices and biodiversity outcomes. Despite these limitations, the applied methodology is suitable for identifying general patterns and supporting the discussion of biodiversity-oriented management approaches in Romanian agriculture.

RESULTS AND DISCUSSIONS

Biodiversity management in agricultural systems is strongly influenced by the way agricultural practices are implemented and adapted to local environmental conditions. Agricultural biodiversity depends on multiple interacting factors, including crop diversity, landscape structure, and the intensity of agricultural inputs, all of which directly affect ecosystem services such as soil fertility, pollination, and biological regulation (Zhang et al., 2007; FAO, 2018; Pretty, 2018).

In Romania, increasing attention has been given to environmentally friendly agricultural practices in response to biodiversity decline and growing environmental pressures on agroecosystems. One relevant indicator for assessing this trend is the evolution of agricultural areas managed under low

environmental impact systems, which reflects the gradual integration of biodiversity-oriented management principles into agricultural practice.

Table 1 presents the evolution of agricultural area managed under low environmental impact systems in Romania between 2007 and 2023.

Table 1. Evolution of agricultural area managed under low environmental impact systems in Romania (2007-2023)

Year	Area (thousand ha)	Share of total agricultural area (%)
2007	131	1.0
2010	182	1.4
2013	288	2.2
2016	326	2.5
2019	451	3.4
2023	596	4.5

Source: Public data, authors' processing.

The data reveal a constant upward trend in the area managed under low environmental impact practices. Between 2007 and 2023, the area increased more than fourfold, indicating a growing interest in agricultural practices that are better aligned with biodiversity conservation objectives. Despite this increase, such areas still account for a relatively small share of total agricultural land, suggesting that biodiversity-friendly management remains a complementary rather than dominant approach within Romanian agriculture.

This trend is illustrated in Figure 1, which highlights the steady expansion of low environmental-impact agricultural areas over the analyzed period.

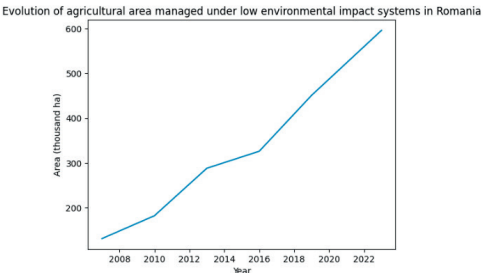


Figure 1. Evolution of agricultural area managed under low environmental impact systems in Romania (2007-2023)

(Source: Public data, authors' processing)

The gradual increase shown in Figure 1 reflects the combined influence of agri-environmental

policy measures, economic incentives, and increasing awareness of environmental issues among farmers. However, the moderate pace of expansion also indicates existing constraints, such as limited access to information, administrative complexity, and economic uncertainty, which may reduce the adoption of biodiversity-oriented practices.

Beyond land-use indicators, evaluating biodiversity management also requires an assessment of pressures related to agricultural inputs. At European Union level, the Harmonised Risk Indicator 1 (HRI1) is used to estimate risks associated with pesticide use (European Commission, 2022; Eurostat, 2024). Lower HRI1 values indicate reduced pressure on agroecosystems and a potentially more favorable context for biodiversity conservation. The evolution of HRI1 values for Romania between 2015 and 2023 is presented in Figure 2. The decreasing trend observed for HRI1 suggests a gradual reduction in pressure related to pesticide use in Romanian agriculture. This evolution may be associated with improved compliance with integrated pest management principles, increased use of preventive agronomic measures, and more targeted application of plant protection inputs. Such developments are relevant for biodiversity management, as reduced pressure on non-target organisms contributes to the preservation of functional biodiversity within agroecosystems.

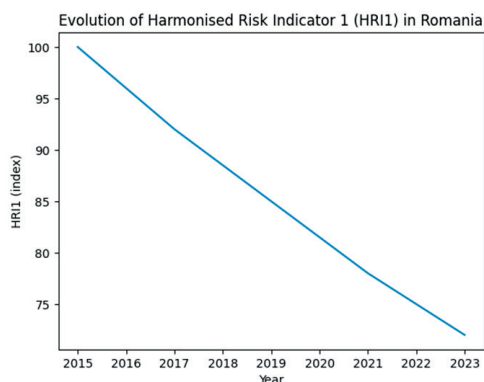


Figure 2. Evolution of Harmonised Risk Indicator 1 (HRI1) in Romania (2015-2023)
(Source: Eurostat, authors' processing)

A comparative interpretation of the two trends indicates that the expansion of low environmental impact agricultural areas is accompanied by a reduction in overall environmental pressure. While a direct causal relationship cannot be established based solely on these indicators, the observed patterns are consistent with the assumption that biodiversity-oriented management practices contribute to improved environmental performance in agricultural systems (FAO, 2018).

Overall, the results emphasize that effective biodiversity management in Romanian agricultural systems relies on the integration of sustainable agronomic practices, landscape-level planning, and supportive policy frameworks. Biodiversity-friendly management should therefore be understood as a gradual and adaptive process that balances agricultural productivity with environmental protection, rather than as a rigid transformation of existing farming systems.

CONCLUSIONS

The management of biodiversity in agricultural systems represents a key challenge for ensuring the long-term sustainability of agriculture in Romania. The analysis highlights that biodiversity conservation is closely linked to the way agricultural practices are implemented and integrated at the field and landscape levels.

The results confirm that agricultural systems characterized by diversified practices and reduced environmental impact have a higher potential to support ecosystem services such as

soil fertility, pollination, and biological regulation. These ecosystem services are essential for maintaining productive and resilient agroecosystems.

The evolution of agricultural areas managed under low environmental impact systems indicates a gradual but consistent increase over the analyzed period. Although these areas still represent a limited share of total agricultural land, their steady expansion reflects a growing interest in biodiversity-friendly management approaches and the increasing role of environmental considerations in agricultural decision-making.

The analysis of the figures presented in the Results and Discussions section shows a clear correspondence between the expansion of low environmental impact agricultural areas and the reduction of environmental pressure related to agricultural inputs. In particular, the trends illustrated in Figures 1 and 2 suggest that biodiversity-oriented management practices are associated with more favorable environmental outcomes, even in the context of predominantly conventional agricultural systems.

The decreasing trend of the Harmonised Risk Indicator 1 (HRI1) further indicates progress toward more responsible input management in Romanian agriculture. While this trend cannot be interpreted as a direct causal effect of biodiversity-oriented practices alone, it supports the assumption that integrated and preventive management approaches contribute to reducing pressure on agroecosystems.

Overall, the findings emphasize that effective biodiversity management in Romanian agricultural systems does not require a radical transformation of existing farming models, but rather their gradual adaptation through sustainable agronomic practices, landscape-level planning, and supportive policy frameworks. Strengthening the implementation of biodiversity-friendly measures and improving farmer participation remain essential for enhancing the environmental performance and resilience of agricultural systems in the future.

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IN SITU AND EX SITU CONSERVATION MEASURES FOR THE THREATENED SPECIES *POTENTILLA ASTRACANICA* JACQ. IN THE REPUBLIC OF MOLDOVA

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Abstract

This study of in situ and ex situ activities is aimed at the effective protection of populations of Potentilla astracanica Jacq. - an endangered Eurasian species that grows spontaneously in countries such as Romania, Bulgaria, Türkiye, Southern Ukraine, and South-Eastern Russia. In the Republic of Moldova, it is at the northern limit of its distribution area and is protected by Law, by inclusion in the Red Book of the Republic of Moldova, 3rd edition. Potentilla astracanica was identified in the local flora only in the southeast and southwest of the country. Our investigations in the species' habitat have demonstrated that the numerical strength of the populations is very low and consists mainly of mature specimens that grow solitary, but also in small groups of 2-3 specimens. In order to establish viable and sustainable populations of Potentilla astracanica, it is necessary to cultivate an effective species through conservation actions in natural habitats, but also by strengthening existing populations. Therefore, a series of measures to improve in situ conditions is imperative, as well as the identification of new specific habitats for its repatriation actions.

Key words: *Potentilla astracanica*, rare taxa, habitat, repatriation, population.

INTRODUCTION

A thorough knowledge of the biological specificity and ecological preferences of rare species, as well as their constant monitoring, allows us to identify the factors that influence their existence and helps us to develop more effective conservation strategies and measures. *Potentilla astracanica* Jacq. is an endangered Eurasian species, which grows spontaneously in countries such as Romania, Bulgaria, Türkiye, Southern Ukraine, and South-Eastern Russia (Figure 1) (POWO). The habitat of the species is represented by steppe slopes, pubescent oak forests, shrubberies, and forest edges, dry, rocky, and sandy places, limestone outcrops, sands, granites, and shales. Subendemic species. Due to the specific habitat, in all these countries the numerical population is reduced, and in Romania, *Potentilla astracanica* is included in the The red list of higher plants in Romania (Oltean, Negrean et al., 1994). In the Republic of Moldova, it is at the northern limit of its distribution area, having a dispersed and fragmented distribution, being found in the South-West of the country on the loess hills of the Emerald Site “Lower Prut,” with three

growing points, and in the South-East of the republic, with a single growing point in the Emerald Site “Lower Nistru”. The low number of individuals and the fragmented distribution on the territory of the Republic of Moldova determined the inclusion of this species in the 3rd edition of the Red Book of the Republic of Moldova, being classified in the Endangered category [EN] (Tofan-Dorofeev, 2015).



Figure 1. The distribution range of the species *Potentilla P. astracanica* Jacq.

Potentilla astracanica Jacq. grows spontaneously in the flora of the Republic of Moldova in glades

and forest edges among phytocenoses of subarid downy oak thickets, scrublands, and slopes with steppe vegetation. Populations of the species have been identified only in the northwest of the Râscăieți commune in the Ștefan-Vodă district and in the communes of Giurgiulești, Văleni, Slobozia Mare, and Cișlița-Prut in the Cahul district (Figure 2).

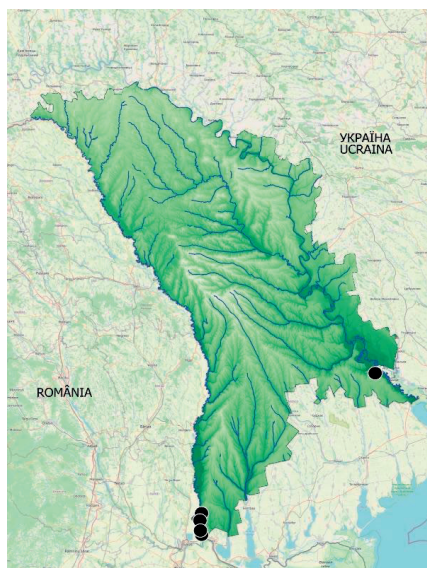


Figure 2. Geographical location of the species *P. astrachanica* Jacq. in the Republic of Moldova

Investigations carried out by us in the habitat of the species have shown that the numerical strength of the populations in all the places of growth is very low and is mainly made up of mature specimens, which grow solitary, but also in small groups. Our monitoring has established that, although the size of the populations is extremely small, the number of individuals remains constant from one year to the next. However, it was found that the condition of the habitats in all sites is precarious due to various pressures, the plants being strongly affected by drought, intensive grazing, but also by soil erosion, which has been increasingly severe in recent years. The biological particularities and environmental requirements of the species, combined with the existing threats to its populations and habitats, determine the need for prompt protection measures. In order to have viable and sustainable populations of *Potentilla astrachanica*, it is necessary to increase the

number of the species through repopulation and conservation activities in its natural habitats, as well as the consolidation of existing populations. Therefore, a series of measures to improve the *in situ* conditions is imperative, as well as the identification of new specific habitats for its repatriation actions. Thus, within the project "Research and *ex situ* and *in situ* conservation of plant diversity in the Republic of Moldova", we aim to offer solutions for the efficient protection of *P. astrachanica* populations by applying *in situ* and *ex situ* activities that consist of actions to strengthen existing populations, but also the creation of new populations in natural habitats and/or by repatriating them to previously known growth sites.

MATERIALS AND METHODS

The present study was conducted during 2023-2025 and was based on the populations of *P. astrachanica*, a rare species on the territory of the Republic of Moldova. The seeds were collected in the summer of 2022 from the natural habitat near Slobozia Mare, Cahul district. The seeds were germinated on filter paper in Petri dishes without prior processing. After germination, they were transferred to chernozem (black soil) mixed with peat in a ratio of 3:1, in seedling trays for one month, after which they were taken out of the greenhouse for growth and acclimatization to outdoor conditions. The exact location of the sites was determined using Google Maps. Population parameters were established on site according to accepted methods (Akeroyd and Jackson, 1995; IUCN Guidelines for Re-introductions, 1998; Godefroid et al., 2011; Gorbunov et al., 2008), and the detailed description is based on collected live plants, but also on data from the specialized literature (Nicolayeva, 1986; Tofan-Dorofeev, 2020). The general distribution, chorology, ecology, and phytocoenology of the species *P. astrachanica* were established by the authors, supported by data from the specialized literature (Gibbs, 1972; Nicolayeva, 1986; POWO). The conservation status of the species was assessed according to the methodology (Combroux & Schwoerer, 2007). Taxon identification was performed using the determiner (Negru, 2009).

RESULTS AND DISCUSSIONS

In the flora of the Republic of Moldova, *P. astracanica* grows on steppe slopes in priority habitats with conservation value represented by steppe meadows – 62C0* Ponto-Sarmatian steppes and habitats with shrub and forest edges – 40C0* Ponto-Sarmatian deciduous shrubs (Interpretation ... 2013). Within the Emerald Site “Lower Prut”, *P. astracanica* grows on a very rugged relief consisting of loess hills with quite steep southwest and west exposures, in some places up to 45 degrees, rising to almost vertical walls, with numerous ravines washed by water and landslides. To reduce the pronounced erosion, some slopes are planted with allogeneic tree species - *Robinia pseudacacia*, *Elaeagnus angustifolia*, and *Gleditschia triacanthos* L. On these slopes, together with *P. astracanica*, a rich flora grows, consisting of significant populations of rare plants, including species included in the Red Book, such as: *Ephedra distachya* L., *Scorzonera mollis* M. Bieb., *Convolvulus cantabrica* L., *Allium guttatum* Stev., *A. inaequale* Janka, *Ornithogalum amphibolum* Zahar. The populations of the species in this location are in an unfavourable conservation status, taking into account the

small number of specimens and the significant pressures affecting the species’ habitat, related to the presence of intensive grazing and its ruderalization. Also, the habitat around the Slobozia Mare locality is subject to a potential negative impact caused by the construction of a viewing platform. The other locations in the Site are used as pastures, but, due to landslides and difficult accessibility, these areas where the species studied grows have recently become impracticable for grazing, which offers some protection to the habitat. The population of *P. astracanica* in the South-East of the Republic of Moldova is located on the steppe hills of the lower Dniester, near the Răscăieți commune, Ștefan-Vodă district, which grows on a slope with a southwest exposure, alongside other valuable species such as *Achillea coarctata* Poir., *Amigdalus nana* L., *Asparagus verticillatus* L., *Allium paniculatum* L., *Hyacinthella leucophaea* (K. Koch) Schur, *Helichrysum arenarium* (L.) Moench, *Crocus reticulatus* Stev. ex Adams. The population of this site is in real danger of extinction caused by the construction of a pilgrimage site and landslides that have created gaps in the vegetation where ruderal and weed species are invading.



Figure 3. *P. astracanica* Jacq. in natural habitat near Slobozia Mare locality, Emerald Site “Lower Prut”

Potentilla astracanica is a herbaceous, perennial plant. The entire plant is densely villous, with long, soft, and patent hairs mixed with short hairs, as well as with long glandular hairs. Erect stems 10-30 cm tall, thick. Lower basal and cauline leaves long-petiolate, 5-foliolate, upper ones subsessile, ternate. Leaflets oblong-ob lanceolate to obovate, serrate. Stipules of the

lower leaves are cuneate, often untoothed; those of the upper cauline leaves are lanceolate. Inflorescence corymbiform. Flowers are short-pedicellate, actinomorphic, bisexual, 20-40 mm in diameter. The lacinia of the inner calyx are triangular-ovate; the lacinia of the outer calyx are triangular-lanceolate, long-acuminate, 2 times longer than the lacinia of the inner calyx, but

with the base of the same-width base. Petals broadly obcordate, by the length of the outer sepals, pale yellow. Androecium composed of about 30 stamens. Carpels numerous, cylindrical. Style conical. Fruit ovoid, broadly carinate, wrinkled nutlets. Pontic hemicytrophite. Xeromesophilic, moderately thermophilic, drought-resistant, photophilous, weakly acid-neutrophil species; prefers xerophytic forest chernozems. It blooms from late April to June; it is pollinated by insects. The fruits ripen towards the end of June to July. Decorative species (Figure 3).

The loss of habitats and the reduction in their surface area, determined by anthropogenic impact but also by climate change in recent decades, have led to the initiation of *ex situ* conservation actions, by collecting seeds and multiplying the species, with the aim of consolidating existing populations. Thus, in the summer of 2023, a series of field investigations were carried out to assess the conservation status of the species, to collect seed material, and to identify potential habitats for the creation of new populations. The population around the commune of Slobozia Mare was taken into study as the “donor population” from which seeds were taken; geographical coordinates: 45°35'35.3"N, 28°09'50.0"E. It occupies an area of about 500 m² and consists of about 30 generative specimens that grow isolated and in small groups of 2-3 phytoindividuals. The habitat of the species is represented by primary steppe communities with *Stipa capillata* L., *Stipa lessingiana* Trin. et Rupr., *Stipa ukrainica* P. Smirn., *Festuca valesiaca* Gaudin, with companion species such as *Poa bulbosa* L., *Euphorbia cyparissias* L., *Linum austriacum* L., *Medicago falcata* L., *Muscari neglectum* Guss. ex Ten., *Falcaria vulgaris*, *Teucrium chamaedrys* L., *Sideritis montana* L., *Thymus marschallianus* Willd., *Gagea ukrainica* Klokov, *Allium paniculatum* L., *Allium rotundum* L., *Eryngium campestre* L., *Marrubium peregrinum* L., including rare species *Hyacinthella leucophaea* (K. Koch) Schur, *Helichrysum arenarium* (L.) Moench, *Achillea coarctata* Poir., *Iris pumila* L., *Crocus reticulatus* Stev. ex Adams, *Amygdalus nana* L. In the spring of 2024, the previously collected seeds were germinated in Petri dishes on filter paper soaked in distilled water. 100 seeds were

placed in each Petri dish, and after 7 days 93% of the seeds germinated (Figure 4). The seedlings were transferred to a substrate of black soil and peat in a ratio of 3:1. As the seedlings developed, they were placed in the shade and watered regularly until they adapted. After 30 days of growth and adaptation, the plantlets were transferred to the experimental sector of the National Botanical Garden (Institute) "Alexandru Ciubotaru" (GBNI).

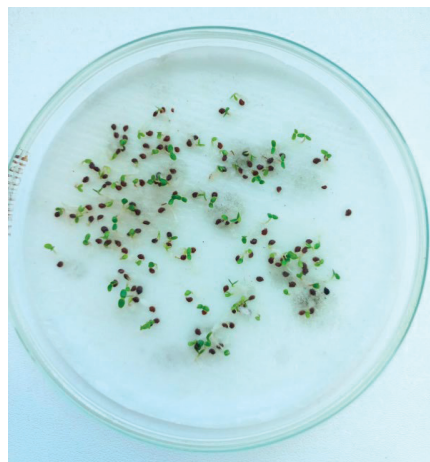


Figure 4. *P. astracanica* seed germination

The plants developed rapidly, and in the summer of 2025 several specimens bloomed (Figure 5). In the autumn of the same year, 20 specimens were extracted with a soil ball and translocated to a natural habitat previously identified during field expeditions. The habitat chosen for the creation of a new population was selected taking into account the distribution area of the species, the history of its detection in nearby locations or, at least, in the given area, the presence of compatible species, and the area with protection status. Thus, the location chosen by us is part of the Emerald Site “Lower Nistru”. Thus, we opted for a clearing in a pubescent oak forest, near the village of Grădinița, Căușeni District, geographical coordinates: 46°39'02.5"N, 29°35'31.5"E.

The selected habitat is in the proximity of the *P. astracanica* population from the vicinity of the Rascaieti commune and represents a pubescent oak clearing (species-specific habitat) with Ponto-Sarmatian steppe vegetation that corresponds to the Habitat of Community

Interest – 91AA* Ponto-Sarmatian Forest vegetation with pubescent oak. In the gaps formed by oaks, the vegetation cover is dominated by *Stipa lessingiana* Trin. et Rupr., *Stipa penata* L., *Melica transilvanica* Schur, *Thymus marchallianus* Willd., *Salvia nutans* L., *Hypericum perforatum* L., *Betonica officinalis* L., including rare species protected by law: *Amigdalus nana* L., *Asparagus verticillatus* L., *Adonis vernalis* L., *Pulsatilla montana* (Hoppe) Reicheinb., *Crocus reticulatus* Stev. ex Adams., *Iris hungarica* Waldst. et Kit. (Figure 6).



Figure 5. *P. astrachanica* in the experimental plot of the "Al. Ciubotaru" National Botanical Garden (I)

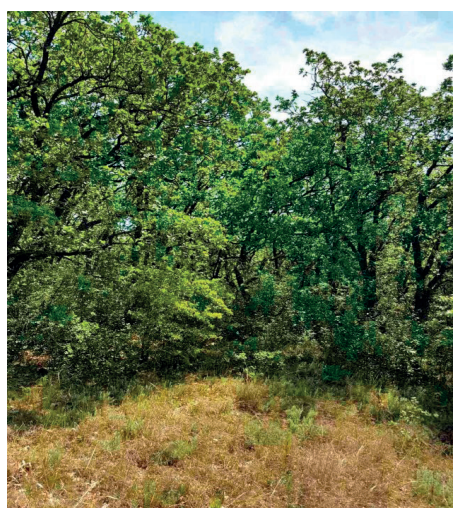


Figure 6. Habitat selected for the creation of a new population of *P. astrachanica*

In the following period, the newly established population will be monitored to assess the capture rate, growth, and survival of individuals, as well as possible interactions with environmental factors and other species in the habitat. Most likely, we will complete the population with new specimens in the following period to consolidate the population and increase the number of individuals. Our goal is to integrate the species into the respective habitat and contribute to the long-term conservation of the species under study.

At the same time, for the *ex situ* conservation of the *P. astrachanica* species, in the spring of 2025 an experimental collection was established on the plot designated for conservation studies within the GBNI. This collection represents an important element in the conservation plan, with the role of ensuring the maintenance and multiplication of the biological material of the species under controlled conditions, outside its natural habitat. The plants grown in this collection can be used to consolidate existing populations of the *P. astrachanica* species, but their main role is to serve as "seed-source plants." Seeds will be periodically harvested from these specimens, which can be preserved in the gene bank, or will be used to produce new seedlings under *ex situ* conditions. Thus, we will have planting material at hand for actions to reintroduce the species into the natural habitats from which it has disappeared in recent decades, thereby contributing to the restoration of natural populations and the restoration of the balance of affected habitats. We also intend to strengthen existing natural populations by introducing new specimens obtained under *ex situ* conditions in order to increase the density and stability of these populations. At the same time, research will be oriented towards identifying and analysing the limiting factors that have determined the decline of *P. astrachanica* populations in natural habitats. These factors may include habitat degradation, changes in land-use designation, anthropogenic pressure, competition with other species, especially alien ones resulting from tourism, and, last but not least, the influence of climate change. In this context, studies will aim to both assess the ecological conditions of the species' historical habitats and identify the management measures necessary for their restoration and protection.

The results of this research will contribute to the development of effective conservation and management strategies aimed at ensuring the long-term survival of the species in the spontaneous flora.

CONCLUSIONS

P. astracanica is a rare species in the Republic of Moldova and is included in the Red Book of the Republic of Moldova, 3rd ed., with the category Endangered [EN]. Most of the populations are located in the South-West of the country, while in the South-East there is only one population. The populations of the species are not very large in terms of area, and the main threat factors for the *P. astracanica* species are the destruction of the habitat caused by various anthropogenic factors, such as overgrazing and uncontrolled tourism, as well as soil erosion and climate change in recent years. It is necessary to respect the protection regime in the Emerald sites where the species grows, as well as to reinforce and strengthen existing populations. As a result of the study, the habitat of the species in the vicinity of Slobozia Mare was investigated, its condition was assessed, which has a high potential for conservation actions. A new population was established in the South-East of the country, which will be monitored over the next 10 years to assess the success of this initiative. An experimental plot was established within the GBNI for conservation and research under *ex situ* conditions, as well as for its use as a seed source, which will allow us to systematically collect data on phenology, productivity, and seed viability, contributing to the development of protocols for the conservation and multiplication of the *P. astracanica* species. The seeds obtained can be subsequently used for reintroduction or fortification of existing populations, or even in ecological restoration programs.

ACKNOWLEDGEMENTS

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RESEARCH ON THE VALORIZATION OF WATERMELON RIND IN MIXTURES WITH APPLE VARIETIES FOR DIVERSIFYING THE FOOD ASSORTMENT

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Abstract

*On a large scale worldwide, watermelons and apples are highly appreciated by consumers. Watermelon (*Citrullus lanatus*) is an excellent source of vitamins A, B1, B6, and C. It is consumed due to its health benefits and low caloric value. Watermelon rind mainly contains carbohydrates rich in pectic substances. The present study demonstrated the possibility of valorizing watermelon rind in combination with apples through the development of watermelon rind and apple jam, representing both a challenge and an opportunity for diversifying the range of preserved food products in the food industry. Laboratory-scale experimental trials demonstrated that such valorization enables the production of food products with valuable nutritional compounds. In line with current trends aimed at meeting consumer demand for healthy and sustainable products, the natural ingredients used-apples and watermelon rind- made it possible to obtain products without the addition of food additives, as these ingredients can act as natural substitutes. Apples are an excellent source of nutrients, with a high content of antioxidants, dietary fiber, and vitamins.*

Key words: apple varieties, by-product, source of nutrients, valorization, watermelon rind.

INTRODUCTION

Watermelon rind is a by-product that is most often discarded. In order to valorize this material, numerous studies have been conducted and continue to demonstrate its potential for a wide range of applications. Thus, Juan Pablo Capossio (2022) obtained watermelon rind flour, which was used in bakery products, leading to modifications in several quality indicators, including acidity, pH, water-holding capacity (WHC), oil-holding capacity (OHC), and processing time.

Ho et al. (2016) investigated the effect of hot-air oven drying at 40°C and 60°C, as well as freeze-drying, on the nutritional quality of watermelon rind powder, with particular emphasis on carbohydrate content and functional properties. In addition, Ho et al. (2016) used this powder in the production of noodles, and the analysis of their physical, chemical, and sensory properties showed high consumer acceptability for noodles containing watermelon rind powder.

Bread enriched with watermelon rind has the potential to be introduced to the market as a

functional food, as reported by Badr et al. (2015). Shivapour (2020) highlighted watermelon rind powder as a promising additive for improving the quality characteristics of toasted bread, with the study focusing on changes in sensory attributes such as moisture and color. Souad et al. (2012) demonstrated the feasibility of using watermelon rind in the preparation of jams with various flavors, including vanilla, pineapple, strawberry, lemon, and unflavored formulations.

The study conducted by Waghmare (2015) emphasized the potential use of watermelon rind as a source of pectin, in combination with isabgol husk (*Plantago ovata*), for the development of a novel laxative product.

Apples are highly complex fruits in terms of their biochemical composition, and apple juice is one of the most widely consumed fruit juices worldwide. It is preferred by both adults and children due to its characteristic taste and nutritional health benefits. Ghinea et al. (2022) analyzed cloudy apple juice in order to highlight the importance of polyphenols, which are present in higher concentrations compared with

clarified juice. The final products were stored for 7, 14, and 21 days at 20°C to evaluate their physicochemical and sensory properties after processing. Apple juice extraction was performed using a blender and a cold-press juicer. The results demonstrated that the apple juice samples varied depending on both the apple variety and the processing method used. Immature apples are used to obtain a natural source of acidity (apple-based acidulant). In the food industry, this ingredient is added for the preservation of fruits and vegetables (Cruciorescu, 2024).

Apple pomace is a residual biomass generated after apple processing and, on an industrial scale, can be used as a source of food ingredients (Rana, 2015). Lisičar et al. (2020) utilized apple pomace as a substrate for the production of pectin, biofuel (pellets), and concentrated apple pomace extracts, thereby valorizing this residual by-product into value-added products and creating links between different industries.

Sharma et al. (2014) demonstrated that pectin can be extracted from this by-product and described the extraction methodology. Similarly, Gullón et al. (2007) identified apple pomace as a promising raw material for lactic acid production. Perussello (2017) reported the extraction of valuable compounds from apple pomace using novel extraction techniques involving enzymes, electric fields, ultrasound, microwave heating, pressurized liquids, and supercritical/subcritical fluids. The study also discussed the advantages and disadvantages of conventional extraction methods required for the recovery of major compounds, namely pectin and polyphenols.

Apple pomace infusions have been proposed as a direct application of this by-product. Additionally, the volatile composition of beers produced with and without sugar was evaluated to mimic consumer preferences. To achieve this objective, three different valorization strategies were assessed: evaluation of apple aroma quality generated during the juice evaporation stage from different production seasons and its definition as a by-product; recovery of additional apple aroma through hydrodistillation from wet apple pomace; and stabilization of apple pomace as a dried product for infusion preparation (Coelho, 2021).

From apple juice processing waste, a functional ingredient (powder) with a complex biochemical composition and antioxidant potential was extracted, which can be used to improve bakery and pastry products (Catană et al., 2018). Gorjanović et al. (2020) presented apple pomace flour obtained through an innovative technological process (dehydration for 5 h at $T \leq 55^\circ\text{C}$, milling to 300 μm), used as a source of bioactive compounds with antioxidant, anti-obesity, and antidiabetic effects.

Corey et al. (2011) demonstrated the possibility of valorizing by-products resulting from apple juice production. These by-products were freeze-dried with the addition of green tea extract and stored for up to 45 days at 30°C under low and intermediate humidity conditions. Kinetic models were developed to describe color changes and reductions in selected monomeric and polymeric flavonoid content.

Rascon et al. (2009) investigated the pectin fraction of apple fruits by analyzing its composition and selected functional physicochemical properties, with the aim of using pectin as a potential texturizing agent in the food industry.

Apples and apple-derived products have beneficial effects on consumer health. Recent studies have shown that these products may be associated with improved outcomes related to age-related cognitive decline, diabetes, weight management, bone health, pulmonary function, and gastrointestinal protection (Dianne, 2011; Boyles, 2000).

In the present study, a comparison is made between jam produced from watermelon rind with apples and a product obtained from apples processed into apple juice. We valorized watermelon rind by obtaining an innovative product, “Watermelon Rind Jam with Apples”, and the comparison between the products highlights consumers’ preferences for different products.

MATERIALS AND METHODS

The fruits used in this study were obtained from ICDP Pitești - Mărăcineni. The apple varieties included in the study were Idared and Golden Reinders. These raw materials were processed to obtain the following products: watermelon rind jam with apples and apples in apple juice.

For the preparation of the novel product, apples in apple juice, the apple juice was obtained from

a blend of apple varieties - Florina, Idared, and Golden Delicious - harvested from the experimental field of ICDIMPH Horting Bucharest. According to the technological instructions, all processing steps within the apple juice production flow were strictly followed (Figure 1).

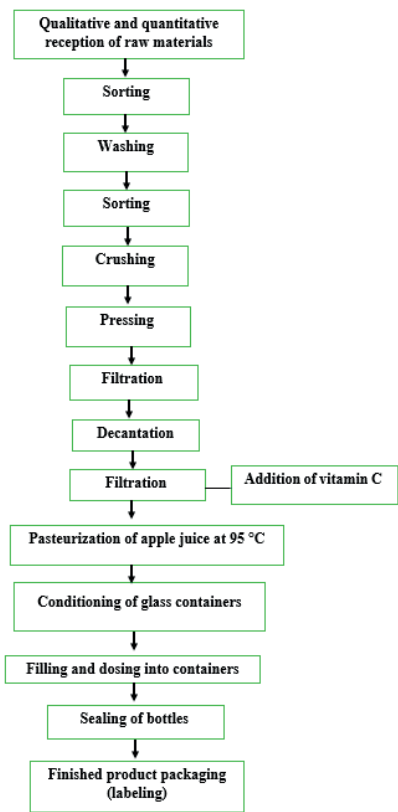


Figure 1. Technological process of apple juice production

The apple juice used in the production of apples in apple juice replaces the syrup traditionally obtained from sugar and water, thereby increasing the nutritional value of the final product. Under laboratory conditions, the Idared and Golden Reinders apple varieties were evaluated from an organoleptic perspective, and physico-chemical results were taken into account to establish the product formulation. A solid-to-liquid ratio was determined based on the net weight, and calculations were performed to determine the final dry matter content of the product -apples and apple juice (Figure 2). The soluble dry matter balance was carried out in accordance with the legislation.

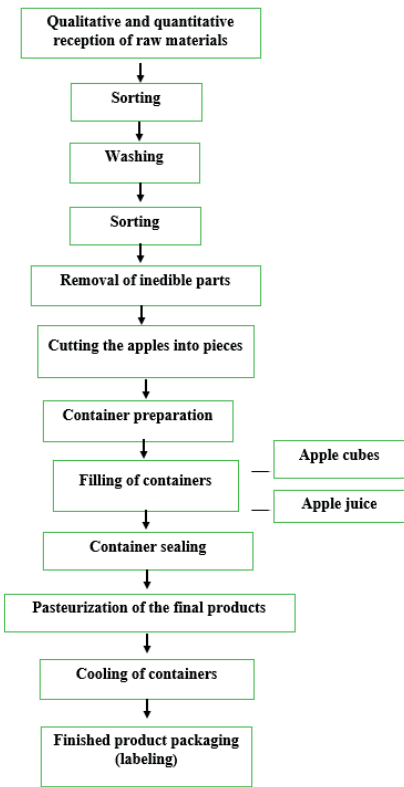


Figure 2. Technological Flow Chart for the Production of Apples in Apple Juice

The product Apples in Apple Juice is obtained from apples cut into pieces and covered with apple juice, packaged in hermetically sealed containers, and pasteurized (Figure 3).



Figure 3. Stages in the Production Process of Apples in Apple Juice

The focus of this study is on the valorization potential of watermelon rinds in combination with apple pulp. The watermelon rind, as a key ingredient in the product *Watermelon Rind Jam with Apples*, meets consumer demands for a novel product, regardless of age. Transforming watermelon rinds into stable and safe food products represents a technological and economic opportunity, with the potential to

generate innovative products with nutritional value. The sequence of operations in the technological flow is illustrated in Figure 4, showing the preparation process of this product. The first stage involves the reception of raw materials and verification of quality criteria (maturity level, fruit integrity, absence of mechanical and microbiological defects) and the weighing of batches. Sorting aims to eliminate damaged fruits, affected portions, or those showing signs of disease or pest infestation. The washing stage plays a crucial role in preventing contamination during subsequent processing.

After preparation, the raw materials- watermelon rind and apples- are cut into different forms, followed by the blanching operation. Blanching is an essential step for reducing the initial microbial load, inactivating oxidative enzymes, and preventing browning. Watermelon rind was blanched at 85-90°C for 3-5 minutes, while apple pieces were blanched at 80-85°C for 2-3 minutes. Additionally, blanching softens the plant tissues moderately, stabilizes color and texture, and facilitates efficient syrup diffusion.

After blanching, the watermelon rinds are immersed in a sugar syrup to allow syrup penetration into the plant tissues. Syrup concentration and diffusion time are essential parameters for achieving the desired final product texture. Apple pulp is then incorporated into the syrup obtained in the previous step, which ensures the harmonization of the flavor profile, reduces resource losses, and increases process efficiency.

The next stage involves concentrating the product by boiling until the desired final dry matter content is reached. Boiling was carried out until a final soluble solids concentration of 67°Brix was reached, as determined refractometrically. Glass containers are conditioned through sterilization to prevent microbial growth. Filling is followed by hermetic sealing, which prevents contamination and maintains the vacuum required for preservation. Pasteurization is then applied to ensure long-term product stability and is adjusted according to the product's characteristics. Pasteurization of the finished product was carried out at 100°C for 10 minutes. Cooling and labeling of the finished product are the final steps of the production flow.

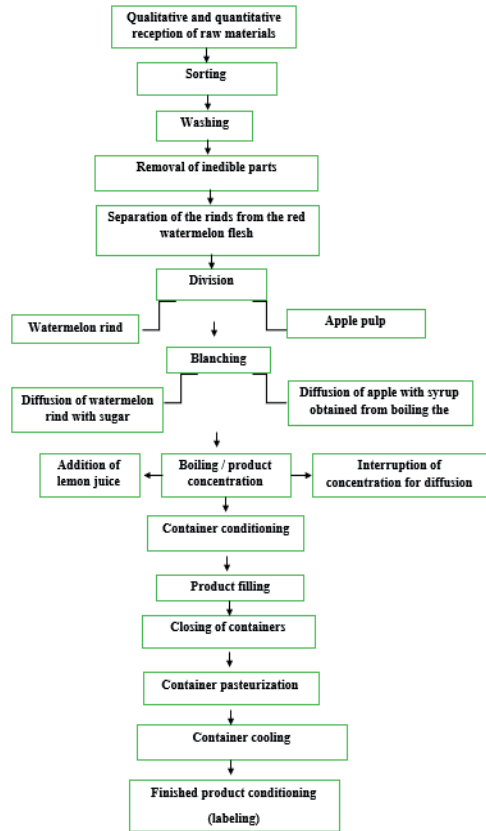


Figure 4. Technological Flow Chart of Watermelon Rind Jam with Apples

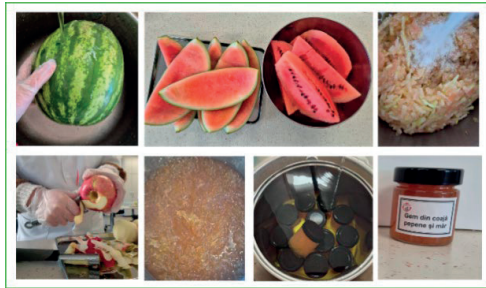


Figure 5. Stages in the Production Process of Watermelon Rind Jam with Apples

RESULTS AND DISCUSSIONS

The apples were analyzed from an organoleptic perspective in their fresh form, evaluating appearance, colour, aroma, and taste. Additionally, characteristics related to impurities, total sugar content, and vitamin C are detailed for the Golden Reinders variety in

Table 1 and for the Idared variety in Table 2. The results of the valorised products are presented in Table 3 for the finished product, *Apples in Apple Juice*, and in Table 4 for *Watermelon Rind Jam with Apples*.

The Idared apple variety has a higher sugar content, which explains its sweeter taste and better juiciness compared to Golden Reinders, which is less sweet. Golden Reinders contains more than twice the vitamin C (0.17 g/100 g) compared to Idared (0.08 g/100 g). This suggests a slightly higher nutritional value for Golden Reinders from the perspective of antioxidant contribution.

Table 1. Initial Analysis Results - Golden Reinders Variety

Characteristics	Maximum allowed	Methods of analysis	Results
Appearance	In accordance with	STAS 756-1/1985	Whole fruit, large size, slightly elongated shape, thin peel
Colour	In accordance with	STAS 756-1/1985	Greenish
Smell	In accordance with	STAS 756-1/1985	Specific
Taste	In accordance with	STAS 756-1/1985	Specific
Impurity content, %	In accordance with	STAS 756-1/1985	Absent
Total sugar content, g/100 g	In accordance with	SR 2213-12:2009	9.23
Vitamin C content, g/100 g	In accordance with	Farm. Rom. ed a X-a/ 93	0.17

Table 3. Results of the Finished Product - Apples in Apple Juice

Product characteristics	Analysis method	Results for apples in apple juice (Idared variety)	Results for apples in apple juice (Golden variety)
Moisture content, g%	SR 8613-1:2009	84.46	83.34
Sodium chloride, g%	SR ISO 3634:2009	0.04	0.11
Ash content, g%	SR 8613-1:2009	0.19	0.25
Total fat content, g%	SR 8613-1:2009	0.24	0.21
Saturated fatty acids, g%	SR 8613-1:2009	0.00	0.00
Sugars, g%	SR 2213-12:2009	10.81	8.34
Protein content, g%	SR 8613-6:2009	0.90	0.58
Carbohydrate content, g%	Norma met. 0702/2002	14.21	15.62
Energy value /caloric value, kcal per 100 g of product / kJ per 100 g of product	Norma met. 0702/2002	62.60/265.75	66.69/283.17

Idared juice is sweeter, consistent with the sugar content of the raw fruits (~14.31% in Idared vs.

Table 2. Initial Analysis Results - Idared Apple Variety

Characteristics	Maximum allowed	Methods of analysis	Results
Appearance	In accordance with	STAS 756-1/1985	Whole fruit, large size, spherical to slightly elongated shape, thin peel, juicy
Colour	In accordance with	STAS 756-1/1985	Red
Smell	In accordance with	STAS 756-1/1985	Specific
Taste	In accordance with	STAS 756-1/1985	Specific
Impurity content, %	In accordance with	STAS 756-1/1985	Absent
Total sugar content, g/100 g	In accordance with	SR 2213-12:2009	14.31
Vitamin C content, g/100 g	In accordance with	Farm. Rom. ed a X-a/93	0.08

The data in Table 3 highlight notable differences between the two apple juices, both in terms of composition and nutritional content. Regarding the initial fruit characteristics, particularly water content, the juice obtained from Idared apples exhibits a slightly higher water content of 84.46%, which aligns with the greater juiciness of the raw fruits, compared to Golden Reinders juice, which contains 83.34%.

The Golden Reinders juice shows a slightly higher sodium content (0.11%) than Idared juice (0.04%), which may result from natural variation. The mineral content is also slightly higher in Golden Reinders juice (0.25%), suggesting a better mineral profile for this variety.

9.23% in Golden Delicious). This difference remains after processing, with Idared juice

containing 10.81% sugar and Golden juice 8.34%.

Although Idared juice has a higher sugar content, Golden Delicious juice exhibits a slightly higher total carbohydrate content (15.62% vs. 14.21% in Idared), due to its higher content of polysaccharides and non-fermentable carbohydrates.

Table 4. Results of the Finished Product: Watermelon Rind Jam with Apples

Characteristics	Maximum permitted	Methods of analysis	Results
Appearance	According to	STAS 756-1/1985	Dense, homogeneous, uniformly viscous mass, free of impurities
Color	According to	STAS 756-1/01985	Light brown
Taste	According to	STAS 756-1/01985	Characteristic
Aroma	According to	STAS 756-1/01985	Characteristic
Moisture, %	According to	SR 2213-4:2007	17.94
Total inverted sugar, %	According to	SR 2213-12:2009	51.68
Total fats, g/100 g	According to	SR 2213-11:2007	0.37
Saturated fats, g/100 g	According to	SR 2213-11:2007	0.00
""Protein, %	According to	SR 2213-17:2009	0.13
Fiber, %	According to	Norma met.07/02/2002	0.32
Total carbohydrates, %	According to	Norma met.0702/2002	78.61
Sodium chloride, g/100 g	According to	SR91:2007	0.41
Total ash, %	According to	SR 2213-6:2009	2.60
Caloric/energy value, kcal/100 g product / kJ/100 g product	According to	Norma met. 07/02/2002	318.93/ 1354.83

The appearance of the *Watermelon Rind Jam with Apples* shows pieces of watermelon rind and julienned apple suspended in a gelled mass, with no signs of fermentation or mold. The color is consistent with the ingredients used. From an organoleptic perspective, the jam complies with STAS 3750/90, and both taste and aroma are pleasant and characteristic, without any off-flavors or off-odors.



Figure 6. Sensory Evaluation of Watermelon Rind Jam with Apples

The *Watermelon Rind Jam with Apples* was analyzed microbiologically, showing yeast and mold counts below 10 CFU/g, according to SR ISO 21527-2:2009, and Enterobacteriaceae counts below 10 CFU/g, according to SR ISO 21528-2:2017. The microbiological analysis of the products was carried out six months after the product was obtained. This product was developed and tested within the doctoral thesis on watermelon valorization. The products were evaluated based on five characteristics using a scale from 1 to 5. In Figure 7, the mean scores obtained from the sensory assessments are presented. *Apples in Apple Juice* received a score of 24.04, while *Watermelon Rind Jam with Apples* scored 23.76. Both products were rated as “Very Good”, according to Table 5.

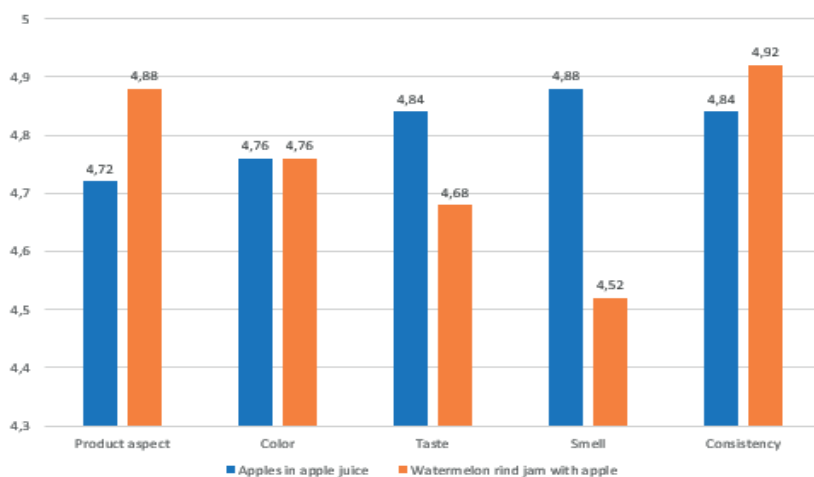


Figure 7. Mean Results of Sensory Evaluation for the Finished Products

Table 5. Classification of Scores from Sensory Analysis

Rating	Score Range
Very Good	23,1 – 25,00
Good	20,1 – 23,00
Satisfactory	16,1 – 20,00
Unsatisfactory	12,1 – 16,00
Bad	5,1 – 12,00
Very Bad	0 – 5,00

In the current study, the products belong to different groups. During the sensory evaluation, they obtained scores that reflect consumer preferences. The sensory analysis was carried out by a panel consisting of 10 semi-trained evaluators aged between 20 and 55 years, including both female and male participants. In the evaluation of appearance, Watermelon Rind Jam with Apples received a score of 4.88, making it slightly more visually appealing than Apples in Apple Juice, which scored 4.72. This small difference indicates an aesthetic advantage for the jam, due to its color and texture.

Both products received the same score for color (4.76), reflecting a similar appreciation from the panelists.

Both products were well rated for taste. Apples in Apple Juice, classified as a compote-type product, scored 4.84 and was perceived as pleasant, balanced, refreshing, and natural. The jam received a score of 4.68, and was appreciated by the panelists as a dessert product (Figure 7).

Each product has a characteristic aroma, with

both scores close to the maximum value. Apples in Apple Juice was considered superior in terms of aroma, which had a significant impact on the overall impression of the product.

Regarding consistency, evaluators perceived the jam as having a more appropriate texture (gelation, structure, liquid-solid ratio), with the jam scoring higher (4.92) compared to the apples in juice (4.84).

CONCLUSIONS

The primary characteristic of a food product is its nutritional value, as reflected in the results presented in Tables 3 and 4.

Raw watermelon rinds are plant-based resources with limited potential; however, when combined with the mentioned apple varieties, they can be transformed into safe, sensory-appealing food products. It is recommended to process watermelon rind in combination with both Golden and Idared apple varieties.

The differences between the products- *Watermelon Rind Jam with Apples* and *Apples*

in Apple Juice- are relatively small, as shown by sensory evaluation. *Apples in Apple Juice*, obtained from Golden Reinders and Idared apples, is perceived as pleasant, balanced, refreshing, and natural. The nutritional value of this product is enhanced by the juice obtained from a blend of Florina, Idared, and Golden Delicious apples.

Watermelon Rind Jam with Apples was also well-received by tasters and is recommended for consumption in various culinary applications within the food service and gastronomic industry.

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IN VITRO REGENERATION OF GLACIAL RELICT *VIOLA PALUSTRIS* L. SUPSP. *PALUSTRIS* AS GROUNDWORK FOR SUSTAINABLE USE AND GERMPLASM CONSERVATION

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Abstract

Climate change and increasing anthropogenic pressures have accelerated the degradation of European peatlands, leading to the decline of specialist wetland flora. *Viola palustris* L., a glacial relict and indicator of peatland ecosystem health, is currently listed as critically endangered in Romania. To support future conservation and sustainable use of this species, we developed an in vitro regeneration protocol based on aseptic germination of immature embryos and subsequent shoot and root development on optimized culture media. A custom sterilization procedure effectively eliminated fungal contamination typical of wetland-derived material, enabling reliable culture initiation. Although germination percentages were modest, regenerated plantlets exhibited normal morphology, vigorous growth, and strong multiplication capacity, particularly on media supplemented with balanced cytokinin–auxin–gibberellin combinations. Root induction with indole-3-butyric acid produced dense, healthy root systems, and acclimatized plants remained vigorous under ex vitro conditions. This protocol provides a practical foundation for establishing germplasm collections, supporting peatland restoration efforts, and facilitating the horticultural or phytochemical valorization of *V. palustris*.

Key words: anthropic pressure, endangered plants, ex situ conservation, *Viola palustris*.

INTRODUCTION

Marsh violet - *Viola palustris* L.- (Figure 1) is a polycarpic perennial plant, generally glabrous, stemless with thin rhizomes, which in summer emerges aerial stolones. Leaves are simple, 3-4 grouped in basal rosettes, ascending to erect, glabrous, with a petiole of 4-15 cm and sub-median bracteoles. Leaf blade of 2-6 cm is reniform, wider than long, and with an obtuse tip. The flowers are odourless, measure about 1-1.5 cm, have ovate to lance-ovate sepals, and pale lilac-coloured petals (Dihoru & Negrean, 2009)

V. palustris is a glacial relict (Dihoru & Negrean, 2009; Szatmari & Hurdu, 2022), cold-adapted, and occurs in niche habitats hosted in eutrophic and transition marshes. During the last centuries, the wetland areas have significantly diminished under the combined pressure of both climate change and human activities (Swindles et al., 2019). Consequently, the quality of wet habitats is altered and the species range is declining. Strong related to its habitat, *V. palustris* is considered an indicator of peatland health; a decline in its populations or the loss of

subpopulations signals significant changes in wetland and peatland habitats (Zabicka et al., 2023). In Romania, the species has scarce populations, being reported mainly from a few peatlands in Suceava and Maramureș counties. In national red lists and books, is currently assessed as *Critically Endangered* (Dihoru & Negrean, 2009; Hurdu et al., 2022). Despite population decline at the national level to date, no conservation measure was taken.

To restore peatland ecosystem services, the Institute of Biology Bucharest of the Romanian Academy began implementing various projects in 2015. In the PeatRO2 project, focused on the restoration of degraded mires and peatlands in the North-East 1 region of Romania, various restoration activities were conducted in peatland habitats from Suceava County. In addition to restoration activities, some *ex situ* conservation attempts were undertaken for a range of endangered plant species recorded at damaged sites, including the marsh violet.

Apart from its conservative importance, marsh violet represents an unexploited valuable genetic source as a cultivated plant in wet landscaping (rain gardens). In sympatric

European populations of *V. palustris*, interspecific hybrids with other violet species were identified (Zabicka et al., 2023), which recommends marsh violet as a valuable genetic source for amelioration in floriculture.

Moreover, in its natural habitat, marsh violet has a key role in ecosystem trophic relations. Marsh violet is a larval food plant for the small pearl-bordered fritillary (*Boloria selene*), a threatened butterfly species whose decline is strongly correlated with marsh violet population decline (Kendon et al., 2021).

In addition, recent studies have shown that wild violas are a valuable source of bioactive compounds supporting a wide range of biological functions due to their antibacterial, anti-inflammatory, and antioxidant effects (Zeng et al., 2025).

Considering the complex importance of marsh violet, the goal of the present study was to develop an efficient protocol for *in vitro* multiplication as a preliminary step for its germplasm *ex situ* conservation and availability for further natural habitat restoration, floriculture, or bioactive compound production.

MATERIALS AND METHODS

Plant material

Plant material sampling was allowed under collection permit No. 4621 issued on 02.04.2022 by the *Commission for the Protection of Natural Monuments*, Romanian Academy.



Figure 1. *Viola palustris* L. habitus - anthesis and fructification (Photo by Anca Manole)

Samples consisting of immature fruits (capsules) were collected between June and July

2023 during field trips conducted at the site *Tinovul din spatele Școlii Coșna*, Suceava County (47° 22' 16.80" N, 25° 10' 38.76" E).

Capsules were air-dried until the outer membrane detached from the immature embryos, allowing for inspection of morphological characteristics and evaluation of seed viability. Seeds were collected from all immature fruits and evaluated for use in *in vitro* cultures.

Seed selection was performed by visual inspection in regard to size variance, tissue damage, and other morphological variations, with healthy seeds being selected for regeneration.

To ensure aseptic conditions, all sample handling and procedures were performed in the controlled environment of a laminar airflow cabinet.

Sterilization

A custom sterilization protocol was developed for seed surface sterilization, involving a series of protocol steps:

1. Pre-washing: seeds were placed under running tap water for 30 minutes, with the addition of 5 ml of detergent (Domestos) to reduce surface residues.
2. Ethanol washing: seeds were immersed in a 70% (v/v) ethanol solution for 1 minute for antimicrobial membrane permeabilization.
3. NaDCC washing: seeds were immersed in a 2% (w/v) NaDCC (Sodium dichloroisocyanurate) for 10 minutes of exposure, while stirring the vial for uniform exposure.
4. Rinsing: seeds were rinsed 5 times with sterile double-distilled water to ensure all traces of NaDCC were removed.

After successful sterilization, seeds were inoculated onto different variants of culture media to ensure survivability and reduce desiccation.

Culture Media

Culture media variants M1-M4 and M6 were prepared using the same base of basal salts and vitamins, following an adapted Murashige & Skoog (MS) medium recipe (Murashige & Skoog, 1962). Composition of the media was adjusted with supplemental sucrose, 3% (w/v), and the media were solidified by adding 0.35% (w/v) agar. In the case of M6 the composition was also supplemented with the addition of activated charcoal, 1% (w/v).

The pH was measured and adjusted to 5.8 ± 0.10 , using stock solutions of 1N HCl and 1N NaOH. Media sterilization was done by autoclaving at 121°C for 20 minutes. After the media cooled to 50°C, selected plant growth regulators (PGRs) were added to complete the media variants.

Culture medium M5 was prepared using a Woody Plant (McCown & Lloyd, 1981) basal medium (WPM), supplemented with 1 mg/L Kinetin, with supplemental sucrose, 3% (w/v), and the media were solidified by adding 0.35% (w/v) agar.

Table 1. Variants of culture media (mg/L)

Media/ PGRs	BAP	GA ₃	Kin	IAA	IBA	NAA
M1	1	0.25	-	0.5	-	-
M2	-	1	-	0.1	-	-
M3	1	-	-	0.1	-	-
M4	-	-	-	-	1.5	-
M5	-	-	1	-	-	-
M6	5	-	-	-	-	1

***In vitro* Germination**

Three replicates of 10 seeds were inoculated on variants of M6 medium to assess germination and regeneration capacity.

The culture media with inoculated seeds were stored and incubated in a growth chamber at $24 \pm 2^\circ\text{C}$, photoperiod with a 16/8 h light/dark cycle and a light intensity of $40\text{--}50 \mu\text{mol m}^{-2} \text{s}^{-1}$. Germination rates were recorded after 7 and 14 days, while shoot length was measured at 60 days.

Total germination capacity was evaluated using formulas for the calculation of indicators of seed germination:

GE - Germination energy (%) = $(\text{Number of germinated seeds for } 7\text{d} \div \text{Total number of seeds}) \times 100$

GR - Germination rate (%) = $(\text{Number of germinated seeds for } 14\text{d} \div \text{Total number of seeds}) \times 100$

Shoot multiplication

After sufficient growth and development, tissue explants represented by nodal segments from the *in vitro* seedlings were used as explants for shoot multiplication.

Explants were inoculated in 350 mL culture jars containing 40 ± 5 mL of media. Culture environmental conditions were maintained as those described for germination.

Parameters such as the number of shoots, internode length, and vigour of explants were evaluated after 30 days. Subculture intervals were extended first to 60 days, then 90 days, as shoot proliferation and vigour increased.

Root induction

Rooting was induced by transferring elongated shoots to a basal MS medium supplemented with 1.5 mg/L indole-3-butyric acid (IBA).

Root development was monitored every 15 days, and the parameters recorded included root length, number of roots, as well as root morphology.

Acclimatization

Regenerated plants were transferred to *ex vitro* conditions in a solid substrate mixture of soil and perlite.

After a period of 14 days, the plant remained vigorous and presented no morphological or physiological abnormalities.

After 30 days, plants remained healthy and began producing new vegetative growth.

RESULTS AND DISCUSSIONS

Seed germination

The custom sterilization protocol developed for *V. palustris* seeds ensured that most contaminants from the seed surface and within the tissue were reduced or eliminated. NaDCC is especially effective against bacteria and fungi, which were the main risks of culture contamination.

Longer exposure times of the sterilizing agent yielded no contamination due to fungal infection across all replicates in the germination phase. This emphasizes the necessity of adapting sterilization protocols for removing fungal contaminants, especially from plants harvested from wet environments.

After sterilization, the seeds were inoculated on M6 medium variant with added active charcoal. Each 7 days, indicators for germination were calculated.

The germinative energy for all replicates varied between 10-20% in the initial timespan of a

week. Germination rate after 14 days was calculated between 30-40%. Total germination percentage after 30 days was calculated at 33.33% (Figure 2).

After germination had occurred, the plantlets began generating multiple shoots and increased rapidly in size.

Auxin presence in the medium, in small concentrations, induced rhizogenesis parallel to shoot generation. The plantlets developed a few small adventitious roots, white to pale green in colour.

Although germination rates were low, the shoots exhibited strong viability, and the developmental response was favourable in the presence of salts in MS media. Growth and development of the plantlets was uniform, with well-differentiated morphology.



Figure 2. *Viola palustris* L. plantlet germinated *in vitro* on MSR medium, 14 days after germination (Photo by Mihnea Vladimirescu)

The germinated plantlets exhibited no abnormal morphological characteristics. Shoot proliferation and elongation occurred rapidly; regenerated plantlets generated numerous axillary shoots from a basal nodal segment. Shoots developed long internodes with small to medium leaves.

Shoot growth and multiplication

Plantlets regenerated from immature embryos were transferred as individuals or used for explants on variants M1-M3 of culture media with different PGR concentrations to evaluate the best composition for germplasm growth and storage (Table 2).

Table 2. Shoot multiplication response on different media variants

Medium	PGR Composition	Multiplication Rate	Morphological Response
M1	BAP 1 mg/L + GA ₃ 0.25 mg/L + IAA 0.5 mg/L	High	Vigorous shoots, balanced growth
M2	GA ₃ 1 mg/L + IAA 0.1 mg/L	Low	Thin stems, few axillary shoots, small leaves
M3	BAP 1 mg/L + IAA 0.1 mg/L	Moderate	Multiple shoots but less vigorous than M1
M5	Kinetin 1 mg/L	Low-Moderate	Slow growth, reduced senescence
M6	BAP 5 mg/L + NAA 1 mg/L + activated charcoal	Not used for multiplication	High cytokinin inhibited germination

M1 medium variant provided the highest growth and multiplication rates among the tested variants (Figure 3). The PGR mix of cytokinin, auxin, and gibberellin used in this variant induced a balanced morphological response. Plantlets and explants generated axillary shoots within a month after inoculation. Shoots began to proliferate and grow over the following months. Subculture interval was optimized from 1 month in the initial stages of growth to 3 months following accelerated growth on this medium variant.



Figure 3. *Viola palustris* L. plantlets regenerated and multiplied on M1 medium variant (Photo by Mihnea Vladimirescu)

M2 variant offered low multiplication rates. Presence of gibberellin in this variant appeared to reduce the growth of plantlets, having reduced stem diameter, a low number of axillary shoots and leaves, and smaller leaf sizes.



Figure 4. *Viola palustris* L. plantlets regenerated and multiplied on M3 medium variant (Photo by Mihnea Vladimirescu)

M3 variant offered similar results to M1 variant (Figure 4), but reactivity was lower and growth balance was skewed. Multiple axillary shoots were present but were not as vigorous as M1. M5 medium variant based on WPM was used as an intermediate storage medium base. The kinetin supplement to this variant was used due to its properties as an anti-stress agent. Although shoot generation still occurred frequently, the senescence-retarding effect of kinetin induced a slower progression of life cycles and reduced loss of chlorophyll and yellowing.

Table 3. Tukey HSD post-hoc for shoot length

Comparison	Mean diff (cm)	p-value	Cohen's d
M1 vs M2	3.55	0	3.26
M1 vs M3	2.11	0	1.72
M2 vs M3	-1.44	0	-2.15

Group means (cm, across 12 replicates):

- M1: 4.64
- M2: 1.09
- M3: 2.53

M1 produced substantially longer shoots than both M2 and M3, and M3 also clearly exceeded M2 in shoot length (Table 3).

Root induction

Rhizogenesis was induced separately from growth and development of the main plantlet tissues.

Grown plantlets were placed on M4 medium • with 1.5 mg/L indole-3-butyric acid (IBA). After 7 days, multiple short lateral roots began sprouting, and after 14 days, the roots began forming a lattice on the sides and bottom of the culture jar, with long, fibrous, and thin roots present throughout the medium. After a month,

all plantlets had developed healthy, dense, and wide-spread root systems (Figure 5).



Figure 5. *Viola palustris* L. plantlet with regenerated roots (Photo by Mihnea Vladimirescu)

Table 4. Tukey HSD post-hoc for root length

Comparison	Mean diff (cm)	p-value	Cohen's d
M1 vs M2	4.32	0	2.06
M1 vs M3	3.33	0	1.49
M2 vs M3	-0.99	>0.05	-0.73

Group means (cm, across 12 replicates):

- M1: 6.14
- M2: 1.82
- M3: 2.81

M1 induces significantly longer roots than both M2 and M3, with large effect sizes. The difference between M2 and M3 is moderate in magnitude ($d \approx 0.73$) but does not reach significance at $\alpha = 0.05$ under Tukey correction (Table 4).

Table 5. ANOVA summary across media (M1, M2, M3)

Dependent variable	F	df (between, within)	p	η^2
Shoot number	0.00	2, 33	1.00	0.00
Shoot length	36.77	2, 33	0.0000	0.69
Root number	0.00	2, 33	1.00	0.00
Root length	16.35	2, 33	0.0000	0.50

- Shoot number and root number: no effect of medium (values are structurally identical across media).
- Shoot length: very strong medium effect (large $\eta^2 \approx 0.69$).
- Root length: strong medium effect ($\eta^2 \approx 0.50$).

Shoot number and root number did not differ among media (one-way ANOVA, $F(2,33) = 0.00$, $p = 1.00$ for both traits (Table 5). In contrast, shoot length was strongly affected by medium ($F(2,33) = 36.77$, $p < 0.001$, $\eta^2 = 0.69$), with M1 producing significantly longer shoots

than M2 and M3 (Tukey HSD, $p < 0.001$), and M3 exceeding M2 ($p < 0.001$) (Table 2). Root length also differed significantly among media ($F(2,33) = 16.35$, $p < 0.001$, $\eta^2 = 0.50$), with M1 yielding longer roots than both M2 and M3 (Tukey HSD, $p < 0.001$), while the difference between M2 and M3 was not significant (Table 3).

Acclimatization

The acclimatization phase demonstrated that *in vitro*-regenerated *V. palustris* plantlets possess strong adaptive capacity when transferred to *ex vitro* conditions. Rooted plantlets were transplanted into a soil-perlite substrate and maintained under high humidity during the initial transition period. After 14 days, the plants remained vigorous and exhibited no signs of stress, confirming the functional integrity of the root systems developed *in vitro*. As noted in the original observations, “after a period of 14 days, the plant remained vigorous and presented no morphological or physiological abnormalities,” indicating successful adaptation to non-sterile conditions.



Figure 6. Acclimatized *Viola palustris* L. plantlet after 30 days (Photo by Mihnea Vladimirescu)

By 30 days, all acclimatized individuals continued to grow actively, producing new leaves and stoloniferous structures characteristic of healthy marsh violet plants. The statement that “after 30 days, plants remained healthy and began producing new vegetative growth” underscores the stability of the regenerated material and the suitability of the rooting protocol for generating robust plants capable of surviving environmental transition.

These results confirm that the *in vitro* regeneration system developed in this study can

reliably produce acclimatized plants suitable for *ex situ* conservation, future reintroduction efforts, or horticultural applications. The high survival rate during acclimatization highlights the physiological normality of the regenerated plantlets and supports the broader applicability of the protocol for conservation and restoration programs targeting *V. palustris*.

Discussions

The present study represents an important step toward establishing an efficient *in vitro* regeneration system for *V. palustris*, a species of high conservation concern whose natural populations are declining due to peatland degradation. The successful initiation of aseptic cultures from immature embryos demonstrates that *in vitro* techniques can overcome the challenges associated with propagating wetland species, which often carry high microbial loads. The effectiveness of the custom sterilization protocol - particularly the use of NaDCC - confirms its suitability for plant material collected from humid, microbe-rich environments. This aligns with previous findings that chlorine-based disinfectants are especially effective for species originating from wet habitats (Jadranin et al., 2024; Kendon et al., 2021; Li & Gallagher, 1996).

Germination percentages were modest, with germinative energy ranging from 10-20% after seven days and total germination reaching 33.33% after 30 days. These values are comparable to those reported for other *Viola* species, which frequently exhibit physiological dormancy or embryo immaturity at the time of seed dispersal (Wang et al., 2023; Kuroda & Sawada, 2022; Varsamis et al., 2025). Nevertheless, once germination occurred, plantlets displayed vigorous growth and normal morphology, indicating that the MS-based medium provided adequate nutritional support. Shoot multiplication varied considerably among media variants. M1, which combined cytokinin, auxin, and gibberellin, produced the most balanced and vigorous shoot proliferation. This suggests a synergistic effect between the three PGR classes, promoting both cell division and elongation. The reduced performance of M2, containing gibberellin and low auxin, indicates that gibberellin alone is insufficient to sustain robust shoot formation in *V. palustris*. M3

produced acceptable but less vigorous growth, highlighting the importance of fine-tuning cytokinin–auxin ratios for optimal morphogenesis. These findings are consistent with previous studies on *Viola* species, where balanced PGR combinations were essential for stable shoot proliferation (Zhang et al., 2025; Babu et al., 2025; Capaci et al., 2024; Kendon et al., 2021). Root induction on IBA-supplemented medium was rapid and uniform, with dense, fibrous root systems forming within two weeks. This response aligns with the well-established role of IBA in promoting adventitious rooting in herbaceous species. The successful acclimatization of regenerated plants further demonstrates the physiological stability of the *in vitro*-derived material and confirms the suitability of the protocol for producing viable plants for *ex situ* conservation or restoration.

The statistical analysis of shoot and root development across the three culture media provides clear quantitative confirmation of the morphological trends observed during the experiment. As noted earlier in the manuscript, “M1 medium variant provided the highest growth and multiplication rates among the tested variants” and the ANOVA results strongly support this qualitative assessment. Shoot number and root number did not differ among media, reflecting the fact that each replicate produced a similar number of organs regardless of treatment. This uniformity is consistent with the species’ intrinsic developmental pattern, in which the initiation of new shoots and roots appears to be less sensitive to hormonal variation than subsequent elongation processes. In contrast, both shoot length and root length showed highly significant differences among media, indicating that elongation growth is strongly regulated by the specific hormonal composition of the culture medium. Shoot length exhibited a very large effect of medium ($F(2,33) = 36.77$, $p < 0.001$, $\eta^2 = 0.69$), with M1 producing markedly longer shoots than either M2 or M3. Tukey post-hoc comparisons revealed that all pairwise differences were significant, and the effect sizes were exceptionally large (Cohen’s $d > 1.7$ for M1 vs M3 and > 3.2 for M1 vs M2). These results confirm that the balanced cytokinin–auxin–gibberellin combination in M1 strongly promotes both cell division and elongation,

leading to vigorous shoot development. This aligns with previous observations in the genus *Viola*, where synergistic interactions between cytokinins and gibberellins have been shown to enhance shoot elongation and reduce apical dominance.

Root elongation followed a similar pattern, with a significant medium effect ($F(2,33) = 16.35$, $p < 0.001$, $\eta^2 = 0.50$). Again, M1 supported the most extensive root growth, producing roots more than twice as long as those in M2 and substantially longer than those in M3. The contrast between M1 and M2 was particularly pronounced ($d \approx 2.06$), reflecting the inhibitory effect of gibberellin-only supplementation in M2 on root elongation. Although M3 performed better than M2, the difference between these two media was not statistically significant, suggesting that the cytokinin–auxin balance in M3 was insufficient to fully stimulate rhizogenesis. These findings reinforce the conclusion that auxin availability – particularly in combination with cytokinin – is essential for robust root development in *V. palustris*, a pattern consistent with classical models of auxin-driven root induction.

Taken together, the statistical results demonstrate that M1 is the most effective medium for promoting vigorous, balanced growth of *V. palustris* plantlets. The strong elongation responses observed in both shoots and roots indicate that this medium provides an optimal hormonal environment for early morphogenesis, supporting the earlier qualitative observation that “growth and development of the plantlets was uniform, with well differentiated morphology”. The lack of variation in organ number across media suggests that while initiation of shoots and roots is relatively stable, their subsequent elongation is highly plastic and strongly influenced by exogenous growth regulators.

These findings have important implications for conservation and propagation efforts. Efficient elongation of both shoots and roots is essential for producing robust plantlets capable of surviving acclimatization and eventual reintroduction into peatland habitats. The superior performance of M1 therefore positions it as the most suitable medium for large-scale multiplication of *V. palustris* germplasm. Moreover, the strong effect sizes observed in the

statistical analysis highlight the sensitivity of this species to hormonal balance, underscoring the need for carefully optimized media when developing *ex situ* conservation protocols.

The integration of quantitative statistical evidence with morphological observations strengthens the reliability of the proposed regeneration system and confirms its suitability as a foundation for future conservation, restoration, and sustainable utilization of this glacial relict species.

Overall, the results highlight the potential of *in vitro* culture as a conservation tool for *V. palustris*. The species' ecological importance - as a peatland indicator and larval host for *Boloria selene* - underscores the need for reliable propagation methods. The protocol developed here provides a foundation for establishing germplasm collections, supporting the restoration of degraded peatlands, and enabling future horticultural or phytochemical applications. Further work should focus on optimizing germination rates, assessing the genetic stability of regenerated plants, and scaling up propagation for conservation programs.

CONCLUSIONS

The present study provides the first documented protocol for the *in vitro* regeneration of *V. palustris* L. subsp. *palustris* in Romania and establishes a methodological foundation for future *ex situ* conservation and sustainable use of this glacial relict species. By combining aseptic germination of immature embryos with subsequent shoot multiplication and rooting, we successfully generated viable plantlets capable of acclimatizing to *ex vitro* conditions.

The custom sterilization protocol, which included NaDCC treatment, proved highly effective in eliminating fungal contamination typical of wetland-derived plant material, enabling reliable initiation of *in vitro* cultures. Although germination percentages remained modest, the regenerated plantlets displayed vigorous growth and normal morphology. Among the tested media, variant M1 supported the most balanced and robust shoot proliferation, while IBA-supplemented medium efficiently induced dense and healthy root systems.

The successful acclimatization of regenerated plants demonstrates the applicability of this protocol for establishing germplasm collections, supporting restoration efforts in degraded peatlands, and enabling future horticultural or phytochemical applications. Given the species' conservation status and ecological importance, the developed *in vitro* system represents an essential step toward safeguarding *V. palustris* genetic resources and enhancing its availability for research, restoration, and sustainable utilization.

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IN VITRO CULTURE OF *ALTHAEA OFFICINALIS* L. (BIG MALLOW): OPTIMIZATION OF GERMINATION, SHOOT MULTIPLICATION AND CALLUS INDUCTION

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Abstract

Althaea officinalis L. is a medicinal plant valued for its mucilaginous polysaccharides and bioactive compounds. Efficient in vitro propagation systems are essential for producing uniform plant material and supporting conservation and phytochemical studies. This work aimed to optimize in vitro germination, shoot multiplication, and callus induction using MS medium supplemented with different plant growth regulators. Seeds sterilized with ethanol and mercuric chloride achieved 90% germination on basal MS medium within eight days. Supplementation with GA₃ enhanced seedling elongation. Shoot multiplication was most efficient on MS medium containing BAP and IAA, producing vigorous shoots with extended subculture intervals. Rooting was successfully induced with IBA. Callus formation occurred on MS medium supplemented with either BAP + NAA or 2,4 D + BAP; the latter produced a compact white callus. Regenerated plants acclimatized successfully in a mixture of soil:perlite 1:1. The results confirm that *A. officinalis* responds strongly to in vitro culture conditions and provide a reliable protocol for micropropagation and callus production.

Key words: *Althaea officinalis*, micropropagation, gibberellic acid, BAP, callus induction.

INTRODUCTION

Althaea officinalis L. (big mallow) is a perennial medicinal species widely used in traditional and modern phytotherapy. Its therapeutic value is attributed to mucilaginous polysaccharides, polyphenols, flavonoids, and volatile compounds with antioxidant, anti-inflammatory, and wound healing properties (Al Snafi, 2013; Pașca et al., 2016; Albahri et al., 2023). The mucilage fraction, composed of glucuronic acid, galacturonic acid, rhamnose, and galactose, is of particular pharmacological interest (Ionkova & Alfermann, 1994).

Phytochemical analyses have identified numerous compounds in *A. officinalis*, including tetradecane, α pinene, hexadecane, 2 hexenal, dodecane, β ionone, and trans geranylacetone (Shahsavari & Derikvand, 2022). Given its medicinal importance, developing efficient in vitro propagation systems is essential for producing uniform plant material, conserving germplasm, and supporting biotechnological applications.

Previous studies have examined seed germination (Younesikelati et al., 2016), artificial seed technology (Naz et al., 2018), and

shoot proliferation using BAP (Mujib et al., 2017; Naz et al., 2015). However, protocols vary considerably, and further optimization is needed to improve reproducibility and plant vigor.

The present study aimed to refine in vitro culture techniques for *A. officinalis*, focusing on seed germination, shoot elongation and multiplication, and callus induction.

Despite previous studies, no comprehensive protocol integrating germination, elongation, multiplication, rooting, and callus induction has been reported for *A. officinalis*. This study aims to fill this gap.

MATERIALS AND METHODS

Plant Material

Seeds of *Althaea officinalis* L. (big mallow) were obtained from a commercial supplier (www.semintelegumefflori.ro; lot PL530/12/210/3473A). Seeds were visually inspected and selected based on uniformity, absence of mechanical damage, and consistent size. Only intact, healthy seeds were used for in vitro culture experiments. All procedures were conducted under aseptic conditions in a laminar

airflow cabinet to prevent microbial contamination.

Surface Sterilization Procedure

A multistep sterilization protocol was applied to ensure complete decontamination of the seed surface:

Pre-washing:

Seeds were rinsed thoroughly under running tap water for 10 minutes to remove dust and surface debris.

Initial rinse:

Seeds were washed twice with sterile distilled water.

Ethanol treatment:

Seeds were immersed in 70% (v/v) ethanol for 30 seconds to disrupt microbial membranes.

Mercuric chloride treatment:

Seeds were transferred to a 0.1% (w/v) HgCl_2 solution supplemented with three drops of Tween-80 as a surfactant. Exposure time was 3-5 minutes, with gentle agitation to ensure uniform contact.

Final rinses:

Seeds were rinsed 4-5 times with sterile distilled water to remove all traces of HgCl_2 .

Sterilized seeds were immediately inoculated onto culture media to prevent desiccation.

Culture Media Preparation

All media were prepared using Murashige and Skoog (MS) basal salts and vitamins. Media were supplemented with 3% (w/v) sucrose and solidified with 0.7% (w/v) agar. The pH was adjusted to 5.7 ± 0.1 prior to autoclaving at 121°C for 20 minutes. Plant growth regulators (PGRs) were added to the media after cooling to approximately 50°C .

In Vitro Germination and Seedling Elongation

Sterilized seeds were placed individually on basal MS medium to assess germination capacity. To evaluate the effect of gibberellic acid (GA_3) on seedling elongation, three additional media were prepared:

MS + 0.5 mg/L GA_3

MS + 1.0 mg/L GA_3

MS + 1.5 mg/L GA_3

Seeds were incubated in a growth chamber at $24 \pm 2^\circ\text{C}$ under a 16/8 h light/dark photoperiod with a light intensity of approximately $40\text{--}50 \mu\text{mol m}^{-2} \text{ s}^{-1}$. Germination percentage was recorded

after eight days, and shoot length was measured after five weeks. Each treatment included $n = 10$ replicates, with one seed per culture vessel.

Shoot Multiplication

Nodal segments (0.8-1.0 cm) excised from in vitro-grown seedlings were used as explants for shoot multiplication. Three hormonal combinations were tested:

MS + 3 mg/L BAP + 0.5 mg/L IAA

MS + 3 mg/L BAP + 1 mg/L GA_3

MS + 3 mg/L BAP + 1 mg/L GA_3 + 0.5 mg/L IAA

Explants were cultured in 350 mL culture jars containing 40 mL of medium. Cultures were maintained under the same environmental conditions described for germination. Shoot proliferation, the number of shoots per explant, internode length, and overall vigor were evaluated after four weeks. Subculture intervals were gradually extended to eight weeks as shoot vigor increased. Each treatment included $n = 10$ explants.

Root Induction

Elongated shoots (3-4 cm) were transferred to MS medium supplemented with 1.5 mg/L indole-3-butyric acid (IBA) to induce rooting. Root initiation and development were monitored over a two-week period. Parameters recorded included rooting percentage, the number of roots per shoot, and root morphology. Rooting experiments were conducted with $n = 10$ shoots per treatment.

Callus Induction

Callus formation was induced from hypocotyl and cotyledon explants excised from in vitro seedlings. Two hormonal combinations were tested:

Cytokinin-auxin balance:

MS + 1 mg/L BAP + 1 mg/L NAA

High-auxin induction:

MS + 5 mg/L 2,4-D + 2.5 mg/L BAP + 2 g/L casein hydrolysate

Explants were placed on callus induction media and incubated in the dark at $24 \pm 2^\circ\text{C}$ for the first 10 days to promote dedifferentiation. Cultures were then transferred to low-light conditions ($10\text{--}15 \mu\text{mol m}^{-2} \text{ s}^{-1}$). Callus characteristics

(color, texture, compactness, and growth rate) were recorded after three weeks.

Acclimatization of Regenerated Plants

Rooted plantlets were gently removed from the culture vessels, and agar residues were washed off with lukewarm sterile water. Plants were transferred to Magenta boxes containing a soil:perlite mixture (1:1). During the first week, humidity was maintained by covering the containers with transparent lids. Gradual ventilation was introduced to harden the plants. After two weeks, acclimatized plants were transferred to greenhouse conditions and monitored for survival and growth.

RESULTS

Seed germination and elongation

The sterilization protocol applied to *A. officinalis* seeds proved highly effective, yielding a 100% contamination-free rate across all replicates. This ensured that subsequent developmental responses were attributable solely to the culture conditions rather than microbial interference. When inoculated onto basal MS medium, seeds initiated visible swelling within the first 48 hours, followed by radicle emergence. By day eight, germination reached 90%, demonstrating that *A. Officinalis* seeds possess strong intrinsic viability and respond favourably to MS basal salts without the need for exogenous growth regulators (Figure 1).



Figure 1. *Althaea officinalis* plantlets germinated *in vitro* on basal MS medium eight days after inoculation

Seedlings developed uniformly, producing well-formed hypocotyls and cotyledons. The early establishment of vigorous plantlets provided a reliable source of explants for subsequent micro-propagation and callus induction experiments.

Effect of GA₃ on shoot length

Supplementation of MS medium with gibberellic acid (GA₃) significantly influenced seedling elongation. After five weeks of culture, clear differences in shoot length were observed among treatments (Table 1). The 0.5 mg/L GA₃ treatment produced the most pronounced elongation, with shoots reaching an average of 13.5 cm (Figure 2).



Figure 2. *Althaea officinalis* seedling elongated to 13.5 cm on MS medium supplemented with 0.5 mg/L GA₃ after five weeks

Seedlings exhibited elongated internodes, expanded leaves, and an overall vigorous appearance.

At 1.0 mg/L GA₃, shoot elongation remained enhanced (12 cm) but was slightly reduced compared to the 0.5 mg/L treatment. Interestingly, the highest concentration tested (1.5 mg/L) resulted in noticeably shorter shoots (8 cm), suggesting that excessive GA₃ may inhibit elongation or induce physiological stress. Seedlings at this concentration displayed thicker stems and smaller leaves, indicating a shift in the growth balance.

Table 1. Effect of gibberellic acid on shoot length after five weeks

GA ₃ concentration (mg/L)	Shoot length (cm)
0.5	13.5
1.0	12.0
1.5	8.0

These results confirm that *A. officinalis* is highly responsive to GA₃, but optimal elongation occurs at lower concentrations. The highest elongation occurred at 0.5 mg/L GA₃.

Shoot multiplication

Nodal segments excised from *in vitro* seedlings responded strongly to cytokinin-rich media. The

combination of 3 mg/L BAP with 0.5 mg/L IAA produced the highest multiplication rate, with explants generating multiple axillary shoots within four weeks. Shoots were compact, with short internodes and a dense foliar system, characteristics desirable for micropropagation. When GA₃ (1 mg/L) was added to BAP, shoot proliferation still occurred, but the morphology shifted toward elongated shoots with fewer lateral branches. This suggests that GA₃ promotes elongation at the expense of lateral bud activation. The medium containing BAP + GA₃ + IAA produced intermediate results, with moderate elongation and acceptable shoot numbers (Figure 3 a, and c).



Figure 3. Shoot multiplication on MS medium supplemented with:
 (a) 3 mg/L BAP + 0.5 mg/L IAA;
 (b) 3 mg/L BAP + 1 mg/L GA₃ + 0.5 mg/L IAA;
 (c) 3 mg/L BAP + 1 mg/L GA₃.

A notable observation was a progressive increase in the subculture interval. Initially, explants required transfer every four weeks; however, as shoots adapted to the optimized medium, the interval extended to eight weeks due to their vigorous and sustained growth. This represents a practical advantage for large-scale propagation, reducing labor and resource inputs.

Rooting

Root induction was successfully achieved on MS medium supplemented with 1.5 mg/L IBA. Within two weeks, shoots developed thick, well-branched root systems. Roots were white, robust, and exhibited a strong geotropic response. The rooting percentage approached 100%, indicating that *A. officinalis* is highly amenable to auxin-induced rhizogenesis (Figure 4).



Figure 4. Root induction on MS medium supplemented with 1.5 mg/L IBA

Rooted plantlets displayed no signs of hyperhydricity or physiological disorders, confirming the suitability of the chosen auxin concentration.

Callus induction

Two types of callus were obtained:

- Green, friable callus on MS + BAP (1 mg/L) + NAA (1 mg/L) (Figure 5).
- Compact white callus on MS + 2,4 D (5 mg/L) + BAP (2.5 mg/L) + casein hydrolysate (2 g/L). The latter produced the most vigorous callus mass (Figure 6).

Callus formation varied markedly depending on the auxin-cytokinin balance in the medium. On MS medium containing 1 mg/L BAP and 1 mg/L NAA, explants produced a green, friable callus with visible meristematic nodules. This callus

type expanded moderately and retained chlorophyll, suggesting partial differentiation.

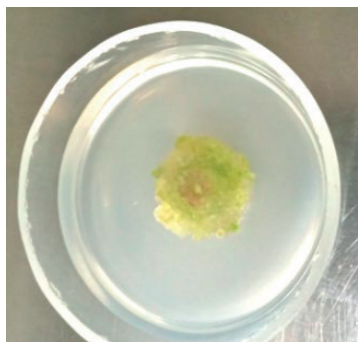


Figure 5. Green callus induced on MS medium containing 1 mg/L BAP and 1 mg/L NAA

In contrast, the medium supplemented with 5 mg/L 2,4-D, 2.5 mg/L BAP, and 2 g/L casein hydrolysate induced a compact, white callus with rapid proliferation. The callus mass increased substantially within three weeks, forming dense, homogeneous tissue. Casein hydrolysate appeared to enhance callus vigor, likely due to its amino acid content.



Figure 6. Compact white callus induced on MS medium supplemented with 5 mg/L 2,4-D, 2.5 mg/L BAP, and 2 g/L casein hydrolysate

The distinct callus morphologies indicate that *A. officinalis* tissues can be directed toward different developmental pathways depending on hormonal composition, offering flexibility for future applications such as secondary metabolite production or somatic embryogenesis.

Acclimatization

Regenerated plants were transferred to Magenta boxes containing a soil:perlite mixture (1:1) exhibited high survival rates (Figure 7).



Figure 7. Acclimatized *Althaea officinalis* plant in a soil:perlite (1:1) substrate.

Within two weeks, plantlets produced new leaves and displayed strong root anchorage. No chlorosis or wilting was observed, indicating a successful transition from in vitro to ex vitro conditions.

Plants maintained vigorous growth over the following month, confirming the robustness of the micropropagation protocol and the physiological stability of the regenerated individuals.

DISCUSSIONS

The present study provides a comprehensive evaluation of the in vitro behavior of *Althaea officinalis* L., demonstrating that this species responds strongly and consistently to controlled culture conditions. The high germination rate obtained on basal MS medium (90%) confirms the intrinsic viability of the seeds and supports previous findings that *A. officinalis* seeds do not require complex hormonal supplementation for successful germination (Younesikelati et al., 2016). The absence of contamination following the sterilization protocol further ensured that developmental responses were attributable solely to the culture environment.

The influence of gibberellic acid on seedling elongation was particularly evident. GA₃ at 0.5 mg/L produced the most vigorous elongation, while higher concentrations resulted in progressively shorter shoots. This dose-dependent response aligns with the

well-established role of GA₃ in promoting cell elongation and internode expansion. Similar patterns have been reported in other species, such as *Diets bicolor*, where moderate GA₃ concentrations enhanced germination and seedling vigor, while excessive levels inhibited elongation (Silva et al., 2020). The reduced shoot length observed at 1.5 mg/L GA₃ in our study may reflect a hormonal imbalance that disrupts endogenous growth regulation, suggesting that *A. officinalis* is particularly sensitive to supra-optimal GA₃ levels.

Shoot multiplication was strongly influenced by the cytokinin–auxin balance. The combination of 3 mg/L BAP and 0.5 mg/L IAA produced the highest multiplication rate and the most compact, morphologically stable shoots. This result is consistent with previous studies reporting that BAP is the most effective cytokinin for inducing axillary shoot proliferation in *A. officinalis* (Naz et al., 2015; Mujib et al., 2017). The addition of GA₃ to BAP-containing media resulted in elongated shoots with fewer lateral branches, indicating that GA₃ shifts the developmental balance toward elongation rather than proliferation. This observation reinforces the importance of carefully adjusting cytokinin–auxin ratios to achieve the desired morphogenic response.

Root induction using 1.5 mg/L IBA was highly successful, producing thick, well-branched root systems. IBA is widely recognized as one of the most effective auxins for rhizogenesis in dicotyledonous species, and the strong rooting response observed here confirms its suitability for *A. officinalis*. The absence of hyperhydricity or physiological abnormalities further indicates that the hormonal environment was well-balanced and that the shoots were physiologically competent for rooting.

Callus induction varied significantly depending on the hormonal composition of the medium. The combination of BAP and NAA (1 mg/L each) produced a green, friable callus, suggesting partial differentiation and the presence of chlorophyll-retaining tissues. In contrast, the medium containing 2,4-D, BAP, and casein hydrolysate produced a compact, rapidly proliferating white callus. This response is consistent with the strong callus-inducing capacity of 2,4-D, which promotes dedifferentiation and cell proliferation (Hosseini

et al., 2017; Görmez et al., 2019). The addition of casein hydrolysate likely enhanced callus vigor by providing amino acids and peptides that support rapid cell division. The distinct callus morphologies observed in this study highlight the developmental plasticity of *A. officinalis* tissues and suggest potential applications in secondary metabolite production, somatic embryogenesis, or genetic transformation.

Acclimatization of regenerated plants was highly successful, with plantlets exhibiting rapid adaptation to *ex vitro* conditions. The use of a soil-to-perlite mixture (1:1) provided adequate aeration and moisture retention, supporting root establishment and shoot development. The high survival rate indicates that the *in vitro*-derived plants were physiologically stable and capable of transitioning to natural growth conditions without stress symptoms.

Overall, the results of this study demonstrate that *A. officinalis* is an excellent candidate for *in vitro* propagation and callus culture. The optimized protocol presented here offers several advantages: high germination rates, efficient shoot multiplication, reliable rooting, and robust callus induction. These findings contribute to the growing body of knowledge on medicinal plant biotechnology and provide a solid foundation for future research aimed at large-scale propagation, conservation, and secondary metabolite production in *A. officinalis*. Further studies may explore the biochemical profiles of callus tissues, the potential for somatic embryogenesis, and the influence of alternative growth regulators or elicitors on metabolite accumulation.

CONCLUSIONS

The present study establishes a reliable and efficient *in vitro* propagation system for *Althaea officinalis* L., a medicinal species of considerable pharmacological importance. The high germination rate obtained on basal MS medium demonstrates that the species does not require complex hormonal supplementation for early development, making it highly suitable for *in vitro* culture. The addition of gibberellic acid significantly enhanced seedling elongation, with 0.5 mg/L GA₃ identified as the optimal concentration. This finding is particularly relevant for laboratories aiming to generate

elongated seedlings for explant preparation or physiological studies.

Shoot multiplication was most effectively achieved on MS medium supplemented with 3 mg/L BAP and 0.5 mg/L IAA, producing compact, vigorous shoots with extended subculture intervals. This optimized hormonal combination offers a practical advantage for large-scale propagation by reducing labor and resource inputs. The successful rooting of shoots on MS medium containing 1.5 mg/L IBA further confirms the suitability of this protocol for generating complete plantlets ready for acclimatization.

Callus induction was strongly influenced by the auxin–cytokinin balance. The combination of 2,4-D, BAP, and casein hydrolysate produced compact, rapidly proliferating white callus, highlighting the potential of *A. officinalis* tissues for applications such as secondary metabolite production, somatic embryogenesis, or genetic transformation. The ability to generate distinct callus types depending on hormonal composition underscores the developmental plasticity of this species and opens avenues for future biotechnological studies.

The high survival rate of regenerated plants during acclimatization demonstrates the physiological stability and robustness of the *in vitro*-derived material. This confirms that the optimized protocol is suitable not only for laboratory propagation but also for producing healthy plants capable of thriving under *ex vitro* conditions.

Overall, the results of this study provide a comprehensive and reproducible protocol for the *in vitro* propagation and callus induction of *A. officinalis*. The optimized system offers significant potential for germplasm conservation, large-scale propagation, and future research on the biosynthesis of valuable secondary metabolites. Further investigations may focus on the biochemical characterization of callus tissues, the induction of somatic embryos, and the use of elicitors to enhance the production of pharmacologically active compounds.

Taken together, these results provide a solid foundation for future applications in medicinal plant biotechnology, including large-scale propagation for industry, the conservation of valuable germplasm, and the targeted

production of bioactive metabolites through callus-based or cell-suspension systems.

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GENETIC POLYMORPHISM AND PRODUCTION CAPACITY OF SOME ROMANIAN-NATIVE WILD ISOLATES OF *PLEUROTUS OSTREATUS*

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Abstract

Pleurotus ostreatus is a globally significant edible mushroom. Its ecological versatility and rapid growth make it essential for sustainable agriculture, waste valorization, and functional food production. The aim of this study is to explore the genetic variability and fruiting potential of seven Romanian-native wild isolates of *P. ostreatus* compared with some commercial strains of *P. ostreatus*, *P. columbinus*, and *P. citrinopileatus* from the RDIVFG Vidra collection. Genetic variability was assessed using the RAPD technique. Results showed a high degree of genetic divergence among isolates, highlighting intraspecific variability and clear genetic structuring within the analyzed *P. ostreatus* isolates. In mushroom farm testing for fruiting potential, the seven wild isolates of *P. ostreatus* were outperformed in mushroom production by the commercial *P. ostreatus* strains PoM-421 and PoM-77. However, differences were not very large in terms of quantity and, especially, quality. Among the wild isolates, the highest average yield was recorded at Po5C, 567.4 g of mushrooms per 2.6 kg of lignocellulosic substrate (21.82%). This value was only 20.4 g lower than the PoM-77 control, revealing a statistically insignificant difference.

Key words: *macromycetes*, *oyster mushroom*, *RAPD*, *wild isolates*.

INTRODUCTION

The genus *Pleurotus* includes numerous species of ligninolytic basidiomycetes with great ecological, nutritional, and biotechnological importance and with medicinal potential; the best-known and most cultivated of them being *Pleurotus ostreatus* (Jacq.) P. Kumm (Mastranzo-Pérez et al., 2025). The performance achieved in cultivating these mushrooms both in commercial and household systems is due to rapid mycelial growth, wide adaptability to substrates, efficient lignocellulose degradation, and the ability to convert low-value agricultural residues into biomass rich in high-quality proteins (Rusu et al., 2022; Juárez-Hernández et al., 2023; Zăgrean et al., 2024; Miśkiewicz et al., 2025). Advances in the application of breeding programs for *P. ostreatus* mushrooms, as well as for other cultivated species, have led to the introduction on the market of valuable commercial strains that meet the demand for higher yields and superior quality (Barh et al., 2019). However, commercial strains represent only a limited number of genotypes and often

lose their performance after several consecutive subcultures and/or a long period of storage on the culture medium, causing reduced yield (Melanouri et al., 2022). A valuable aid can come from wild strains, which constitute important genetic material adapted to their ecosystems of origin and, as a result, require characterization and preservation. Collecting specimens of *P. ostreatus* basidiocarps grown in different areas from the spontaneous mycobiota and obtaining viable mycelial isolates from them, as in the case of other edible macrofungal species, provides the surplus genetic variability necessary for introducing or improving useful traits, with the aim of achieving higher yields and/or phenotypic traits demanded by production and market (Barh et al., 2019; Cruz-Villegas et al., 2024; Cai et al., 2025). The integration-preservation of these in the strain collection (fungal germplasm bank) and the evaluation of their biological and cultural potential constitute favorable premises for diversifying the cultivated assortment by increasing the number of new indigenous varieties or strains capable of utilizing the large

quantities of recyclable natural materials resulting from existing agro-forestry and livestock operations (Zăgrean et al., 2025). The classical taxonomy of fungi uses exclusively morphological, cultural, and biochemical characteristics for the identification and evaluation of such wild isolates from different geographical areas. Their discriminatory ability is not perfect, as it presents certain limitations. Most morphological and agronomic characteristics are quantitative traits that can be easily affected by environmental variation (Li et al., 2019). Nevertheless, these criteria are still widely used, being considered acceptable, and always constituting valuable, guiding complementary elements that support the determination of a precise systematic classification at the species/genus level, ultimately based on molecular genetics techniques. Molecular analysis is necessary to clarify the taxonomic and phylogenetic relationships within certain groups of *Pleurotus* (Cruz-Villegas et al., 2024). The molecular markers could directly reflect the genetic diversity at the DNA level. Among the molecular markers used to verify identity confusions of *P. ostreatus* strains and to assess genetic diversity at the species level are amplified fragment length polymorphism (AFLP), restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), inter-simple sequence repeats (ISSR), and combinatorial tubulin based polymorphism (cTBP) (Popa et al., 2017; Li et al., 2019).

In the present study, seven wild isolates of *P. ostreatus* were obtained from various regions of Romania and evaluated for fruiting potential in comparison with two commercial strains of the same species. In parallel, the RAPD molecular technique was used to assess the genetic diversity and phylogenetic relationship among the wild strains, along with three additional commercial strains belonging to *P. ostreatus*, *P. columbinus*, and *P. citrinopileatus*, respectively. The aim of this study was to assess the fruiting performance and genetic diversity of these Romanian-native wild *P. ostreatus* strains in comparison with established commercial strains, in order to identify new genotypes with valuable agronomic potential for cultivation and strain improvement.

MATERIALS AND METHODS

Biological material. Specimens of *P. ostreatus* mushrooms were collected from the spontaneous mycobiota of natural habitats located in different areas of Romania, specifically in four counties and Bucharest: Po1Br (forest, Brebu, Prahova Co.), Po2T (forest, Tigveni, Argeş Co.), Po3M (forest, Mogoşoaia, Ilfov Co.), Po4V (meadow, Vidra, Ilfov Co.), Po5C (forest, Comana, Giurgiu Co.), Po6M (forest, Mogoşoaia, Ilfov Co.), Po7B (park, sector 5, Bucharest).

Preliminary identification and taxonomic classification based on morphological characteristics of the collected mushroom specimens were carried out in accordance with the data provided by the studied specialized literature (Sălăgeanu & Sălăgeanu, 1985; Şesan & Tănase, 2004; Kuo, 2017).

From the collected carpophores, seven wild mycelial isolates were obtained using the tissue culture method: tissue blocks of 2-3 mm, aseptically excised from the inner part of the cap trama, were cultured on Petri dishes with malt extract agar medium (MEA) containing 17 g/L malt extract broth (VVR Chemicals) and 20 g agar, pH 6.5 and incubated in the dark at 24-26°C for 14-16 days. The obtained wild isolates were clonally multiplied on MEA Petri plates and subsequently propagated in the laboratory, with the resulting mycelium intended partly for preservation in the Collection and partly for use in evaluation and characterization work based on *in vitro* morphophysiological and cultural criteria (Zăgrean & Şovărel, 2025 - previous study). Other mycelial subcultures of the wild *P. ostreatus* isolates were intended for this assessment of intraspecific genetic variability at the molecular level and fruiting potential in the testing mushroom growing unit.

In order to evaluate the fruiting capacity of the seven wild isolates, two commercial strains from the RDIVFG Vidra Collection, namely PoM-421 and PoM-77, were used for comparison.

Seeding spawn has been prepared on a granular support: pre-boiled wheat grains (moisture 65%) and fined with 6% CaSO₄ and 2% CaCO₃ were distributed in 400 g portions into 500 ml Erlenmeyer flasks and sterilized in an autoclave for 90 minutes at 123°C. The flasks with sterilized granular substrate were inoculated in a

laminar flow hood using pure cultures on agar medium from the mycelia of the experimental isolates. After inoculation, they were incubated at 24-26°C in the dark for 16-18 days until the total colonization of the granular support.

Substrate preparation, inoculation and incubation. The culture substrate was prepared from chopped wheat straw and ground corn cobs, moistened to saturation with water overnight. The excess water was removed, and CaSO₄ (6%), CaCO₃ (2%) and natural nutritional supplements consisting of wheat bran, cornmeal and sunflower seed cake (3% w/w, each) were added. The homogenized lignocellulosic material, adjusted to 65-68% moisture, was divided into 1.3 kg portions in polypropylene-autoclavable bags fitted with cotton plugs, which were then sterilized for 90 minutes at 123°C. After cooling, they were inoculated under a laminar flow hood with the experimental strains' spawn at a rate of 5% w/w. Subsequently, the bags with inoculated substrate (6 bags for each strain) were placed for incubation at 24-26°C, in the absence of light. At the end of the incubation period (3-4 weeks), the bags with colonized substrate were transferred into PVC culture vessels (4 liters) and moved to the test mushroom growing room to verify their fruiting capacity. The individual replicates of each variant were arranged in a completely randomized design.

Mushroom cultivation. To initiate the formation of mushroom primordia, the temperature in the growing room was reduced to 15-16°C; relative humidity was maintained at 92-94%, light was provided for 8 hours daily at 600-700 lux, and CO₂ concentration was kept below 1000 ppm by introducing fresh air. During the production period, the air temperature was maintained at 16-19°C, the CO₂ level under 1200 ppm, relative humidity was lowered to 82-85%, and light was provided for 10 hours daily at 700-800 lux. The yield of mushrooms was evaluated for the first harvest flush.

Statistical analysis. Experiments were carried out in triplicate (1 repetition = 2 bags x 1.3 kg substrate/bag = 2.6 kg substrate) and results were expressed as means ± standard deviation (SD). The data were analysed by analysis of variance (ANOVA) using Microsoft Office Excel 2010. Checking the significance of the differences between variants was done based on

limit differences (DL 5%, DL1%, DL 0,1%) and by Duncan's multiple range test at $p < 0.05$.

Molecular analysis. The seven autochthonous *P. ostreatus* strains were analysed compared to three authenticated reference strains: *P. ostreatus* PoM-421, *P. citrinopileatus* Pc95, and *P. columbinus* Pc98. For studying the genetic polymorphism of the Romanian-native *P. ostreatus* wild strains, Random Amplified Polymorphic DNA (RAPD) analysis was performed using oligonucleotide primers from Carl Roth GmbH + Co. KG (Karlsruhe, Germania) (Table 1).

Table 1. Primers used for RAPD analysis

Primer	Sequence	Annealing temperature
Roth 01	5'-CTG GCC CTT C-3'	34 °C
Roth 02	5'-TGC CGA GCT G-3'	
Roth 06	5'-GGT CCC TGA C-3'	
Roth 09	5'-GGG TAA CGC C-3'	
Roth 13	5'-CAG CAC CCA C-3'	

*Legend: A- adenine; C- cytosine; G- guanine; T- thymine.

Amplification was performed in a final reaction volume of 20 µL per sample. The reaction mixture included 1X reaction buffer with MgCl₂, 0.2 mM deoxynucleotides, 5 pmol/µL of primer, 0.4 U of Dream Taq DNA Polymerase (Thermo-Scientific LSG) and 20 ng template DNA. The amplification program initiated with a denaturation step at 94°C for 5 min, followed by 45 cycles of 1 min at 94°C for denaturation, 45 s at 34°C for primer annealing, 90 s at 72°C for extension, then a final step of elongation for 7 min at 72°C. The PCR products were analysed in 1.2% agarose gel supplemented with ethidium bromide, in 0.5 X Tris-Borate-EDTA (TBE) as migration buffer (VWR International GmbH). To estimate the size of the amplicons, the samples were compared with HyperLadder 100 pb, and 1Kb (Meridian BioScience) respectively. Samples were migrated by horizontal gel electrophoresis at 70 V for 2 hours and 30 min. The electrophoretic profiles were visualized under UV light using the BioDoc-It Imaging System (Ultra-Violet Products Ltd.). The molecular fingerprints generated by the electrophoretic profiles of RAPD reactions were visually compared. Amplicons having the same length were considered a locus and scored in a binary matrix, where each PCR product was recorded as present and coded as 1, or absent as 0, across genotypes. The data were used to

generate a phylogenetic tree using the TreeCon software (version 1.3b) for Windows based on the Lei & Li (1979) genetic distance estimation method and the Unweighted Pair-Group Method using arithmetic Averages (UPGMA) clustering analysis. The Shannon-Weaver index (H') was also calculated within the *P. ostreatus* group to evaluate the intraspecific genetic diversity, as well as for the whole set of analysed strains to evaluate the interspecific genetic diversity.

Therefore, the Shannon-Weaver (H') index per locus (Spellerberg & Fedor, 2003) was computed using the following formula:

$$H'_{locus} = -(p_{1,i} \ln + p_{0,i} \ln)$$

where:

$p_1 = n_1 / \text{total number of loci}$;

$n_1 = \text{number of present loci}$;

$\ln = \text{natural log}$;

$p_0 = n_0 / \text{total number of loci}$;

$n_0 = \text{number of absent loci}$;

$0 < H'_{locus} < \ln(2)$.

While the total H' and mean H' were as follows:

$$H'_{total} = \sum_{i=1}^n [-(p_{1,i} \ln + p_{0,i} \ln)]$$

$$\bar{H}' = \frac{H'_{total}}{n}$$

where:

$i = \text{locus}$;

$n = \text{number of total detected loci}$;

$H'_{total} \leq n \times \ln(2)$.

The Polymorphic Information Content (PIC), which quantifies how informative each locus is for distinguishing among genotypes, was also calculated for all polymorphic loci generated by the five RAPD primers. This analysis provided an estimate of the overall discriminatory power of the marker set to differentiate the studied *Pleurotus* spp. and *P. ostreatus* strains (Singh et al., 2014). The PIC value was computed using the formula:

$$PIC = 1 - p^2 - q^2$$

where:

$p = \text{frequency of band presence (binary encoded as 1)}$;

$q = \text{frequency of band absence (binary encoded with 0)}$;

Within the *P. ostreatus* group, the Percentage of Polymorphic Bands (PPB) were also evaluated as follows:

$$PPB = \frac{\text{Number of polymorphic bands}}{\text{Total number of loci}} \times 100$$

These indices were used collectively to characterize the genetic variability revealed by the RAPD profiles and to determine the discriminatory efficiency of the marker system across the examined *Pleurotus* genotypes.

RESULTS AND DISCUSSIONS

All seven wild strains of *P. ostreatus* and the two commercial ones supported primordia formation and mushroom production under the conditions of our experiment. The time required for complete colonization of the substrate (incubation period), time elapsed from the inoculation of the culture substrate until the visible appearance of fruiting primordia (earliness), yield, and biological efficiency are important parameters of the cultivation cycle of *P. ostreatus* in particular and of edible mushrooms in general, and they are strongly dependent on the interaction between the culture substrate and the tested strain/species (Hoa et al., 2015; Melanouri et al., 2022; Timm et al., 2025). In our study, incubation period and earliness varied among the strains tested. Almost all the tested strains showed statistically significant individual differences ($p < 0.05$) regarding the time of substrate colonization (Table 2).

Table 2. The incubation period and earliness in cultivation of a variety of *P. ostreatus* strains - 7 wild strains and 2 commercial ones

Strain	Strain type	Incubation* (days)	Earliness* (days)
Po1Br	Wild	23.83 ± 1.32 c	30.17 ± 1.15 a b c
Po2T	Wild	29.33 ± 1.26 g	41.33 ± 1.04 g h
Po3M	Wild	26.17 ± 1.53 f	38.83 ± 1.04 g h
Po4V	Wild	24.00 ± 0.50 c	33.17 ± 0.76 d e
Po5C	Wild	24.17 ± 0.29 c d	31.83 ± 1.76 c d
Po6M	Wild	24.83 ± 0.58 d e	34.17 ± 0.76 e f g
Po7B	Wild	24.00 ± 0.50 c	33.67 ± 0.76 d e f
PoM-421	commercial	20.83 ± 1.15 a	28.67 ± 0.29 a
PoM-77	commercial	22.83 ± 0.29 b	29.83 ± 1.26 a b

*Mean values with standard deviations from 3 replicates.

Mean values of each parameter within the same column, not sharing common letters, are significantly different ($p < 0.05$)

Table 2 shows that when cultivated on a substrate of wheat straw and corn cobs supplemented with wheat bran, corn meal, and sunflower seed cake, the two commercial strains of *P. ostreatus* colonized the substrate faster

than the wild ones, namely in 20 days (PoM-421) and 22 days (PoM-77), with significant differences ($p < 0.05$). Among the wild strains, the fastest colonization was reported for the strain Po1Br (23 days), followed closely, with a non-significant difference, by Po4V, Po7B and Po5C (24 days). They showed good adaptability and rapid mycelial colonization, suggesting high nutrient utilization efficiency and active lignocellulolytic enzyme systems (Cai et al., 2025). Po2T colonized the entire substrate the latest (29 days), with a significant delay compared to the others. Results of the present study are in accordance with the findings of Melanouri et al. (2022), who, in a study on the cultivation of *P. ostreatus* and *Pleurotus eryngii* on 10 agro-industrial residues and the influence of the strain/species-substrate interaction, obtained complete colonization of *P. ostreatus* strains in between 16 and 38 days.

Intraspecific variability also decisively influenced the earliness of the seven wild strains of *P. ostreatus*, with the appearance of primordia occurring between 30 and 41 days after spawning: first primordia appeared in isolate Po1Br (30 days), followed by the others Po5C (32), Po4V (33 days), Po6M and Po7B (34 days), with Po3M (39 days) and Po2T (41 days) being later (Tabel 1). The commercial strains formed primordia earlier than the wild-type ones. The first primordia appeared in the PoM-421 strain 29 days after spawning, followed by PoM-77 (30 days), this possibly correlates with their faster ability to colonize the lignocellulosic substrate and the corresponding shortening of the incubation period.

Consistent results regarding the effect determined by genetic factors on the earliness of *P. ostreatus* strains have also been reported by others. The study by Koutrotsios et al. (2017) on the cultivation of 16 commercial and wild *P. ostreatus* strains on pasteurized wheat straw revealed a wide variation in earliness, with earliness time ranging from 21 to 71 days.

Bekiaris et al. (2025) obtained results slightly different from ours: by cultivating 5 strains of *P. ostreatus* on substrates obtained from agro-industrial by-products and agricultural waste enriched with various supplements, they reported the staggered appearance of their primordia between 14 and 18 days after spawning.

All experimental wild isolates developed healthy basidiomata, predominantly forming tiered clusters, with normal individual morphology typical for the species *P. ostreatus*: large, fleshy, shell-shaped, slightly concave caps, with margins usually entire and turned up in the young stage; cap cuticle colored gray with darker shades on some (Po4V and Po7B), lighter on others, and with small radial striations (Po1Br, Po3M, Po6M, and PoM-421); nude hymenophore, with long gills on the underside of the cap, decurrent, cream-beige in color; stem with an excentric, rarely lateral insertion, medium short, slightly curved, fused towards the base in clusters (Figure 1).

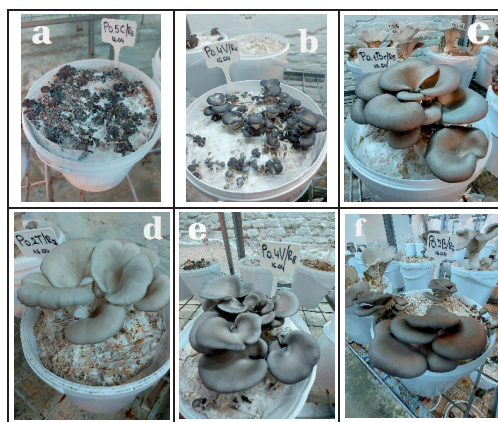


Figure 1. Wild strains of *P. ostreatus* cultivated in plastic bags/vessels: primordia (a, b) and basidiocarps ready for harvest (c-f)

Intraspecific variability also had a considerable effect on mushroom yield and biological efficiency of the nine strains of *P. ostreatus* tested. In Tables 3 and 4 it can be seen that the highest production of the first flush of mushrooms was recorded for the commercial strain PoM-421, with an average of 612.9 g of mushrooms per 2.6 kg of substrate (2 culture bags/vessels), a value corresponding to a harvest yield of 23.57%, surpassing the other commercial strain, PoM-77, designated as the control, by a non-significant surplus of 25.1 g (+0.96%). The control PoM-77 ranked second with a production of 587.8 g, equivalent to a yield of 22.61% (22.61 g of fresh mushrooms harvested per 100 g of culture substrate, or more illustratively, 22.61 kg of mushrooms per 100 kg of culture substrate).

Table 3. Mushroom yield and biological efficiency in a variety of 7 wild isolates and 2 commercial strains of *Pleurotus ostreatus* (1st flush)

Strain	Strain type	Yield*		Biological efficiency (BE)
		(g)	(%)	
Po1Br	wild	482.9 ± 10.76	18.57 ± 0.42	56.28 ± 1.25
Po2T	wild	394.5 ± 17.99	15.17 ± 0.70	45.98 ± 2.11
Po3M	wild	464.1 ± 7.66	17.85 ± 0.30	54.09 ± 0.92
Po4V	wild	439.7 ± 12.91	16.91 ± 0.50	51.24 ± 1.51
Po5C	wild	567.4 ± 15.55	21.82 ± 0.60	66.13 ± 1.81
Po6M	wild	410.3 ± 18.77	15.78 ± 0.72	47.82 ± 2.19
Po7B	wild	390.5 ± 16.75	15.02 ± 0.44	45.52 ± 1.95
PoM-421	commercial	612.9 ± 21.38	23.57 ± 0.82	71.43 ± 2.48
PoM-77	commercial	587.8 ± 20.16	22.61 ± 0.78	68.51 ± 2.38

*Mean values with standard deviations from 3 replicate
Yield (g): weight of fresh mushrooms (g) per 2.6 kg of cultivated substrate (2 bags, each with 1.3 kg substrate)
Yield (%): average weight of fresh mushrooms (g)/2.6 kg of cultivated substrate x 100
Biological efficiency (BE%): average weight of fresh mushrooms (g)/weight of dry substrate (g) × 100

Table 4. The influence of strain on the yield in a variety of 7 wild isolates and 2 commercial strains of *Pleurotus ostreatus* (1st flush)

Strain	Strain type	Average yield*			Significance of difference
		(g)	%	±d (g)	
Po1Br	wild isolate	482.9	82.15	-104.9	ns
Po2T	wild isolate	394.5	67.11	-193.3	0
Po3M	wild isolate	464.1	78.95	-123.7	ns
Po4V	wild isolate	439.7	74.80	-148.1	ns
Po5C	wild isolate	567.4	96.52	-20.4	ns
Po6M	wild isolate	410.3	69.80	-177.5	0
Po7B	wild isolate	390.5	66.43	-197.3	0
PoM-421	commercial strain	612.9	104.27	25.1	ns
PoM-77 (control)	commercial strain	587.8	100	-	-

*Yield (g): weight of fresh mushrooms (g) per 2.6 kg of cultivated substrate (2 bags, each with 1.3 kg substrate)
DL 5% 149.48
DL 1% 205.88
DL 0.1% 283.45

Among the wild isolates, Po5C achieved the highest average yield, namely 567.4 g of fresh mushrooms per 2.6 kg of substrate (21.82%), just 20.4 g less than the control PoM-77, a statistically non-significant difference. It was followed, in decreasing order of yield harvested in the first flush, by isolates Po1Br with 482.9 g of mushrooms (18.57%), Po3M with 464.1 g of mushrooms (17.85%), and Po4V with 439.7 g of mushrooms (16.91%), also with non-significant negative differences compared to the control. The lowest mushroom productions were recorded for the strains Po2T with an average of 394.5 g of mushrooms (15.17%) and Po7B with 390.5 g of mushrooms (15.02%), with significant negative differences of -193 g and -197.3 g of mushrooms, respectively, compared to the control PoM-77.

In general, substrates that gave higher yields also gave higher values of BE (Melanouri et al., 2022). The experimental results of the present study showed that the highest BE values were recorded in the commercial strains PoM-421 (71.43%) and PoM-77 (68.51%), while among the wild types, Po5C (66.13%) stood out, followed by Po1Br (56.28%) and Po3M (54.09%). These wild strains have proven to have a better conversion efficiency of lignocellulosic substrate, which makes them recommended for future testing.

Based on the molecular analysis of the tenth *Pleurotus* strains studied, a total of 79 loci were distinguished after five RAPD amplifications. Using the Roth 01 primer, 15 loci were differentiated (Figure 2), whereas with the ROTH 02 primer, 20 loci (Figure 3).

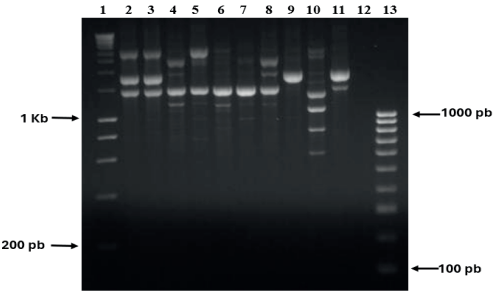


Figure 2. The RAPD electrophoretic profile obtained with the Roth01 primer. Sample order: line 1 – 1Kb ladder; line 2 – Po1Br; line 3 – Po2T; line 4 – Po3M; line 5 – Po4V; line 6 – Po5C; line 7 – Po6M; line 8 – Po7B; line 9 – PoM-421; line 10 – Pc95; line 11 – Pc98; line 12 – Negative Control; line 13 – 100 bp ladder

When using the Roth 01 primer, the electrophoretic profiles of the Po1Br and Po2T strains proved to be identical. However, some variations can be noted in the electrophoretic patterns of the remaining *P. ostreatus* strains, even though certain amplicons are shared among specific loci. However, a substantial difference was noticed between *P. ostreatus* strains and *P. columbinus* Pc98, compared to *P. citrinopileatus* Pc95, based on their electrophoretic profiles, the latter having a completely different pattern from the rest of the strains studied (Figure 2). *P. citrinopileatus* Pc95 showed a distinct electrophoretic pattern from the *P. ostreatus* strains and *P. columbinus* Pc98, also when RAPD was performed with the Roth 02 primer (Figure 3).

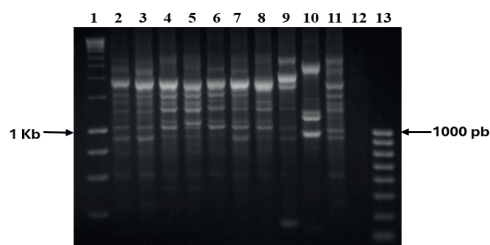


Figure 3. The RAPD electrophoretic profile obtained with the Roth02 primer. Sample order: line 1 – 1Kb ladder; line 2 – Po1Br; line 3 – Po2T; line 4 – Po3M; line 5 – Po4V; line 6 – Po5C; line 7 – Po6M; line 8 – Po7B; line 9 – PoM-421; line 10 – Pc95; line 11 – Pc98; line 12 – Negative Control; line 13 – 100 bp ladder

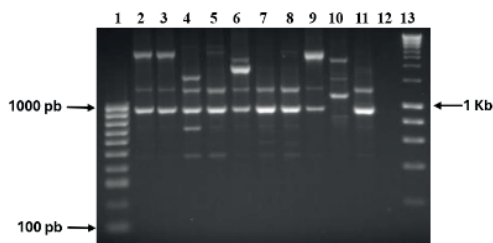


Figure 4. The RAPD electrophoretic profile obtained with the Roth09 primer. Sample order: line 1 – 1Kb ladder; line 2 – Po1Br; line 3 – Po2T; line 4 – Po3M; line 5 – Po4V; line 6 – Po5C; line 7 – Po6M; line 8 – Po7B; line 9 – PoM-421; line 10 – Pc95; line 11 – Pc98; line 12 – Negative Control; line 13 – 100 bp ladder

The electrophoretic profiles generated with the Roth 02 primer indicate that Po1Br and Po2T strains share a nearly identical pattern and display high similarity with the Po6M strain. Apart from some variations in the intensity of individual bands, the *P. ostreatus* Po3M, Po4V, Po5C and Po7B strains also display almost identical electrophoretic patterns among them when using the Roth 02 primer. While the *P. ostreatus* reference strain (Po-421) differed significantly from the rest of the native *P. ostreatus* strains.

Analysing the RAPD electrophoretic patterns obtained with the Roth 06 primer, it was observed that Po1Br and Po2T strains have no detectable amplicons across all loci. The *P. ostreatus* Po3M, Po4V, Po5C, Po6M, Po7B strains, and the *P. columbinus* Pc98 reference strain, displayed a remarkable similar pattern, revealing the same diagnostic loci. *P. ostreatus* PoM-421 reference strain showed a comparable pattern with the previously mentioned strains, but lacking one of the common amplicons. Regarding the *P. citrinopileatus* Pc95 reference strain, it displayed a completely distinct pattern, sharing no common loci with the other strains examined.

When using the Roth 09 primer, the electrophoretic profiles of the amplification products from the Po1Br and Po2T strains proved to be very similar to those of the *P. ostreatus* PoM-421 reference strain, the recorded differences being in terms of the intensity of the bands obtained. Likewise, the Po4V, Po6M and Po7B strains showed some similarities among them. In contrast, the Po3M and Po5C strains differed considerably between themselves and from the rest of the strains of the same species (Figure 4).

As in the previous RAPD reactions, the *P. citrinopileatus* Pc95 strain showed a discordant note in terms of the electrophoretic profile when using the Roth 09 primer. A single amplicon from this strain shared a similar size with an amplification product from Po5C strain. In contrast, *P. columbinus* Pc98 presented an electrophoretic profile similar to the native *P. ostreatus* strains Po4V, Po6M and Po7B (Figure 4).

When using the RAPD primer Roth13, the electrophoretic profiles of the Po1Br and Po2T strains proved to be identical. Some similarities can also be observed between the electrophoretic profiles of the PoM-421 and Po7M strains, with some differences between the loci for which amplification products were obtained. Substantial differences, compared to the rest of the *P. ostreatus* strains, were noted in the Po3M and Po4V strains, which revealed distinctive electrophoretic patterns (Figure 5).

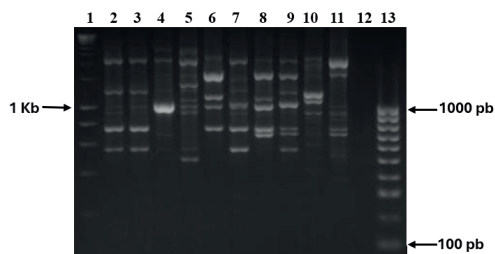


Figure 5. The RAPD electrophoretic profile obtained with the Roth13 primer. Sample order: line 1 – 1Kb ladder; line 2 – Po1Br; line 3 – Po2T; line 4 – Po3M; line 5 – Po4V; line 6 – Po5C; line 7 – Po6M; line 8 – Po7B; line 9 – PoM-421; line 10 – Pc95; line 11 – Pc98; line 12 – Negative Control; line 13 – 100 bp ladder

P. columbinus Pc98 strain shared some similarities with *P. ostreatus* PoM-421 and

Po7M. However, the electrophoretic profile of *P. citrinopileatus* Pc95 varied noticeably from that of the other studied strains.

Compiling the results from RAPD analysis, the phylogeny of the studied *Pleurotus* spp. strains was determined. The electrophoretic profiles were converted into a binary matrix and processed in TreeCon using UPGMA clustering based on the Nei-Li coefficient, with 100 iterations. The molecular fingerprints generated by the RAPD primers allowed the construction of a phylogenetic tree for the ten *Pleurotus* spp. strains included in the genetic study (Figure 6).

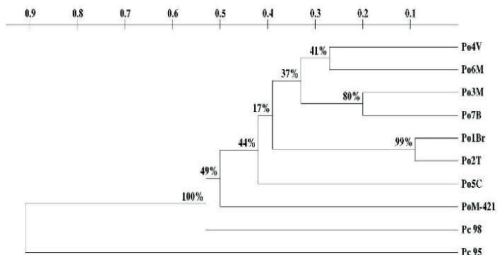


Figure 6. Dendrogram revealing the phylogeny of the studied *Pleurotus* spp. strains based on RAPD analysis with five oligonucleotide primers

The dendrogram revealed no deviations from the expected taxonomic relationship among the *P. ostreatus* strains, with the Romanian native strains clustering together with the reference strain PoM-421. The *P. columbinus* Pc98 strain was grouped closely with this cluster, whereas the *P. citrinopileatus* Pc95 strain appeared more distantly related (Figure 6).

According to the genetic distance values, Po1Br and Po2T exhibit a distance below 0.1, indicating an extremely high genetic similarity (Sharma et al., 2018). Similarly, Po1Br and Po2T, Po3M and Po7B, Po4V and Po6M, together with Po5C show genetic distance values below 0.45 among themselves, suggesting a relatively recent common origin, consistent with their Romanian provenance, despite originating from different locations. The reference strain PoM-421 remained grouped together with the autochthonous *P. ostreatus* strains, showing a moderately low distance from *P. columbinus* Pc98.

Among the five RAPD markers used, the Roth 13 primer revealed the highest number of loci (23), followed by Roth 02 with 20 distinctive amplicons, thereby increasing more

substantially the resolution of the genetic variability analysis.

The combined diversity metrics derived from the RAPD dataset indicate that the ten *Pleurotus* strains exhibit a high level of overall genetic variability across the 79 detected loci. This is reflected in the total H' value of 36.97 and the mean H' of 0.468 (Table 5), that the genetic variation is broadly distributed across many loci, and that the presence/absence patterns are relatively heterogenous. In contrast, when the analysis was restricted to the subset of *P. ostreatus*, the total H' index decreased to 26, while the mean H' dropped to 0.329 (Table 5). This reduction is understandable, as 28 loci were found monomorphic in this group. The remaining 51 polymorphic loci contributed to the observed diversity, although with lower average entropy per locus, indicating more unevenly distributed bands.

Table 5. Integrated diversity metrics

Group/Index	Total H'	Mean H'	Polymorphic loci	PIC	PPB
<i>P. ostreatus</i> group	26.00	0.329	51	0.329	64.56%
All ten strains	36.97	0.468	79	0.468	

The Shannon-Weaver diversity index indicates that the *P. ostreatus* group possesses a moderate genetic variability, whereas the full set of *Pleurotus* strains shows a broad and more evenly distributed diversity.

The mean PIC value of 0.466 obtained for the full set of ten strains suggests that the dataset contains rich and well-distributed genetic information across the ten *Pleurotus* strains. Moreover, a PIC value of such magnitude confirms that the identified loci exhibit moderately high presence/absence diversity, and to each locus this variation is evenly distributed, therefore, increases the entropy and discriminatory power of the marker. However, the mean PIC value of 0.329 found in *P. Ostreatus* group reflects a moderate level of marker informativeness, still providing useful discriminatory power to distinguish the strains within this group.

The PPB value of 64.56% indicates that approximately two-thirds of the RAPD loci (more precisely, 51 of the total 79 loci) are polymorphic within the *P. ostreatus* group. This means that most markers detect some level of genetic variation among the *P. ostreatus* strains,

while the remaining 28 loci are monomorphic and reflect shared genomic markers with *P. citrinopileatus* Pc95 and *P. columbinus* Pc98 strains.

These results all together confirm that the analysed strains possess considerable genetic heterogeneity, and the RAPD marker system used is suitable for assessing the diversity within *Pleurotus* spp.

CONCLUSIONS

In our study, all tested *P. ostreatus* strains, both the seven wild strains and the two commercial ones, supported the formation of primordia and mushroom production when cultivated on a substrate of wheat straw and corn cobs supplemented with wheat bran, cornmeal, and sunflower seed cake.

The group of wild strains showed a well-defined intraspecific variability, which influenced the expression of several important cultivation cycle parameters: substrate colonization capacity, timing of mushroom emergence and their morphological characteristics, yield, and biological efficiency. The two commercial strains of *P. ostreatus* showed better results in these parameters than the wild-type ones because they were obtained through research within applied selection and breeding programs, with the main objective of achieving mushroom yields that are superior in both quantity and quality.

Although the seven wild strains were surpassed in mushroom production by the commercial strains they were compared with in this trial, the differences were not very large, both quantitatively and, more importantly, qualitatively. They developed healthy mushrooms, predominantly forming tiered clusters, with normal individual morphology typical for the species *P. ostreatus*. Among the wild isolates, Po5C strain achieved in the 1st flush the highest yield, namely 567.4 g of fresh mushrooms per 2.6 kg of substrate (21.82%), just 20.4 g less than the control commercial strain PoM-77, a statistically non-significant difference. It was followed by the Po1Br with 482.9 g of mushrooms (18.57%), Po3M with 464.1 g of mushrooms (17.85%), and Po4V with 439.7 g of mushrooms (16.91%), also with non-significant negative differences compared to the commercial strain. These wild strains have

proven to have good efficiency in converting lignocellulosic substrates, which makes them recommended for future testing and application. The RAPD technique used for the molecular analysis of *Pleurotus* mushrooms grouped the indigenous strains of *P. ostreatus* all together at 0.5 genetic distance from the commercial reference strain Po-421. The phylogenetically closer strains are Po1Br and Po2T, and to a lesser extent the strains Po3M and Po7B, respectively, Po4V and Po6M. *P. columbinus* Pc98 proved to be phylogenetically closer to *P. ostreatus* strains, while *P. citrinopileatus* Pc95 was shown to be the most genetically distant from all the other strains.

Although the phylogenetic tree reveals the clustering and the genetic distance among the studied *Pleurotus* spp., suggesting similar strains, the diversity indices show an overall variability in the whole group.

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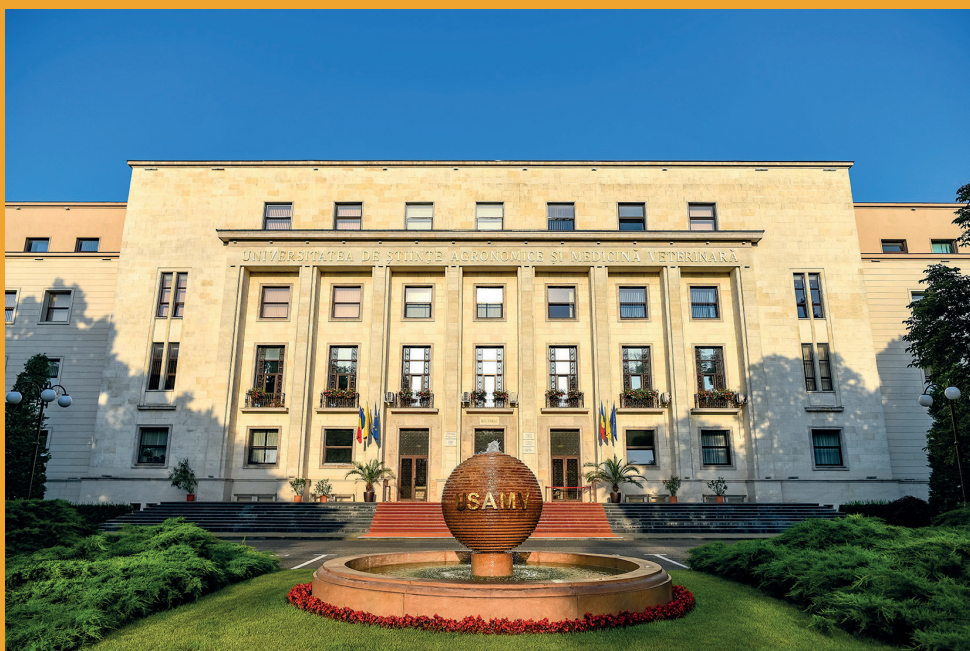
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